AN EVALUATION OF FARMER-SELECTED SPRING WHEAT GENOTYPES FROM CANADA'S FIRST ORGANIC PARTICIPATORY BREEDING PROGRAM

BY

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ABSTRACT

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Despite organic spring wheat's (*Triticum aestivum* L.) economic and cultural importance to Canadian agriculture, breeding for organic production systems remains a challenge. Organic growing environments are different from conventional farms in terms of weed species and abundance, fertility, and soil biology. More specifically, many organic farms where most of the organic wheat in Canada is grown (Alberta, Saskatchewan, and Manitoba), are deficient in soil test phosphorus (P). To address these complex challenges, the unorthodox breeding model, participatory plant breeding (PPB), has been proposed. An organic PPB wheat program has been practiced across Canada over the past decade, providing an unprecedented opportunity to explore the influence that selection environment diversity has on the agronomic performance under organic management. Field trials testing 25 PPB genotypes against 6 check cultivars across 12 environments demonstrated three PPB genotypes and one check cultivar to be top yield performers. A second experiment compared a modern cultivar and a landrace cultivar used as parental material in the PPB program, as well as the product of two farmer-selected PPB genotypes by farmers in different geographic locations from the same cross. The genotypes were tested under P limited and P-amended organic conditions, to investigate resilience against P limited conditions. There were no significant differences in yield among genotypes. Farmer genotypes were similar to the modern parent cultivar for protein concentration and lodging severity, and similar to the landrace parent in plant height and kernel mass. More detailed measurements pertinent to phosphorus use, physiology, and uptake efficiency demonstrated that two different phosphorus uptake and use efficiency mechanisms may be occurring between the farmer genotypes. Overall, this research provides evidence that early generation farmer selection is an effective breeding strategy to create distinct genotypes with phenotypic characteristics that are beneficial for organic production systems in Canada. More research is needed to

determine how PPB initiatives can better serve organic production systems with a focus on specific site selection at the early generation phase in combination with parental material that may enhance pest resistance and greater phosphorus uptake efficiency. A proposed model of future PPB breeding schemes with special attention to selection environment is presented.

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FOREWARD

This thesis is written the format of Frontiers in Plant Science and follow the guidelines of Faculty of Graduate Studies, University of Manitoba and supplementary guidelines of Department of Plant Science, University of Manitoba, Winnipeg, MB, Canada. The thesis is structured in manuscript style format and consists of an introduction to the thesis and five chapters. Chapter 1 is a literature review as well as a hypothesis and theory chapter, which incudes relevant background information for the research chapters. Chapters 2 to 4 are research chapters. Chapter 5 is a general discussion which includes practical recommendations and future research stemming from the research and conclusions. Chapter 1 was published in Frontiers of Plant Science in 2023 beginning at section 1.3.

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The contributions of all authors are as follows:

Michelle Carkner: Conceptualization, wrote the original manuscript draft and completed revisions based on peer-review feedback.

Xiaopeng Gao: Contributed to writing portions of the manuscript and editing.

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Michelle Carkner: Conceptualized and conducted experiments; collected, curated, and analysed data; interpreted results; wrote original manuscript drafts.

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INTRODUCTION

Phosphorus is an essential macronutrient for plants, and its proper management in agricultural systems is crucial for continued sustainable food production. However, many organic farms in the Canadian prairie region (the provinces of Alberta, Saskatchewan, and Manitoba, and the Peace River region in British Columbia) are extremely low in available soil P (Entz et al., 2001; Knight et al., 2010) due to geographically restrictive access to manure. Plant breeding has been proposed as a one strategy to overcome this challenge (Lynch and Brown, 2001; Ojeda-Rivera et al., 2022).

Despite organic agriculture's economic and cultural importance to Canadian agriculture, breeding for organic production systems remains a challenge. It is well established that the characteristics of the early generation selection environment impacts the final performance (Falconer, 1952; Wolfe et al., 2008; Crespo-Herrera and Ortiz, 2015). However, most wheat breeding in Canada occurs on conventionally management land, and therefore, genotypes produced by Canadian breeding programs are not the best fit for organic production systems. Organic production management creates growing environments that are different from conventional farms in terms of weed population species and abundance, fertility (specifically, nitrogen and phosphorus), soil biology, and greater spatial heterogeneity of fertility and weeds (Bond and Grundy, 2001; Welsh et al., 2009; Braman et al., 2016; Carkner et al., 2020).

Unpredictable weather events and seasonal extremes brought on by climate change has prompted the importance of incorporating genetic diversity into food crops, with the goal to enhance production stability and buffer against the extremes. Currently, the registration system and global markets reward breeding programs on the uniformity of plants within a population, which some argue leaves the food system vulnerable (Kahiluoto et al., 2019).

To address these complex challenges, unorthodox breeding strategies have been proposed and successfully shown to maximize genetic gains as well as incorporate crop diversity (Desclaux et al., 2012;

Ceccarelli, 2015). Decentralized crop breeding, also referred to as target environment selection, is not new (Falconer, 1952). Target environment selection differs from current plant breeding practices in that the selection and test environments do not take place in a centralized, research station setting. Instead, the genotypes are selected and tested in the specific fields in which it is meant to be grown.

Taking target environment selection a step further, for the past 30 years breeders around the world have included farmers in the parental selection and the early generation selection process on their land known as 'Participatory Plant Breeding' (PPB) (Almekinders and Elings, 2001a). Participatory plant breeding programs have gained momentum in the last 30 years, in particular under low-input, challenging environments in developing countries (Ceccarelli, 1994; Almekinders and Elings, 2001a; Ceccarelli et al., 2001). However, PPB programs across the Global North are expanding, with organic agriculture as the principle target environment (Colley et al., 2021).

Canada's first PPB program in wheat was established in 2011 in partnership with plant breeders with Agriculture and Agri-Food Canada, and the University of Manitoba's Natural Systems Agriculture lab. The program worked with over 75 farmers across Canada, generating over 50 wheat genotypes from multiple crosses selected on a diversity of farms.

The overall objective of the following Ph.D. thesis is to evaluate the phenotypic characteristics of generated PPB genotypes under a diverse set of organic testing environments on the Canadian prairies. The following thesis is then sub-divided into two objectives: (i) Evaluate the agronomic performance and yield stability of PPB genotypes compared to registered checks under multi-environment trials and, (ii) compare the agronomic and phosphorus physiology of PPB genotypes and parent material under contrasting soil P-levels. Specific objectives of this thesis were:

1. To establish the target traits required for P-efficient wheat ideotype breeding for low-P, organic production systems (Chapter 1; Literature Review).

2. To compare the agronomic performance of farmer genotypes against registered wheat cultivars under diverse organic growing environments in Alberta, Saskatchewan, and Manitoba (Chapter 2).
3. To examine the yield stability and broad adaptation of farmer genotypes and registered wheat cultivars under diverse organic growing environments in Alberta, Saskatchewan, and Manitoba (Chapter 2).
4. To evaluate the trade-offs of abiotic and biotic stresses and genotypes' phenotypic expression in targeted environments within an organic breeding program (Chapter 2).
5. To determine the agronomic performance differences between:
    a. Contrasting cultivars used as parental material in a PPB program
    b. Farmer genotypes from their parental material
    c. Compare full-sibling farmer genotypes to each other (Chapter 3)
6. To investigate traits that facilitate phosphorus uptake, phosphorus yield efficiency, and physiological phosphorus partitioning between:
    a. Contrasting parental cultivars used in a PPB program
    b. Farmer genotypic differences from the parental material
    c. Farmer genotypes with the same parents but different selection histories (Chapter 4).

CHAPTER 1. LITERATURE REVIEW

1.1. Organic production systems

Certified organic farms follow specific standards issued by the Standards Council of Canada and the Canadian General Standards Board (Canadian General Standards Board, 2015). Organic production systems are prohibited from using synthetic chemicals for the purpose of fertility, weed control, and pest control (Canadian General Standards Board, 2021). Adherence to the standards results in growing environments that are wholly different from conventional production systems. Organic grain farmers rely on grazing animals, animal manure, and green manures for fertility needs and use tillage and strategic crop rotations to control weeds (Nelson et al., 2010). The Canadian prairies are made up of Alberta, Saskatchewan, Manitoba and the Peace River region of British Columbia. Organic farms on the prairies have lower soil fertility (Entz et al., 2001; Knight et al., 2010), higher weed populations (Benaragama and Shirtliffe, 2020) than on conventional farms, and long-term experiments have demonstrated greater biological activity in well-managed organic systems compared with conventional systems (Braman et al., 2016).

Organic environments are diverse and variable depending on the crop rotation, tillage activity, organic amendments, weed density, and weed species present (Carkner and Entz, 2017; Isaac et al., 2021). Crop performance can vary temporally and spatially due to these heterogenous environments (Murphy et al., 2007; Lammerts Van Bueren et al., 2011; Messmer et al., 2012). Under conventional management, the environment is controlled as much as possible; fertility, weeds, and diseases are controlled with synthetic fertilizer, herbicides, and fungicides for the benefit of the crop. It has been argued that the opposite is true for organic environments and crops require flexibility and adaptability to the environment in which it is grown (Lammerts Van Bueren and Myers, 2012). The unique environment in organic production is one argument for a dedicated breeding approach where early generation selection takes place under organic conditions (Reid et al., 2009). The benefit of such direct selection under organic

environments for developing genotypes suited to organic production is now well established (Brancourt-Hulmel et al., 2005; Murphy et al., 2007; Reid et al., 2009; Kirk et al., 2012). Despite this knowledge, there is still a dearth of organic wheat breeding initiatives across Canada, and organic farmers on the Canadian prairies are currently relying on cultivars selected and evaluated under conventionally managed environments. The parameters identified as beneficial in organic production include increased height (Huel and Hucl, 1996; Mason et al., 2008; Kaut et al., 2009), early plant vigour (Mason et al., 2007a; Kissing Kucek et al., 2021b), larger kernel mass (Lammerts van Bueren et al., 2002), greater biomass accumulation (Mason et al., 2008), improved kernel production efficiency (Wiebe et al., 2017), higher nutrient uptake (Lammerts Van Bueren and Myers, 2012), and enhanced disease resistance (Lammerts van Bueren et al., 2002).

1.2. Participatory breeding for organic production systems

Decentralized plant breeding initiatives, sometimes referred to as, "client-oriented plant breeding" (Witcombe et al., 2005; Vincourt and Carolo, 2018), originated in the Global South to serve farmers in low-input production systems (Ceccarelli and Grando, 2007). However, decentralized breeding initiatives are expanding into the Global North, with the majority focused on breeding for organic agriculture (Colley et al., 2021). There are two types of decentralized breeding schemes, as outlined by Witcombe et al. (2005):

(1) Participatory plant breeding (PPB) where farmers participate in making selections in the early generation plant material of a breeding program, and

(2) Participatory varietal selection (PVS) where farmers test, under farm management, an appropriate range of advanced generation plant material from a formal breeding program.

While PVS is an important aspect of decentralized breeding, this review will discuss variations of PPB programs exclusively. Decentralized breeding schemes for wheat in the Global North has taken place

in Canada (Entz et al., 2018), Italy (Petitti et al., 2018; Bocci et al., 2020), United States (Murphy et al., 2005; Dawson et al., 2008; Sandro et al., 2022) and France (Rivière et al., 2013; Goldringer et al., 2020; van Frank et al., 2020). Participatory plant breeding is often used colloquially to describe any breeding program with integration from an outside stakeholder (eg. farmer, baker, processor, consumer) separate from the formal breeding process. However, it is important to distinguish that all decentralized breeding programs vary in the level of outside stakeholder integration, how and where selections are made, and how and where advanced genetic material is evaluated.

1.2.1. Variation of logistical processes among PPB programs

Some decentralized breeding programs involve external stakeholders in every step: parental selection, early generation selection, and final evaluation included on-farm test environments (Chapter 2). Other programs incorporate stakeholders only in the selection process, and others include stakeholders only at the testing stage (Sandro et al., 2022). Strategies for parental choice vary; all past programs selected parents (whether modern or landrace cultivars or breeding populations) based on traits useful for organic agricultural systems and markets, but farmer input varied. For example, Entz et al. (2018) initially used crosses from an existing breeding program that consisted of modern parents with traits that were projected to be useful for organic production, then used crosses of parents that were locally adapted, and farmer-recommended (Chapters 2 and 3). Sandro et al. (2022) reported using parents based on baking quality, resistance to diseases, and yield under organic conditions with no indication of farmer input. Working in Italy, Bocci et al. (2020) used crosses derived from 256 parents without farmer input. However, the majority of programs used locally adapted landraces, and modern cultivars with good performance under organic conditions recommended by farmers (Dawson et al., 2011; Rivière et al., 2013; Petitti et al., 2018; van Frank et al., 2020; Kissing Kucek et al., 2021a).

The selection process within each PPB program varies as well. Programs in Italy and France have employed on-farm mass selection, relying only on natural selection over a number of years (Goldringer et

al., 2001; Petitti et al., 2018; Bocci et al., 2020; van Frank et al., 2020). Mass selection was also used within a program in the United States, however, farmers intentionally planted the early-generation population under heavy weed competition and selected the surviving plants (Kissing Kucek et al., 2021a). In France, Rivière et al. (2013) used a combination of mass selection and farmer-led single head selection. The breeding process described by Sandro et al. (2022) resembles PVS, as genotypes derived from single head selection were subjected to multiple disease nurseries on research stations, then evaluated on a farmer's field at the advanced breeding stages. In some cases, where specific disease resistance is required, on-station screening for disease that would otherwise be impossible on-farm can be beneficial prior to testing. In Canada, farmers directly selected a sub-set (300-500 head selection) from a larger population for three years (Entz et al., 2018).

In all PPB programs, testing the performance of the resulting genotypes occurs within a combination of research stations and farmers' fields, and usually in comparison with multiple check cultivars from local formal breeding programs. Some research objectives are expressly interested in local adaption, therefore, a comparison of the PPB genotypes is then compared to modern cultivars on the farm the genotypes were selected from (Rivière et al., 2013; van Frank et al., 2020). Other research has been conducted across wide geographic ranges on farms and research stations to test for broader adaptability (Entz et al., 2018; Goldringer et al., 2020; Sandro et al., 2022).

1.2.2. On-farm selection impacts on performance

Multiple studies have reported beneficial agronomic gains from on-farm selection initiatives. When farmers actively selected from genotypes grown on their own farms, early vigour, spike size, greater plant height, lodging resistance, and greater peduncle length were reported (Rivière et al., 2013; Entz et al., 2018; Goldringer et al., 2020; Kissing Kucek et al., 2021a). On-farm selection studies have reported greater yield and quality stability spatially and temporally under organic management than commercial checks (Bocci et al., 2020; Goldringer et al., 2020; van Frank et al., 2020; Sandro et al., 2022). Taller plant

height than commercial checks was one of the most common impacts of on-farm selection reported in the literature, and was often correlated with enhanced weed competitiveness (Entz et al., 2018; Goldringer et al., 2020; Kissing Kucek et al., 2021a). Lodging resistance in combination with greater plant height was inconsistent, Goldringer et al. (2020) showed greater plant height and lodging resistance and straw biomass, while Entz et al. (2018) reported greater height and more lodging among farmer genotypes than commercial checks. Rivière et al. (2013) demonstrated that mass selection on-farm was positively correlated with greater seed mass and grain weight per spike, but plant height and peduncle length weren't consistently positively correlated with selection activities. Kissing Kucek et al. (2021a) demonstrated that on-farm selection under heavy weed pressure resulted in enhanced weed competitive ability by 11.46%.

1.2.3. The Canadian Participatory Plant Breeding Program

The Canadian Organic PPB program involves farmers in early generation selection with goal of producing wheat genotypes better suited to organic production. To date, over 50 farmers have participated in the program in British Columbia, Alberta, Saskatchewan, Quebec, Ontario, Prince Edward Island, and Nova Scotia. The Canadian wheat PPB program is a collaboration with Agriculture and Agri-Food Canada (Breeder; Dr. Stephen Fox), University of Manitoba (Principal Investigator; Dr. Martin Entz, Breeder; Anne Kirk), and the Bauta Family Initiative on Canadian Seed Security. An illustrative model of how the PPB model differs from centralized, formal breeding models is shown in Figure 1-1. Entz et al. (2018) tested Manitoba farmers' PPB wheat genotypes against commercial checks under organic conditions in Manitoba. They reported similar, and in some environments, greater yield than commercial checks. Farmer genotypes were generally taller, more susceptible to lodging, and later maturing. Additionally, the study demonstrated that farmers selecting from the same cross can significantly impact the phenotypic traits of genotypes.

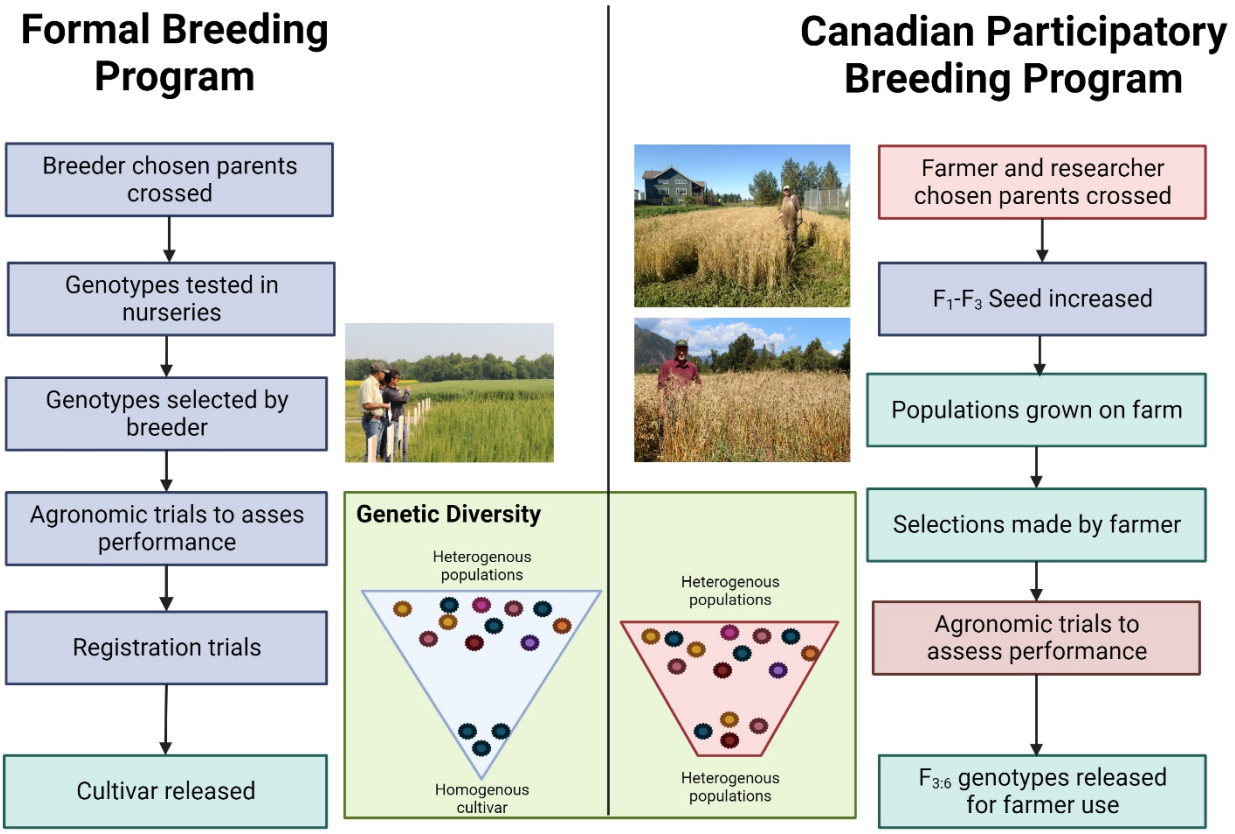

Figure 1-1. A schematic illustration of the Canadian Participatory Plant Breeding (PPB) Program compared to a formal breeding program. Blue boxes represent work done on research stations, green boxes represent work done on farms by farmers, and red boxes represent a collaboration between researchers, breeders, and farmers. The green box demonstrates the genetic diversity of the plant material that results from formal breeding programs versus the Canadian PPB program. This figure was created using BioRender. Photo credits: Michelle Carkner.

1.3. Ideotype breeding for crop adaptation to low phosphorus availability on extensive organic farms

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1.4. Abstract

Organic farmers in extensive production regions, such as the Canadian prairies have a particularly difficult challenge of replenishing soil reserves of phosphorus (P). Organic grains are exported off the farm while resupply of lost P is difficult due to limited availability of animal manures and low solubility of rock organic fertilizers. As a result, many organic farms on the prairies are deficient in plant-available P, leading to productivity breakdown. A portion of the solution may involve crop genetic improvement. A hypothetical 'catch and release' wheat ideotype for organic production systems is proposed to (i) enhance P uptake and use efficiency but (ii) translocate less P from the vegetative biomass into the grain. Root traits that would improve P uptake efficiency from less-available P pools under organic production are explored. The need to understand and classify 'phosphorus use efficiency' using appropriate indices for organic production is considered, as well as the appropriate efficiency indices for use if genetically selecting for the proposed ideotype. The implications for low seed P and high vegetative P are considered from a crop physiology, environmental, and human nutrition standpoint; considerations that are imperative for future feasibility of the ideotype.

1.5. Introduction

1.5.1. Phosphorus challenge on organic farms

Phosphorus (P) management is a particular challenge for Canadian organic farms on the prairies. While most conventional farming systems heavily rely on inputs of synthetic nitrogen (N) fertilizers, organic farms often maintain N levels through growing legumes within the green manure and forage phase of a crop rotation. However, replenishing P is more difficult on organic farms as crop harvest removal of grain or biomass continues to shrink the soil nutrient reservoir (Morrison and Kraft, 1994). Several on-farm studies have reported low soil test phosphorus status on Canadian organic farms (Entz et al., 2001; Martin et al., 2007; Roberts et al., 2008; Knight et al., 2010). Entz et al. (2001) surveyed 14 organic farms in Manitoba, Saskatchewan, and North Dakota, USA, and reported an average soil test phosphorus of 15 kg P ha^{-1}, which was substantially lower than the Manitoba average value for agricultural lands (> 20 kg P ha^{-1}) (Entz et al., 2001). After 13 years of organic production at the Glenlea Long-term Rotation Study site in Manitoba, soil available P fractions rapidly declined in the organic forage rotation (Welsh et al., 2009; Carkner et al., 2020). Additionally, low soil test phosphorus has also been reported on organic farms which lack livestock in Saskatchewan (Knight et al., 2010), and organic dairy farms in Ontario (Roberts et al., 2008). Low available P has been shown to decrease organic grain production in the long-term (Carkner et al., 2020), and limits the productivity of legumes in commercial green manure crops (Thiessen Martens et al., 2021).

Phosphorus is an essential plant macronutrient, as it contributes as a critical structural component of nucleic acids and plays a key role in energy transfer (Marschner, 1995; Grant and Flaten, 2019). Currently, the approved P fertilizer options for organic use are manure and rock phosphate. However, manure is often prohibitively expensive to purchase and transport, especially for large, stockless organic farms on the Canadian prairies where animal manure sources are geographically separated from cropland (Schneider et al., 2019). Moreover, phosphorus in rock phosphate is generally unavailable in the year of

application due to its low solubility, especially when applied to calcareous soils with high pH, which is a common characteristic of Canadian organic farms (Martin et al., 2007). Despite the many attempts to increase the availability of rock phosphate through measures such as co-composting, microbial associations, and green manure residue management (Asea et al., 1988; Arcand and Schneider, 2006; Arcand et al., 2010; Ditta et al., 2018; Billah et al., 2020), these methods showed limited effectiveness in improving agronomic response to rock phosphate in organic cropping systems in Canada (Arcand et al., 2010). Additionally, rock phosphate is mined from a non-renewable resource, counterintuitive to the organic philosophy of closing the nutrient cycle on farm (Nicksy and Entz, 2021). Other promising forms of P using unconventional sources are currently being explored on organic farms such as frass from black soldier fly (BSF; *Hermetia illucens*) larvae, anaerobically digested urban food or manure waste, and struvite ($NH_4MgPO_4 \cdot 6H_2O$) which is a mineral extracted from municipal wastewater streams or manure (Nicksy and Entz, 2021; Thiessen Martens et al., 2021). However, these options are prohibitively expensive, or not approved for organic use under the current Canadian Standards (ex. Struvite which is sourced from human waste-water sources (Canadian General Standards Board, 2021). Improving on-farm P uptake/use efficiency of crops and reducing external P imports play a key role for the sustainability of Canadian organic farms.

The Canadian prairies comprises of Alberta, Saskatchewan, Manitoba, and the Peace River region of British Columbia, and represent 50% of all organic land in Canada. This region is known as one of the bread baskets of the world, and wheat is well adapted to grow under cool, wet conditions in central and eastern Manitoba as well as drier, hotter conditions in Saskatchewan and Alberta. Prairie organic farms grow 93% of Canada's organic wheat, reaching nearly 376 000 hectares in 2020 (Canada Organic Trade Association and Prairies Organic Development Fund, 2021). Canada exported approximately 237 000 metric tonnes of organic wheat valued at over $118 000 000 in 2020 (Agriculture and Agri-Food Canada, 2022). Recent premiums for organic grade wheat grain are at 253% of conventional grade wheat grain

prices (Organic Biz, 2023). Wheat is often the cash crop in an organic rotation, providing essential economic value to farmers.

Crop selection and breeding for greater P use efficiency (PUE) and P uptake under low soil test P has been proposed as a potential solution to tighten the P cycle on farm (Rose et al., 2013). Phosphorus management on organic farms brings unique challenges as these farms rely heavily on biologically mediated nutrient supply, that is, mineralizing P from soil organic matter (SOM). Therefore, specific strategies and perspectives are required to optimize P uptake in partnership with crops and reduce off-farm P losses. The development of new crop cultivars that address P challenges on organic farms can contribute significantly to this goal.

1.5.2. Proposal of wheat ideotype to optimize acquisition and utilization on organic farms

One approach to deploying genetic resources to achieve specific breeding goals is to develop a crop ideotype. An ideotype is defined as "a biological model which is expected to perform or behave in a predictable manner within a defined environment" (Donald, 1968). For common bean and maize cultivars in the Americas, Latin America, and Asia (Lynch and Brown, 2001; Wang et al., 2010a; Lynch, 2011; Richardson et al., 2011), an ideotype has been proposed to enhance plant performance under low P conditions that maximizes P uptake through topsoil foraging root architecture, and enhanced soil-P mining strategies. In this paper, we propose a hypothetical wheat ideotype that can maximize P uptake and minimize off-farm P losses via grain P exportation for organic production systems (Figure 1-2). The P-efficient cultivar is characterized by three main features: (i) root topsoil foraging strategies to increase P acquisition, (ii) root mining strategies to mineralize P from organic pools, and (iii) greater P utilisation efficiency (e.g., greater yield per unit P applied) (Richardson et al., 2011). We further propose a reduced translocation of P from shoot biomass into grain should be considered as an important feature for organic production systems. While this concept is not new (Raboy, 2007; Richardson et al., 2011; Rose et al., 2013, 2022; Julia et al., 2018), the importance of P translocation into grain relative to other traits has not been

highlighted when considering overall P use efficiency in cropping systems, especially within the context of organic production systems. In addition, to the authors' knowledge, the implications of lower grain P as a food source beyond the farm gate and as a seed source in organic systems have not been well investigated. The goal of this paper is to explore the potential of incorporating plant traits to increase P acquisition and lower translocation of shoot P into grain P by considering the distinctive nature and needs of organic production systems. Additionally, implications for lower grain P beyond the farmgate and as a subsequent seed source are further explored.

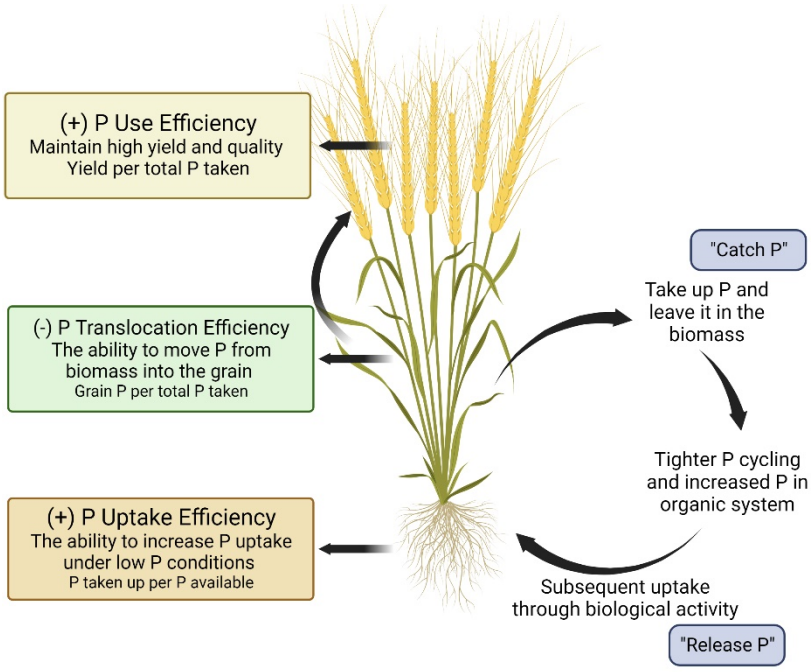


Figure 1-2. A visual model of the 'organic ideotype' of wheat for low phosphorus organic cropping systems. This figure was created with BioRender.

1.5.3. The phosphorus cycle in agroecosystems

The soil P cycle is a complex and dynamic process that involves a range of biological and geochemical transformations influenced by various environmental factors (eg. soil moisture and temperature). Plants can only take up P in the form of HPO_4^{2-} (soil pH 4.0-7.2) or $H_2PO_4^-$ (soil pH >7.2), which are often referred to as plant available P (Pierzynski et al., 2005). Plant available P concentration in

soil solution is typically low, less than 1% of the total P in the soil (Pierzynski, 1991). For optimal plant growth, P concentration in soil solution should exceed 0.2 mg P L^{-1}. However, a P concentration between 0.2-0.3 mg P L^{-1} indicates the potential for eutrophication in water bodies (Pierzynski et al., 2005; Bacelo et al., 2020), emphasizing the need to understand and manage the P cycle in agroecosystems.

The majority of soil indigenous plant available P originates from weathering of apatite (Pierzynski et al., 2005). In agricultural systems, plant available P pool in soils is also enriched by application of synthetic fertilizers or manure. Once P in soil solution exists as free ions, it can react with dissolved iron (Fe), aluminum (Al), manganese (Mn) in acid soils, or calcium (Ca) and magnesium (Mg) in alkaline soils to form phosphate precipitates (Figure 1-3). Plant available P can also be adsorbed onto clays and the oxides of Al and Fe*,* taken up by plant roots, or incorporated into the Organic-P pool as microbial infrastructure and/or organic matter (*immobilization*) (Jakobsen et al., 2005; Pierzynski et al., 2005; Drinkwater et al., 2017). Additionally, microorganisms in the rhizosphere may compete with plants for plant available P in the short-term. However, they also have the potential to release P to plants through the process of *mineralization*. Through continuous biological and geochemical reactions, P available to plants and microorganisms are in a constant flux between mineralization/immobilization and adsorption/desorption processes. For the interest of this paper, biological processes (i.e., mineralization/immobilization, root uptake) will be emphasized while geochemical processes (adsorption/desorption, dissolution/precipitation), although extremely important regarding plant assimilation, microbial recycling, and environmental implications, will be given less attention.

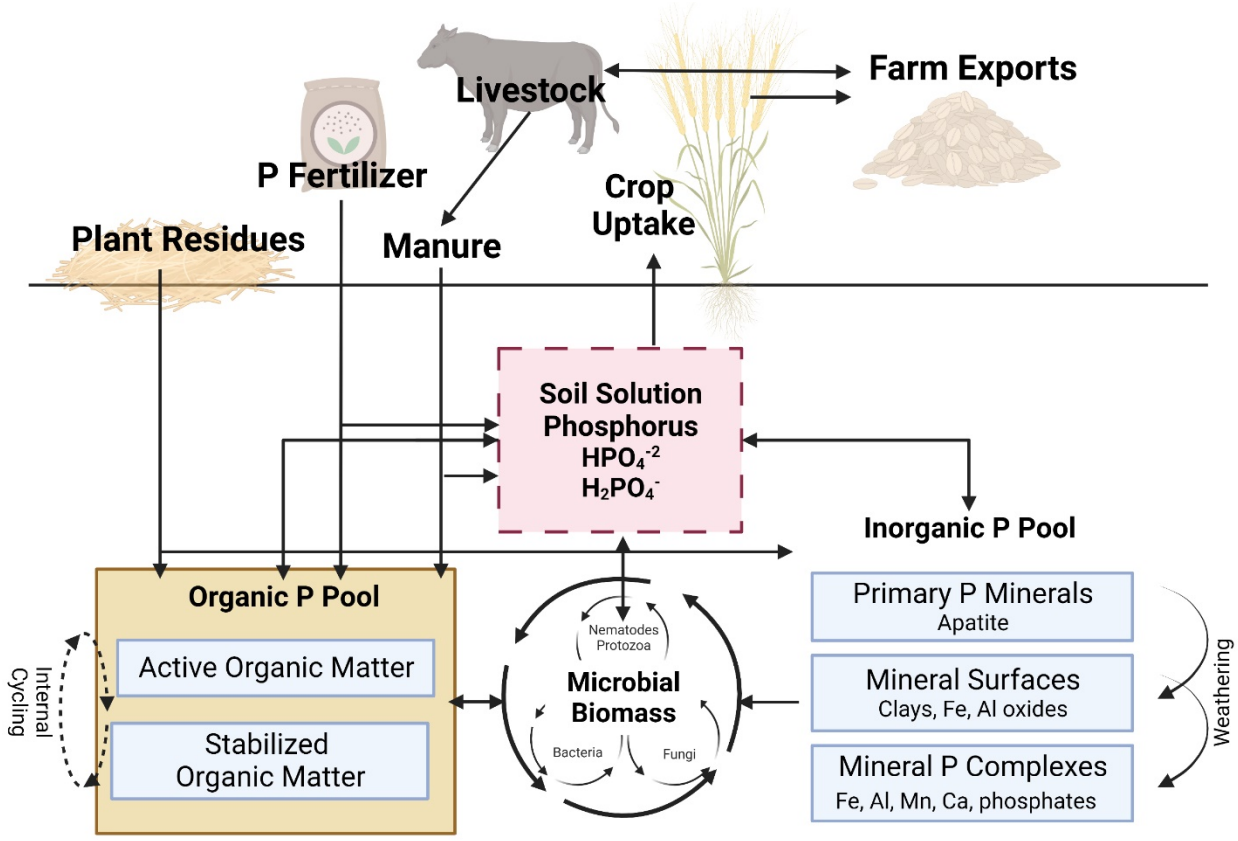


Figure 1-3. A simplified illustration of the phosphorus cycle in agroecosystems. See text for full discussion of cycling processes. P, phosphorus; Fe, iron; Al, aluminum; Mn, manganese; Ca, calcium. Adapted from Kovar and Claasen, 2005. This figure was created with BioRender.

1.5.4. The organic P pool and microbial biomass P

Soil organic P refers to P that is bonded in some way with carbon (C). Soil organic P is initially derived from animal wastes and plant residues and is synthesized by soil organisms. Plants and microorganisms take up and assimilate soil solution P which is then bonded to C through phosphorylation (Condron et al., 2005). Soil organic P consists of various forms including orthophosphate monoesters, inositol phosphates (e.g., phytic acid), phosphoproteins, mononucleotides, sugar phosphates, phospholipids, teichoic acid, aromatic compounds, phosphonates, and organic phosphate anhydrides. Many of these compounds exist in the form of highly stable ring structures, making them resistant to hydrolysis and less accessible to plants (Condron et al., 2005; Jones and Oburger, 2011).

It is estimated that organic P pools make up 30% to 80% of soil total P, depending on the cropping systems (Harrison, 1987; Bhattacharya, 2018). The organic P pool is made of dead material from plant, animal, and microbes. The microbial biomass includes bacteria, fungi, algae, protozoa, nematodes, which make up between 2 to 5% of total soil organic carbon (Brookes et al., 1984). The microbial biomass component within SOM is the 'live' fraction and responsible for mineralization of nutrients such as P (Jakobsen et al., 2005). The abundance and activity of soil microbes are heavily reliant on C inputs, as well as suitable soil moisture and temperature regimes. Microbial biomass P has been reported to account for 2 to 5% of the soil total P and approximately 10 to 15% of the soil organic P (Richardson and Simpson, 2011).

Mineralization of organic P into plant available P is dependent on the size of the microbial P pool, microbial activity, and the time required for the nutrient pool to renew itself (Oberson et al., 2001). Quantifying the size and turnover rate of microbial P during a crop growing season is challenging due to variations in temperature and moisture content. Using ^{33}P isotope tracer in four calcareous soils in Ontario, Schneider et al. (2017) showed that the velocity of microbial P turnover was highest in soil with the lowest available P, despite microbial biomass P concentrations being the same. Using fumigation methodology to assess microbial P content and turnover, Oehl et al. (2001) reported that organic cropping systems had greater microbial biomass P pools and faster turnover rates than conventional systems. Similar results were observed in Canada by Braman et al. (2016). Therefore, the form and rate of P inputs can influence organic P dynamics and availability. Bünemann (2015) reviewed studies on organic P dynamics and reported that the relative contribution of biological and biochemical mineralization of P isotopes to plant available P ranged between 20 and 35% in arable soils, and 50 to 70% in grassland soils. Microbial P dynamics in relation to crop type grown is poorly understood and understudied. To our knowledge, only one study has investigated such a relationship and reported that addition of buckwheat

residues with different types and rates of phosphate rock had little effect on the microbial biomass P in an organic dairy farm in Ontario (Arcand et al., 2010).

Numerous studies have demonstrated that plant P availability is also dependent on N availability in the soil system (Lemaire et al., 2021). For example, Briat et al., (2020) illustrated greater P uptake was coupled with greater N supply. Nitrogen mineralization in a cropping system is largely dependent on factors that also influence microbial P mineralization. Therefore, a whole soil system approach is required to understand soil-P availability, especially under organic management, where crops rely heavily on biologically mediated nutrient supply for both N and P. Furthermore, the role of livestock integration into cropping systems also requires attention. The integration of crop-livestock on organic farms in Canada has multiple benefits ecologically and economically (Entz and Thiessen Martens, 2009; Thiessen Martens and Entz, 2011). Additionally, recent arguments have been made that livestock can not only be a source of nutrients (ie. Manure), but herbivory action has the potential to catalyze nutrient cycling, increasing the microbial pool, and thus creating nutrient pools that cycle more efficiently with less potential for loss (Soussana and Lemaire, 2014; Lemaire et al., 2023). Taken together, achieving efficient P-cycling on organic farms through breeding is important, however, managing the soil system to ensure efficient N-P cycling is equally critical for long-term sustainable production.

1.5.5. Phosphorus uptake in plants and microorganisms

Phosphorus is relatively immobile in soil solution, meaning that plant roots and microorganisms must navigate towards P in the soil for uptake (Kovar and Claassen, 2005). Roots and microorganisms will encounter new available P pools as they move into unexplored soil that has not been depleted. Phosphorus is transported to microorganisms and plant roots by either mass flow or diffusion. Mass flow involves dissolved P moving towards the plant root/microorganism along with water. Phosphorus transport via mass flow accounts for a very small total P absorbed, even when P concentration in the soil solution is high. Diffusion is the process through which P moves from an area of high concentration to low

concentration, accounting for approximately 95% of root P uptake (Kovar and Claassen, 2005). Diffusion is also the main uptake mechanism for microbes such as bacteria and fungi (Jansson, 1988). In plant roots, P uptake from soil is rapid and occurs within cells behind the root tips (Kovar and Claassen, 2005). Phosphorus in the soil solution is much lower than that of the cells within the plant, so P is actively moved across the root membrane by phosphate transport proteins against a concentration gradient (Smith et al., 2003). Phosphate transporters have also been detected in fungi and bacterial organisms (Jansson, 1988).

When P in soil solution is taken up by plant root or microorganism, it creates a 'depletion zone' adjacent to uptake site (Smith et al., 2003), necessitating constantly increased P access. There are two principal strategies to increase P access as 1) greater soil exploration to new zones of higher inorganic P (via better root growth or association with arbuscular mycorrhizal fungi (AMF), and 2) P exploitation via chemical and biological P transformations to increase more available P uptake (York et al., 2013; Fraser et al., 2015) (Figure 1-4). Plants and microorganisms may employ either P exploration or P exploitation, or a combination of both (Richardson et al., 2011).

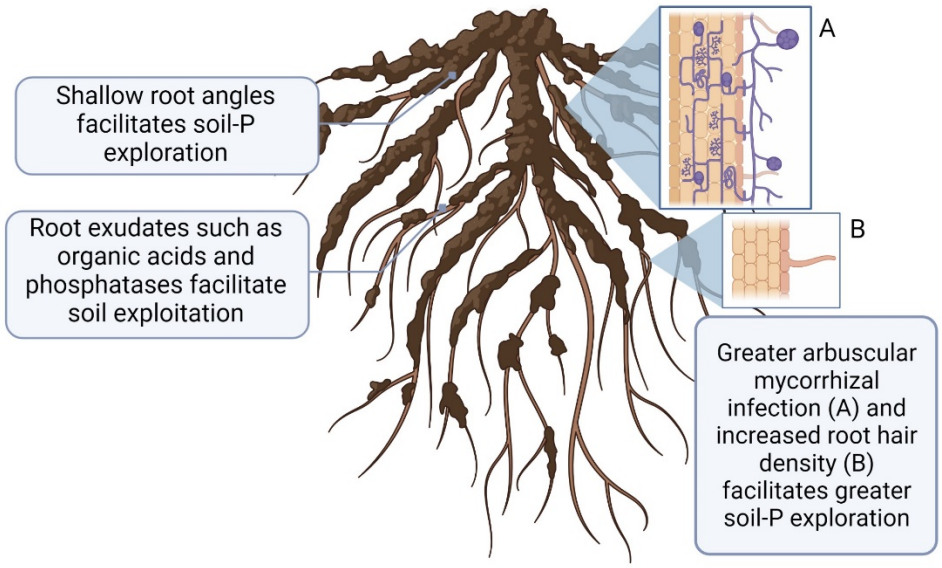


Figure 1-4. A schematic representation of root characteristics associated with greater P uptake adaptations to low soil P availability. This figure was created with BioRender.

1.6. From the ground up: greater P uptake in organically managed soils

1.6.1. Increased physical exploration of soil

Root traits associated with increased P acquisition by explorative strategies have been extensively studied in wheat under field and greenhouse conditions (Mcdonald et al., 2015; da Silva et al., 2016; Wang et al., 2016b; Rabbi et al., 2017; Nguyen and Stangoulis, 2019) and summarized in several review papers (Gahoonia et al., 1999; Lynch and Brown, 2001; Lynch, 2011; Richardson et al., 2011). Greater exploration of the upper soil layer (0-10 cm) by crop root systems is essential due to the generally low P mobility in soils. Root architecture can be divided into the geometric properties that dictate the shape of the root system (root angle, depth, and configuration), and structural properties (pattern of root branching, and growth of root hairs). While some previous works have investigated how root architecture can affect P uptake of bean and corn in response to P deficiency (Lynch and Brown, 2001; Richardson et al., 2011), less research has been done with wheat. The 'topsoil foraging' root architecture phenotype, which is characterized with wide basal and shallow seminal root angles, has been proposed to maximize soil

exploration (Manschadi et al., 2013; Lynch, 2019). Structural characteristics such as greater root hair density, and branching are important for P uptake as they increase root surface area and the volume of soil from which immobile P can be explored, especially under P deficient environments (da Silva et al., 2016). Genotypic differences in root angle have been observed in wheat (Maccaferri et al., 2016; Fradgley et al., 2020; Pariyar et al., 2021), additionally, different root angle responses to contrasting environmental conditions has been widely observed (Manschadi et al., 2013; Chen et al., 2018; Sinha et al., 2018). For instance, greater P uptake in Brazilian wheat genotypes was associated with shallow-angled first and second brace roots (da Silva et al., 2016). Gahoonia et al. (1999) reported that under field conditions barley cultivars with longer root hairs depleted more P from the rhizosphere soil and absorbed more P. Within the same study, wheat genotypes grown in hydroponic media grew longer and greater dense root hairs in response to P deficiency (Gahoonia et al., 1999). Contrary to 'topsoil foraging' characteristics, Manske et al. (2000) proposed that wheat genotypes with more developed root systems may better access indigenous P in deeper soil profiles, which may be advantageous to organic farms. However, further investigation is required to determine the optimal root architecture for P acquisition of crops under organic farming systems.

Association with AMF is another strategy plants may use to increase soil exploration capacity. A mutualistic relationship between the host plant and AMF is characterised by a bi-directional nutrient transfer between the two species. The fungus receives carbon substrates from the host plant, and the plant receives nutrients in return (Harrison, 2005). In addition to their roles in P nutrition, AMF also provides other benefits to the host plant such as greater zinc uptake (Gao et al., 2007), lower grain cadmium levels (Singh et al., 2012) and higher water use efficiency (Li et al., 2019). AMF increases P uptake through multiple strategies. They can enhance the host plants' ability to explore greater soil volume by extending their hyphal network beyond the crops' P depletion zone (Pepe et al., 2018). They can also stimulate the abundance and activity of bacteria in the rhizosphere that excrete alkaline phosphatases to

mobilize organic-P (Zhang et al., 2016, 2018), thus promoting a highly efficient P-affinity system (Harrison, 2005; Kobae, 2019). Root colonization rates by AMF are affected by the plant-available P levels in soils. For example, under high soil Olsen-P conditions (>50 mg P kg^{-1}), plants can access P independently and root AMF colonization decreases (Entz et al., 2004; Schneider et al., 2015). In contrast, under very low soil test P conditions where plant growth is P-limited, root AMF colonization may become parasitic, due to greater carbon acquisition by the fungi relative to the lower P translocation from the fungi to the plant host (Johnson et al., 1997). We confirmed such phenomenon in organic farming systems by showing that root AMF colonization of flax was significantly higher in the low-P organic system relative to the conventional system (Entz et al., 2004).

Genotypic variation in root AMF colonization has been observed in various crop species including wheat (Kirk et al., 2011; Singh et al., 2012; Nahar et al., 2020). However, root AMF colonization depends on both soil environmental conditions and management practices. Production practices used by organic farmers such as cover crops and forages (Lehman et al., 2012; Njeru et al., 2014) and conservative P additions (Schneider et al., 2016) are known to promote root AMF colonization. However, other practices used by organic farmers such as frequent and deep tillage, growing non-mycorrhizal crops, and fallow are known to reduce AMF populations (Gosling et al., 2006). Despite these challenges, satisfactory AMF populations have been found on organic farms (Ryan et al., 1994; Kirk et al., 2011; Chen et al., 2022). At the Glenlea long term rotation study site in Manitoba, Welsh (2007) observed increased diversity and spore abundance of AMF in organic compared to conventional farming systems. Several previous studies have reported a positive relationship between root AMF colonization and crop P uptake efficiency under low P soil conditions (Manske et al., 2000; Nahar et al., 2020). For example, Manske et al. (2000) reported that root AMF colonization rate of 42 wheat genotypes was positively correlated with their P uptake efficiency when grown under P deficient conditions. Similarly, the benefits of AMF to increase P uptake and cause crop growth under low P conditions were frequently reported in other studies (Feng et al.,

2003; Wang et al., 2017). Organic farms would benefit from selecting genotypes with a greater affinity to AMF to overcome the P constraints to crop growth. Therefore, high AMF partnership affinity maybe a valuable quality trait to be included in crop breeding program in organic systems, and efforts to increase the capacity for high throughput evaluation of root AMF association are encouraged.

1.6.2. Accessing where you are: Soil exploitation

Plants have multiple strategies that can increase soil P availability by immobilizing the less soluble P in the inorganic and organic P pools. Kovar and Claassen (2005) reported that plant roots can exudate organic acids into the rhizosphere to solubilize P from Fe and Al complexes in acidic soils and from Ca and Mg complexes in alkaline soils. However, the organic acid compounds in root exudates can vary with crop species and genotypes, and the mechanisms are not well understood (Kovar and Claassen, 2005). Some plants can also excrete phosphatase enzymes into the rhizosphere to enhance mineralization by breaking down carbon and P ring structures within soil organic matter (Li et al., 2004; Nguyen et al., 2019). The following section will explore the potential of soil P exploitation within the context of genotypic variation among grass species and the potential to select/breed such traits for maximizing crop P acquisition.

By comparing six spring wheat genotypes in a hydroponic nutrient solution study, Akhtar et al. (2016) reported a significant relationship between greater plant P uptake and a decrease in solution pH. Also under hydroponic conditions, Gaume et al. (2001) revealed significant differences in organic acid exudation among maize genotypes in response to P deficiency. It was concluded that developing maize genotypes with increased citric and malic acid in root exudates can be an effective strategy to adapt to low P conditions. Nguyen et al. (2019) investigated wheat root exudates under varying P availability in sandy soil and found that a P-efficient genotype ‘RAC875’ released larger amounts of organic acids in root exudates under P deficient compared to adequate conditions. Metabolomics can provide direct measurements of biochemical activities present in cells, tissues, or an organism (Saia et al., 2019), and it has the potential to work in tandem with genomics to screen breeding genotypes for metabolites. For

example, metabolomics can detect organic acids and phosphatases present in root tissues between genotypes under P stress (Nguyen et al., 2019). Clearly, the strategies wheat genotypes use to access P under low P conditions varies widely. The challenge for breeders and physiologists interested in enhancing P acquisition and utilization in genotypes will be to identify the most beneficial traits for their specific breeding goals.

Despite low P levels on organic farms, satisfactory yields can still be produced (Martin et al., 2007). Additionally, research in Ontario (Schneider et al., 2017) and Manitoba (Braman et al., 2016) reported higher organic P content in forage-based soils in organic relative to conventional farming systems, which may explain why organic farms maintain acceptable forage yields despite low soil test P. Organically managed soils are sometimes characterized by more abundant and diverse soil microbial communities (Mäder et al., 2002; Braman et al., 2016), which can lead to greater soil nutrient supply due to the increased mineralization capacity. This leads to questioning the relevance of current soil P tests dictating availability on organic farms, due to richer soil microbial communities (Braman et al., 2016), and the potential for biologically mediated P supply (Welsh et al., 2009).

Unpredictable fertilizer response in agroecosystems has led to a recent argument that researchers and practitioners can no longer rely solely on soil tests as a diagnostic tool for crop fertility (Lemaire et al., 2021). The interactions between plant demand and soils, nutrients to each other, and the role microbial communities play in the rhizosphere also needs to be considered (Briat et al., 2020). Bioassay diagnostic tools evaluating crop uptake for plant P nutrition in organic production systems (Carkner et al., 2020), and the creation of the N Nutrition Index (Lemaire et al., 2008, 2021) have been proposed. Greater understanding of plant-soil interactions and proper diagnostic tools are needed to accurately assess genotypic variation in P uptake.

Given greater biological activity and potentially larger organic P pools on organic farms, increasing cultivars' capacity to access these pools would reduce the reliance on external P inputs (Sattari et al., 2012; Menezes-Blackburn et al., 2018). However, relying only on the soil organic P pool can lead to organic matter mining and decomposition. Therefore, adequate crop residue return is essential on organic farms (Arcand et al., 2016). The proposed P efficient wheat ideotype considers the unique P dynamics in an organic cropping system and the untapped potential of biologically mediated P supply between the root-soil interface. The ideotype would need to possess root characteristics of soil exploration and exploitation to facilitate greater uptake under low P, organic conditions.

1.7. Challenges of using the correct phosphorus use efficiency indices for screening genotypes

Investigating crop cultivars for phosphorus use efficiency (PUE) has been proposed as an effective way to close the P cycle on organic farms (Vance et al., 2003; Schneider et al., 2019). The term PUE is recognized as a combined effect of: (1) increased acquisition and uptake, and (2) increased P utilization (Vance et al., 2003; Veneklaas et al., 2012; van de Wiel et al., 2016; Cong et al., 2020). The term PUE has been used inconsistently throughout literature, and evaluated using different calculations (Bovill et al., 2013, Table 1-1). High P utilization efficiency is defined as growth/biomass production per unit P uptake and is highly associated with the remobilization of P from old to new tissues. In the last decade, little progress has been made in breeding crops with higher P utilization efficiency (Rose et al., 2011; van de Wiel et al., 2016). To select for greater P utilization efficiency, some breeders have chosen to either evaluate cultivars that can maintain high yields under lower soil-P status or increase yields without increasing fertilizer rates. In organic farming, it is critical to develop cultivars that can maintain high yield and quality under low soil available P status. Therefore, an ideotype for organic farming should maximize soil exploration through better root architecture, increased root hair growth and AMF colonization, and enhance soil exploitation through higher root exudates of organic acids and phosphatase.

Table 1-1. Terms and calculations used to assess phosphorus use efficiency (PUE). Adapted from Bovill et al. (2013)

PUE Indicator	Formula	Reference
Agronomic P Use Efficiency	Yield increase/P applied	Hammond et al., 2009
P Use Efficiency (I)	Yield/nutrient supplied	Manske et al., 2001
P Use Efficiency (II)	Shoot biomass/P uptake	Wissuwa et al., 1998
P Use Efficiency (III)	$Yield_{-P}$ / $Yield_{+P}$	Mcdonald et al., 2015
P Use Efficiency (IV)	P Uptake Efficiency*Yield/Total Plant P	Manske et al., 2001
P Uptake Efficiency (I)	Total aboveground P/P applied	Osborne and Rengel, 2002
P Uptake Efficiency (II)	Total P accumulated/root weight or length	Liao et al., 2008; El Mazlouzi et al., 2020
P Uptake Efficiency (III)	Total Plant P/P Supplied	Moll et al., 1982 via Manske et al., 2001
P Acquisition Efficiency	Total Plant P/P Applied	Osborne and Rengel, 2002
P Utilisation Efficiency (I)	Grain yield/Total P Uptake	Manske et al., 2002; El Mazlouzi et al., 2020
P Utilisation Efficiency (II)	Shoot dry weight/Shoot P Concentration	Siddiqi and Glass 1981
P Utilisation Efficiency (III)	P harvest index/grain P concentration	Manske et al., 2001
P Harvest Index	Grain P/Total P	El Mazlouzi et al., 2020b
P Utilisation Efficiency (DM)	Shoot Weight/Shoot P	Mcdonald et al., 2015
P Utilisation Efficiency (GY)	Yield/Grain P	McDonald et al., 2015
Shoot P Utilisation Efficiency (I)	Shoot biomass/P uptake	Su et al., 2006
Shoot P Utilisation Efficiency (II)	Shoot biomass/P uptake (shoots and roots)	Osborne and Rengel, 2002
Biomass Utilisation Efficiency	Biomass yield/P uptake	Batten, 1992
P Efficiency Ratio (I)	Yield/P Uptake	Jones et al., 1989
P efficiency Ratio (II)	Shoot growth at low P/Shoot growth adequate P	Ozturk et al., 2005
Relative Grain Yield	$Yield_{-P}/Yield_{+P}$	Graham, 1984
Root Efficiency Ratio	Total plant P/Root Dry weight	Jones et al., 1992
Apparent Remobilisation of P (%)	Apt_1 - Apt_2 / Apt_1 x 100 Apt_1 = P conc. in shoot at first harvest Apt_2 = P conc. in shoot at second harvest	Hocking and Pate, 1977
Phosphate Acquisition Efficiency	$Shoot_{-P}/Shoot_{+P}$	López-Arredondo et al., 2014

Depending on the calculations used, the selection for P efficient genotypes could be vastly different. Many studies have based PUE calculations on early nitrogen use efficiency (NUE) work, which calculated NUE as the ratio of grain N uptake per unit of N available in the soil (Manske et al., 2001, 2002; Ortiz-Monasterio et al., 2001; Manschadi et al., 2013; Mcdonald et al., 2015; Meier et al., 2022). This can be problematic for assessing PUE as phosphorus availability and behavior in soil-plant systems differs markedly from nitrogen. It is imperative that breeders should select the correct PUE measurements as it relates to their goals, and not rely on PUE precedence in the literature. Similarly, redefining the concept

of NUE towards integrating soil-plant relationships (Ciampitti et al., 2022) and evaluating genotypes on the basis of effective use of N rather than responsiveness to added N has also been argued (Ciampitti and Lemaire, 2022). Selecting genotypes based on their responses to added P through P acquisition efficiency or relative grain yield is inappropriate for organic production systems. For example, a genotype with poor performance under low P conditions and a greater response to P fertilizer might yield well in conventional systems but would not be desirable for organic farming. In contrast, a genotype that has high uptake potential under low or biologically mediated nutrient supply, yield per unit of P uptake, and low P translocation from the vegetative to reproductive organs would be useful. Wheat cultivars with such traits can take up high amounts of soil native P in the current growing season while releasing P for the following crop when wheat residues are returned (Figure 1-2).

Selecting crop cultivars that can simultaneously increase P uptake and utilization efficiency is a challenge since the two traits are intimately linked. Increasing P uptake, and therefore P in biomass often reduces internal utilization efficiency (Veneklaas et al., 2012). Therefore, it is suggested that different genotypes should be targeted for uptake and utilization to optimize the overall PUE. Ultimately, finding a way to combine both traits in a single genotype needs to be considered (van de Wiel et al., 2016). Comparing cultivars' aboveground biomass per unit P uptake at anthesis before P translocation from biomass into grain occurs would be beneficial to maximize P uptake and utilization potential.

1.8. The consequences of low translocation of vegetative P to grain P

The phosphorus harvest index (PHI) is defined as the ratio of grain P to total plant P, and it represents the amount of P translocated from the vegetative biomass into the grain (El Mazlouzi et al., 2020b). Once P is taken up and used for vegetative growth, the remaining P is stored in the vacuoles (Veneklaas et al., 2012). Depending on P supply, pre-anthesis P uptake can contribute up to 81% of grain P accumulation (Batten, 1992; El Mazlouzi et al., 2020b). It has been proposed that selecting crop genotypes with lower PHI would be a beneficial trait to reduce external P inputs (Batten, 1992; Rose et

al., 2013; Vetterlein and Tarkka, 2018; Cong et al., 2020). While high protein content in wheat grain is a market premium, greater grain P is not. Grain P is stored mainly as phytate and to a lesser number, chemical compounds including inorganic phosphate, phospholipids, DNA, RNA, and ATP (Rose et al., 2013). Phytate is poorly digested by monogastric mammals, and often becomes a pollutant to waterbodies from livestock and city wastes (Schneider et al., 2019). Reducing grain P could contribute to decreasing off-farm P exportation. For example, Rose et al. (2010) estimated that a 20% reduction in rice grain P would globally reduce P removal from fields by 0.4 Mt per year.

However, would reducing P translocation to grain adversely affect grain yield and quality? The relationships between PHI and yield are consistently weak (Batten and Khan, 1987; Jones et al., 1989; Rose et al., 2011; Mcdonald et al., 2015), indicating that low P translocation may not affect final grain yield. Movement of carbohydrate and P into grain sink is regulated independently, and it is reported that P movement occurs earlier and faster than carbohydrates (Batten and Khan, 1987; Peng and Li, 2005). Currently, cereal crop breeding efforts are mainly focusing on increasing grain yield while maintaining protein (Wang et al., 2003). However, little is known about how decreasing seed P would play a role. Early studies demonstrate that low seed P can be combined with satisfactory protein levels in wheat, legumes, and oilseed rape (Batten and Khan, 1987; Chitra et al., 1995; Lickfett et al., 1999). For instance, Lickfett et al. (1999) reported a significantly negative correlation between phytate and protein in crop grains. In contrast, we recently observed an inconsistent relationship between grain P and protein levels under organic management, due to P deficiency resulting in low grain P, lower yields, and high protein (Chapter 4). Grain yield and protein are generally negatively correlated (Iqbal et al., 2016), so lower grain P in combination with lower yield would be expected to result in higher protein. Further research is needed to explore the potential impact on grain quality by reducing P translocation from crop biomass into the grain on organic farms with satisfactory yield conditions.

If an ideotype can produce high yield with low grain P concentration, how will this affect seedling vigour when the grain is used as seed? Will low grain P lead to a reduction in early season vigour due to depleted seed P reserves (White and Veneklaas, 2012)? Crop seedlings rely on P reserves for early growth and root establishment, up to three weeks after germination (Grant et al., 2001; White and Veneklaas, 2012). Many studies have reported greater seedling vigour and increase P uptake due to faster root growth when comparing P-rich seeds with P-poor seeds (Thomson and Bolger, 1993; Rose et al., 2012; Lorts et al., 2020). However, source seeds for experiments are usually from P-depleted soils, and it has been argued that poorer seedling vigour may be an artifact of poor seed quality rather than low P concentration (Julia et al., 2018). Some studies with wheat and rice have also reported that seed mass, but not seed P content, influenced seedling and root growth (Derrick and Ryan, 1998; Julia et al., 2018). Additionally, Pariasca-Tanaka et al. (2015) reported significant genotypic by seed-P interactions for seedling vigour in rice, demonstrating that genetic variation may be a tool to manipulate this trait. While average wheat seed P concentration ranges from 3.4-4.5 mg P g^{-1} (Selles et al., 2011), Rose et al. (2013) reported that some genotypes with seed P concentrations as low as 1 mg P g^{-1} did not reduce germination, seedling, vigour, or final grain yield. Finally, Julia et al. (2018) demonstrated that rice seedlings acquire P from outside seed reserves after 2 days after germination. However, their research was conducted in growth media with synthetic P supply. Under organic conditions, where crops rely on biological activity for P nutrition, cold soils in the spring may hinder uptake. Greater understanding of P supply and seedling growth under organic conditions would be valuable to address this issue. Additionally, May et al. (2022) demonstrated that certain cover crops like black medic can increase soil available P over time, which may result in greater seedling nutrition to overcome low seed P reserves. Therefore, with the use of strategic ecological agronomy, there is a potential to select genotypes that can access and store greater P, while translocating less P into the seed for better P cycling. This approach may allow for breeding ideotypes with low seed P levels that do not suffer negative consequences on seedling vigor.

1.8.1. Crop residue potential to increase soil phosphorus availability

After grain harvest, organic farmers often incorporate the remaining crop residue into the soil either in the fall or early spring; this is typical in extensive systems of the Canadian prairies where straw is not collected for animal bedding. The ability of the ideotype to 'release' P back into the soil system through mineralization processes may provide a valuable nutrient source for following crops and for building soil organic matter (Kucey et al., 1989; Arcand et al., 2016), or both.

A core component of the organic ideotype is its ability to release (mineralize) P from plant residue, thus increasing P cycling in the rotation and reducing the reliance on external P inputs. The bioavailability of P in crop residue depends on the the amount and forms of P present. For instance, the biomass of wheat residue (excluding roots) has been estimated up to 7.4 t ha^{-1} (Liu et al., 2019). Damon et al. (2014) provided an extensive literature review on residue contribution to P pools in agricultural soils, reporting that average wheat residue P amounts in southern Australian grain cropping systems are 0.4, 1.8, and 5.4 kg ha^{-1}, under low, medium, and high productivity scenarios, respectively. Tillage may have an influence on P mineralization. For example, wheat residues immobilized 0.2 kg P ha^{-1} under no-till management, and mineralized 0.4 kg P ha^{-1} under conventional tillage during decomposition (Lupwayi et al., 2007). However, the authors indicated that the amount P that was mineralized was too small to contribute significantly to the following crops' P fertility. No-till management may also increase P surface runoff during spring snow melt, causing losses to the system (Grant and Flaten, 2019; Liu et al., 2019). Canadian organic farmers typically incorporate a no-till phase within their rotation (Halde et al., 2015), or leave wheat stubble untilled between harvest and time of spring crop seeding the following year. Therefore, it is essential to consider surface P runoff loss when no-till is being employed on organic farms, particularly when P content in crop residule is high.

1.8.2. Implications for environmental protection and human nutrition

Phosphorus loss from agricultural lands poses a serious threat to water quality of Canadian watersheds. Over the last decade, major Canadian lakes such as Lake Winnipeg and Lake Erie have experienced severe algal bloom outbreaks (Liu et al., 2021). When excessive P from synthetic fertilizers or manure are applied to agricultural lands, they are subject to losses and being transported by surface runoff and drainage in variable proportions of dissolved P and particulate P (Hart et al., 2004). In cold climates like Canada, dissolved P loss associated with snowmelt runoff has been identified as the dominant P transport pathway to water bodies (Jamieson et al., 2003). On some organic farms, especially those located where animal manure is plentiful, manure is often used as a primary N and P source for crops. However, due to the relatively lower N:P ratio in livestock manure relative to crop needs, manure application usually results in accumulation of P in soils and further an environmental concern for water quality. On organic farms where animal manures are less available, and hence more expensive, farmers typically use manure only to satisfy the P deficit, relying on legumes to supply N (Thiessen Martens et al., 2021).

While many previous studies have focused on improving farm management practices such as 4R Nutrient Stewardship and tile drainage (Grant and Flaten, 2019) and regulations on manure production and application, the current paper proposes crop genetic variation as a strategy to maximize crop P uptake and use efficiency. The proposed ideotype for organic farming systems will identify the key traits for PUE including 1) increased root architecture, root hair growth and AMF colonization; 2) efficient phosphate remobilisation strategies; and 3) optimizing biomass P uptake while minimizing allocation to reproductive seeds. Thus, the improved P acquisition and utilization of the ideotype on organic farm can contribute greatly to reducing P loss to water bodies by decreasing P inputs from organic sources. The proposed ideotype also addresses the concerns of producers and policymakers as it simultaneously reduces fertilizer/manure costs and environmental risks.

In addition to the environmental issue, improper P management in crop production systems can negatively affect grain nutritional quality and thereby influence human health. Phytate constitutes 60-80% of total P in most crops and dominates the storage form of P in wheat grains (Gupta et al., 2015). Phytate is often considered an antinutritional compound as it strongly binds to micronutrients such as zinc (Zn) and iron (Fe). Low bioavailability of these minerals in cereal grains can lead to deficiencies in human genotypes who rely mainly on cereal foods for calorie intakes. The phytate to Zn or Fe molar ratio in wheat grain has been generally used to categorize their bioavailability (Bouis and Welch, 2010). Canada is a world-leading wheat producer, and it exports 75% of its wheat products, including to developing countries where people are at high risk of malnutrition (Statistics Canada, 2019). Breeding a wheat ideotype with high PUE and low grain phytate can potentially play an important role in alleviating the global prevalence of micronutrient deficiencies. This can be even more promising for organic farming systems due to the benefits on grain micronutrient accumulation. For example, previous studies from the Glenlea Long-term Rotation Study site showed that wheat produced organically in the perennial rotation had higher Zn than the annual rotation, whereas there was no crop rotation effect when wheat was produced conventionally (Turmel et al., 2009). Further studies are needed to understand the biosynthesis and allocation of phytate throughout the crop life cycle and its influence on bioavailability of micronutrients.

1.9. Conclusion

We propose a hypothetical 'catch and release' wheat ideotype that possesses traits facilitating enhanced P uptake ('catch P' in biomass) under low-P supply, reducing P translocation from the biomass into the grain and thereby returning P back to the cropping system by way of crop residue ('release P'). Finally, the ideotype minimizes off-farm harvest removal for organic production systems, which impacts off-farm P pollution in addition to enhancing micronutrient bioavailability as a food source. The ideotype would carry characteristics such as greater root exploration and exploitation strategies designed to

interact with soil microbial communities. To select for greater "P use efficiency", we argue that current indices used for conventional agriculture are not appropriate, and breeders should use greater uptake efficiency, yield per unit P uptake, and P harvest index to evaluate genotypes.

Early seedling vigour is of particular importance to organic farmers because crops need to compete with early season weed competition. Early season weeds have the largest impact on final yield (Mason and Spaner, 2006). Lower seed P may hinder early seedling vigour due to poorer seed nutrition, but this is unclear. Further research examining the impact of lower seed P and genotypic effects on early vigour under organic conditions would be useful.

Lower seed P provides additional benefits of reducing exports off farm, therefore reducing P entering the wastewater system and polluting major Canadian fresh watersheds. However, residue management needs to be considered to avoid P leaching from high P biomass on farm. Lastly, low seed P is beneficial from a nutritional standpoint, as it leads to improved Zn and Fe bioavailability, potentially playing an important role in the nutritional portfolio of developing countries where Zn and Fe deficiency is prevalent. Taken together, our ideotype attempts to address P challenges on organic farms from a systems perspective, incorporating nutrient cycling dynamics, environmental considerations, and nutrition. As we move into a new paradigm of sustainable food production where external nutrients are becoming scarce and an increasing number of people face malnourishment, multi-pronged approaches will be required to address these challenges.

CHAPTER 2.

PERFORMANCE AND YIELD STABILITY ANALYSIS OF FARMER-SELECTED SPRING WHEAT (*TRITICUM AESTIVUM* L.) GENOTYPES FROM A CANADIAN PARTICIPTORY BREEDING PROGRAM

2.1. Abstract

Differential performance of wheat cultivars under organic and conventional production systems has prompted the establishment of organic breeding programs around the world. Participatory plant breeding (PPB) is a collaborative process between farmers, plant breeders, and researchers to create germplasm specifically bred for organic environments. The objective of this study was to examine the yield performance and stability of genotypes from an organic PPB wheat program under divergent organic environments across the Canadian prairies. To investigate the genotype x environment interaction effects, 25 farmer genotypes and 6 commercially registered cultivars were grown for 3 years (2020-2022) in different locations in Alberta, Saskatchewan, and Manitoba, totalling 12 environments. Using three stability models, the top performers that were most responsive to higher yield environments were three PPB genotypes and one check cultivar (Vesper). Genotype, Genotype by Environment (GGE) Biplot analysis indicated that the PPB genotype, BL23-AS and Vesper demonstrated high yield as well as better yield stability than other genotypes tested. Two registered cultivars, AAC Brandon and Jake, had low yield and low stability, as did PWA10B-LD, a farmer genotype. Yield was positively and strongly correlated with height, anthesis biomass, mature biomass, and kernel number per unit area. We also demonstrate the benefit of using GGE biplot visualization to examine organic test environment discriminatory qualities among genotypes and propose that organic breeding programs would benefit from understanding what environmental conditions positively or negatively contribute to genotypic discrimination to enhance organic breeding progress efficiency. The results provide evidence that early generation farmer selection

is an effective breeding strategy for discovering genotypes with high yield and yield stability across organic production systems in Canada.

2.2. Introduction

Certified organic farms follow specific standards issued by the Standards Council of Canada and the Canadian General Standards Board (Canadian General Standards Board, 2015). Organic production systems are prohibited from using synthetic chemicals for the purpose of fertility, weed control, and pest control (Canadian General Standards Board, 2021). Adherence to the standards results in growing environments that are wholly different from conventional production systems. Organic grain farmers rely on grazing animals, animal manure, and green manures for fertility needs and use tillage and strategic crop rotations to control weeds (Nelson et al., 2010). Organic farms on the prairies have lower soil fertility (Entz et al., 2001; Knight et al., 2010), higher weed populations (Benaragama and Shirtliffe, 2020) than on conventional farms, and long-term experiments have demonstrated greater biological activity in well-managed organic systems compared with conventional systems (Braman et al., 2016).

Organic environments are diverse and variable depending on the crop rotation, tillage activity, organic amendments, weed density, and weed species present (Carkner and Entz, 2017; Isaac et al., 2021). Crop performance can vary temporally and spatially due to these heterogenous environments (Murphy et al., 2007; Lammerts Van Bueren et al., 2011; Messmer et al., 2012). Under conventional management, the environment is controlled as much as possible because fertility, weeds, and diseases are managed for the benefit of the crop. It has been argued that the opposite is true for organic environments and crops require flexibility and adaptability to the environment in which it is grown (Lammerts Van Bueren and Myers, 2012). The unique environment in organic production is one argument for a dedicated breeding approach where early generation selection takes place under organic conditions (Reid et al., 2009). The benefit of such direct selection in target environments for developing organic genotypes is now well

established (Brancourt-Hulmel et al., 2005; Murphy et al., 2007; Reid et al., 2009; Kirk et al., 2012). Despite this knowledge, there is still a dearth of organic wheat breeding initiatives across Canada, and organic farmers on the Canadian prairies are currently relying on cultivars selected and evaluated under conventionally managed environments. The parameters identified as beneficial in organic production include increased height (Huel and Hucl, 1996; Mason et al., 2008; Kaut et al., 2009), early plant vigour (Mason et al., 2007a), larger kernel mass (Lammerts van Bueren et al., 2002), greater biomass accumulation (Mason et al., 2008), improved kernel production efficiency (Wiebe et al., 2017), higher nutrient uptake (Lammerts Van Bueren and Myers, 2012), and enhanced disease resistance (Lammerts van Bueren et al., 2002).

The Canadian PPB program involves farmers in early generation selection and in the hope of producing wheat genotypes better suited to organic production (Entz et al., 2018), and to date, over 50 farmers have participated in the program. Entz et al. (2018) tested PPB wheat genotypes selected by Manitoba farmers, under Manitoba organic environments. The present study considers a wide array of PPB genotypes selected in multiple locations across Canada created between 2013-2020. Our first objective was to evaluate the performance of these geographically diverse PPB wheat genotypes in different organic environments across the Canadian prairies against check cultivars. I hypothesized that PPB genotypes will exhibit significant yield variation across organic environments and PPB genotypes will perform better than commercially registered check cultivars.

Performance stability is defined by Piepho (1999) as the combination of the level of achievement (e.g. yield, protein, test weight, kernel mass) and consistency across a range of growing conditions. Evaluating genotypes across multiple environments is essential to evaluate the genotypic performance and correctly select genotypes that have consistent outcomes under a wide range of growing conditions. This approach is currently used in Canadian wheat breeding programs (Thomas and Graf, 2014).

Performance stability should be emphasized in organic breeding programs as well due to the elevated variability among farms and within farms (Isaac et al., 2021). Several stability indices and calculations have been proposed including using coefficient of variation (CV) (Francis and Kannenberg, 1978), environmental regression coefficient (b_i) (Finlay and Wilkinson, 1963), deviations from the regression line (S^2d_i) (Eberhart and Russell, 1966), GxE regression coefficient (β_i) (Perkins and Jinks, 1968), ecovalence (W_i^2) (Wricke, 1962), and stability variance (σ_i^2) (Shukla, 1972).

The environmental regression coefficient (b_i) as proposed by Finlay and Wilkinson (1963) helps organize genotypes towards one of four performance quadrants. Genotypes can exhibit high, consistent performance; high, inconsistent performance; low, consistent performance; and low, inconsistent performance. The b_i value represents how sensitive the genotype's yield performance is to environmental change, and is referred to as a response parameter (Becker and Leon, 1988). Depending on the breeding goals, each combination of the b_i variable and overall performance may be valued, and genotypes ranked accordingly. A $b_i > 1.0$ indicates genotypes have greater sensitivity to environmental conditions, genotypes with $b_i = 1.0$, have average stability, and genotypes with $b_i < 1.0$ have low sensitivity to environmental change (Finlay and Wilkinson, 1963). Yield potential is often associated with low 'stability', indicating adaptation to favourable environments (Finlay and Wilkinson, 1963). Plant breeders interpret these genotypes as 'most responsive' (Lin et al., 1986). Eberhart and Russel (1966) revised the yield stability measure by proposing that deviations from linear regression (S^2d_i) be used in partnership with b_i. Wheat breeders for conventional environments value high average yield and sensitivity to environmental change (most responsive). This characteristic would indicate greater genetic potential under favorable conditions, which can be obtained with synthetic fertilizer application and pesticide use. Breeding goals for organic environments require adaptation to a wide range of spatial and temporal heterogeneity and maintain yield stability despite greater variability (Dawson et al., 2008; Isaac et al., 2021), which may be achieved through farmer-selection. Our second objective was to determine broad adaptation of PPB genotypes to

identify stable and high yielding genotypes under organic management across the Canadian prairies. We hypothesized that because PPB genotypes were selected under a wide range of organic environmental conditions, they will have greater yield performance and greater stability than check cultivars.

Wheat yield progress has been attributed to greater partitioning of biomass into grains (harvest index), and greater kernel number per unit area (Fischer, 2007). Harvest index (HI), the proportion of grain yield per unit total biomass, has been proposed to be maximized at 62% (Austin et al., 1980), with real world values reflecting between 29-50% (Foulkes et al., 2011; Porker et al., 2020). Given that in-field harvest index has stabilized (Fischer, 2007; Miralles and Slafer, 2007), yield increases will need to come from other factors such as greater biomass accumulation and reducing height. However, shorter cultivars may not be competitive under organic conditions because plants must compete with weeds for light (Mason et al., 2007a). This creates questions around "how to increase yields in organic production?" This question is especially relevant considering findings that the type of grain yield progress due to the selected genetics and physiological changes in conventional breeding programs have not been observed in organic environments (Pswarayi et al., 2014; Herrera et al., 2020). Therefore, another objective of this study was to understand what agronomic qualities are correlated with higher yields under organic production.

Genotype x environment interactions (GEI) have been historically treated as 'noise' and plant breeders have attempted to reduce interactions and amplify genetic effects (Yan and Tinker, 2006). Visually analyzing how genotypes and genotype x environment interactions relate to each other using linear-bilinear models has been proposed as a way to embrace GEI rather than dampen them (Yan and Tinker, 2006). The most popular visual models used in agronomic studies are additive main effects and multiplicative interaction (AMMI) biplots and genotype plus genotype by environment (GGE) biplots (Yan and Rajcan, 2002; Yan and Tinker, 2006; Goyal et al., 2011; Poli et al., 2018; Subedi et al., 2021). Such analysis has included organically grown wheat experiments (Kaut et al., 2009; Kissing Kucek et al., 2019).

Ideal genotypes within a GGE biplot should have high mean yield (PC1 scores) and near zero secondary effects (PC2 scores) (Yan and Tinker, 2006).

Organic test environments have a unique challenge of lower yield potential due to weed competition and low fertility which could mask genetic potential (Cober and Morrison, 2015; Carkner and Entz, 2017; Herrera et al., 2020). Organic GEI studies have reported that environment contributes over 90% of yield variation (Carr et al., 2006; Carkner and Entz, 2017; Kissing Kucek et al., 2019; Weedon and Finckh, 2019), whereas under conventional management environmental variance values range from 41-89% (Park, 1987; Brandle and McVetty, 1988; Mohammadi et al., 2010; Anderson et al., 2011; Weedon and Finckh, 2019). Using yield data from organic, low-input conventional, and high-input conventional trials, Herrera et al. (2020) demonstrated that as external input use is increased, the environmental variance component is reduced. Ideal test environments should have the ability to highly discriminate between genotypes, with high yield potential (Yan and Tinker, 2006). The GGE biplot can be observed as an 'environment-vector' view, based on environment-centered genotype by environment without scaling (Yan and Tinker, 2006). Depending on the angle of the vectors between environments, negative, positive, and uncorrelated relationships can be made among environments. Additionally, the GGE biplot can provide information on how discriminatory test environments are of genotypic variability. Organic test environments need to encapsulate real-world environmental stress observed by organic farmers, while simultaneously yield high enough for genotypic expression of valuable traits. To the author's knowledge, organic environment evaluation for genotype testing has never been investigated in Canada. This motivated our objective to evaluate what environmental qualities create a discriminatory test environment while also delivering stress to the genotypes to realistically reflect organic environments.

Partial least squares (PLS) regression is a useful statistical tool when there are more predictor variables than observations and multicollinearity exist among the predictor variables (Tobias, 1995). High

environmental contribution to yield makes genotypic differences and GEI difficult to interpret. Taken together, partial least square regression has the potential to aid what was driving yield differences across environments. Partial least square regression has been used to elucidate the relationships between wheat quality and meteorological variables (Mkhabela et al., 2018); organic soybean yield (Carkner and Entz, 2017); and regional corn, soybean, and oat yield data (Williams et al., 2008). Our last objective was to use PLS regression to reveal important crop physiology and environmental factors that were significantly contributing to yield variability under organic management across diverse environmental settings.

2.3. Materials and Methods

2.3.1. Genetic material

Farmer-selected PPB genotypes (referenced as 'farmer genotypes' throughout) were sourced from the University of Manitoba participatory wheat breeding program described by Entz et al. (2018). Genetic material was sourced from farmers across multiple agroecological zones in Canada (Table 2-1). Pedigrees of the farmer genotypes and check cultivars are presented in (Table 2-2). Farmer genotypes were tested at the F_6 generation. Check cultivars were chosen based on the popularity among organic farmers, organic breeding history, and positive performance in previously run organic trials.

Table 2-1. Farmer and selection locations of farmer genotypes used in the experiment.

Farmer ID	Location	Latitude (N)	Longitude (W)
AS	Wood Mountain, SK	49°22'16.3"	106°23'06.3"
CG	Pilot Mount, MB	49°12'32.6"	98°55'19.3"
GM	Kleefeld, MB	49°30'10.0"	96°52'07.6''
GW	Metcalfe, ON	45°11'13.3"	75°26'40.7"
HRE	Libau, MB	50°16’11.8”	96°42’45.2”
IG	Brandon, MB	49°45'06.8"	99°52'12.8"
JM	Fort Vermilion, AB	58°22'41.8"	116°02'25.0"
KB	Carman, MB	49°29'53.4"	98°02'14.6"
LD	Les Cedres, QC	45°18'29.8	74°02'19.4"
SC	Melita, MB	49°16'11.3"	100°59'24.3"
SW	Swift Current, SK	50°16'51.4”	107°39'17.5"
TM	Neubergthal, MB	49°04'25.7"	97°28'55.8"
WM	Morinville, AB	53°48'06.9"	113°38'39.8"

Table 2-2. Farmer selected genotypes and registered checks' pedigree. Farmer ID is the identification of the farmer hyphenated with the corresponding genotype.

Genotype Cross	Pedigree	Farmer ID	
BJ08A	BW430/BW897	CG, IG	
BJ10A	ACS 54608/BW342	KB, SC	
BJ11A	ACS 54608/Waskada (*Midge-tolerant)*	CG, KB, SC	
BJ13	BW433/BW430	GW, HRE	
BJ15	BW425/BW430	GW, GM	
BL22A	Vesper (*Sm*1)*/BW461	SW	
BL23	Vesper (*Sm*1)/BF12A*A235	AS, JM	
BL28	AAC Prevail (*Sm1*)/BW431	JM, TM, WM	
BL34A	BD110B-215-8-1-13/Shaw (*Sm*1)	JM, WM, SW	
BL39A	BD110B-215-8-1-13/BW455	WM	
BL41A	BD110B-215-8-1-13/BF12A*A235	AS	
BL43C	BW 486/Shaw (*Sm*1)	TM	
PWA10B	ERA131-R3 / Sable	LD	
Check Cultivars		Year of Registration	Suitable for:
AAC Brandon	Superb/CDC Osler//ND744	2013	Conventional
Vesper (*Sm*1)	A/HWA//*3ACBarrie/6/BW150*2//Tp/Tm/ 3/2*BW252/4/98A190/5/Sup	2010	Conventional
AAC Tradition	98B25-AS6D01/ND744	2016	Organic
Zealand	Alvena/IAS64/ALDAN//URES/3/TNMU/4/T NMU	2016	Organic
Jake	PT764/CDC Stanley	2019	Organic
CDC Kernen	CDC Bounty/FHB4	2012	Conventional

*Genotype contains the *Sm*1 gene that confers resistance to orange wheat blossom midge (*Sitodiplosis modellana* Géhin) which expresses antibiotic properties against larvae of orange wheat blossom midge (Thomas et al., 2005).

2.3.2. Environment Descriptions

Performance of farmer genotypes and check cultivars were evaluated in field trials in five organically managed sites including Edmonton, AB, Oxbow, SK, Roblin, MB, Libau, MB, and Carman, MB from 2020 to 2022 resulting in 12 environments of data (Table 2-3). Carman, Edmonton, and Oxbow soil types were Orthic Black Chernozem (Bowser et al., 1962; Shields et al., 1968; Manitoba Agriculture, Food, and Rural Development (MAFRD), 2015), Libau was a Rego Black Chernozem, and Roblin was an Orthic Dark Grey Chernozem (Manitoba Agriculture Food and Rural Development (MAFRD), 2013).

Soil tests were conducted in either the spring or fall prior to or just after seeding at each site. Soil fertility status is shown in Table 2-4. Weather data was collected by Manitoba Agriculture, Food and Rural Development (MAFRD, 2022), Environment Canada's climate data (Environment Canada, 2022), and 30-year averages (Environment Canada, 2022) are presented (Table 2-5). Libau data was collected from the Selkirk weather station, approximately 8.3 km from the field site (50°17'71" N, 96°79'28" W).

Table 2-3. Location of each experimental site in the present study.

Location (Code)	Environment Year	Latitude (N)	Longitude (W)
Carman (CAR)	2020, 2021, 2022	49°30'03.2"	98°01'54.4"
Libau (LIB)	2020, 2021, 2022	50°14'26.1"	96°43'48.1"
Roblin (ROB)	2021, 2022	51°13'42.3"	101°21'05.0"
Oxbow (OXB)	2021, 2022	49°15'48.4"	102°06'53.5
Edmonton (EDM)	2021, 2022	53°29'38.3"	113°32'47.5

Table 2-4. Soil nutrient status at each experimental site.

Environment	Depth	N[a]	S[b]	P[c]	K[d]	OM[e]	pH[f]
	cm	kg ha^{-1}	kg ha^{-1}	mg g^{-1}	mg g^{-1}	%	
CAR-2020	0-15	47	16	15	336	5.6	6.3
	15-60	158	54				
CAR-2021	0-15	48	11	8	213	3.7	5.8
	15-60	60	20				
CAR-2022	0-15	11	34	7	235	4.8	6.1
	15-60	33	53				
LIB-2020	0-15	24	134	3	297	6	8.2
	15-60	74	403				
LIB-2021	0-15	36	134	6	293	5.5	8.1
	15-60	43	242				
LIB-2022	0-15	30	13	4	268	3.8	8.2
	15-60	110	94				
ROB-2021	0-15	41	27	16	305	7.4	6.1
	15-60	73	60				
ROB-2022	0-15	56	69	159	191	4.3	6.3
	15-60	50	147				
OXB-2021	0-15	49	27	5	374	3.9	7.7
	15-60	242	403				
OXB-2022	0-15	13	13	8	494	3.9	6.6
	15-60	53	60				
EDM-2021	0-15	94	20	49	220	11.9	6.4
EDM-2022	0-15	90	34	80	479	11.6	6.4

[a] Nitrate-N: Extraction in 0.2 M KCl using Cd reduction determination method (Gelderman and Beegle, 2015). Conversion to mass-per-area was determined by the soil analysis lab based on assumptions of regional soil bulk density.

[b] Sulfate-S: Extraction in 0.12 M KCl at room temperature with the turbidimetric determination method (Cihacek et al., 2015). Conversion to mass-per-area was determined by the soil analysis lab based on assumptions of typical regional bulk density.

[c] Olsen-P: Extraction of 0.5 M $NaHCO_3$ at pH 8.5 (Olsen et al., 1954) using the spectrophotometry determination (Frank et al., 2015)

[d] Extraction in 1.0 M NH_4OAc at pH 7.0 with atomic emission spectroscopy (Warncke and Brown, 2015).

[e] Organic matter: Total organic matter by loss of ignition (Combs and Nathan, 2015)

[f] Determined in 1:1 soil:water (Peters et al., 2015)

Table 2-5. Environmental conditions during the growing season (May 1-August 31) at each research site (MAFRD, 2022; Environment Canada, 2022), and long-term averages (Environment Canada, 2022).

Research Site	Precipitation				Heat			
	30-year Average	2020	2021	2022	30-year Average	2020	2021	2022
		----- mm ----				--- Growing Degree Days ---		
Carman	282	232	225	265	1427	1357	1512	1445
% Average		82	79	93		95	105	101
Libau*	291	193	179	366	1433	1548	1568	1458
% Average		66	61	125		108	109	101
Roblin	253	-	232	344	1273	-	1357	1274
% Average			91	135			106	100
Oxbow	243	-	263	342	1449	-	1500	1450
% Average			108	140			103	100
Edmonton	279	-	126	212	1283	-	1491	1463
% Average			45	75			116	114

*Libau location is sourced from Selkirk Manitoba Agriculture weather station

2.3.2.1. Environmental Conditions

Seasonal precipitation accumulation as a percentage of 30-yr average varied significantly among years and environments. Dry and drought conditions coupled with above average temperatures occurred in Libau, Carman, and Edmonton in 2020 and 2021 (Table 2-5). All environments experienced either near normal or wet conditions in 2022. Roblin 2021 reported extremely high volunteer alfalfa (*Medicago sativa* L.) competition, high grasshopper populations, and drought during the critical vegetative period between stem elongation and anthesis. Oxbow 2022 reported heavy wild oat (*Avena fatua* L.) pressure in combination with high precipitation that led to lodging in experimental units.

2.3.3. Experiment Design and Management

Experiments used a randomized complete block design with three replicates, except Carman (2021, 2022), Libau (2021, 2022), and Oxbow (2021) which had four replicates. Plots were seeded at a rate of 350 viable seeds m^{-2} using a disc drill (Fabro Industries, Swift Current) in Edmonton, Libau, Roblin, and Carman. Plots were seeded with a 4-row disc drill with a cone (University of Manitoba, Winnipeg) in Oxbow, SK. All experimental units were 3.04m^2 with 15.2cm row spacing, except Roblin which was 8.44m^2

with 15.2cm row spacing and Oxbow which was 4.27m^2 with 15.2cm row spacing. Two border rows of fall rye (*Secale cereale* L.) were seeded between each experimental unit and experimental unit blocks as well as border plots of wheat to minimize edge effects in Carman and Libau environments. All experimental sites' previous crops were green manure phases, consisting of cereals (barley, oats) and peas in mixed cereal-pea or peas alone. The dates of seeding and harvest operations for each experimental site are given in Table 2-6.

Table 2-6. Seeding and harvest dates of 12 site-years of experiments between 2020 and 2022.

Site-Year	Seeding Date	Harvest Date
CAR-2020	15-May	10-Aug
CAR-2021	27-Apr	29-Jul
CAR-2022	12-May	21-Aug
LIB-2020	8-May	19-Aug
LIB-2021	30-Apr	11-Aug
LIB-2022	26-May	7-Sept
ROB-2021	16-May	3-Sept
ROB-2022	24-May	2-Sept
OXB-2021	29-May	3-Sept
OXB-2022	17-May	30-Aug
EDM-2021	4-June	31-Aug
EDM-2022	9-June	21-Sept

Carman 2022 and Libau 2022 were inter-row cultivated to control Sow Thistle (*Sonchus arvensis* L.) and Canada Thistle (*Cirsium arvense* L.) populations. Oxbow 2021 had heavy wild oat pressure which caused many plots to lodge, eliminating a replicate. Experimental units were harvested using a Wintersteiger plot combine (Wintersteiger, Austria) in Carman, Roblin, and Edmonton. In Libau, experimental units were harvested using a Hege plot combine 58 (Hege model 125, Hege company, Waldenburg, Germany). Experimental units were subsampled (0.61m^2) by hand in Oxbow 2021 and 2022 and sent through a stationary Wintersteiger plot combine for threshing. All samples were dried on forced air beds for 72 hours prior to further cleaning. Grain samples were cleaned using a Carter Day dockage tester (model 31624/W-3301).

2.3.4. Data Collection

Above ground biomass was collected at the anthesis stage (Zadoks stage 64), and at the hard dough stage (Zadoks stage 87) in seven environments (Carman and Libau (2020, 2021, 2022), and Oxbow (2021)) (Zadoks et al., 1974). For both biomass samplings, a 0.15m^2 area was randomly selected from each experimental unit, plants were cut at ground level, dried at 65°C for 72 hours, and weighed. Plant height measurements were taken at all experimental sites and occurred at maturity by measuring the distance from the soil to the top of the spike at two randomly selected areas in each experimental unit. Kernel mass was determined at every experimental site except EDM-2021 by counting 250 seeds with an Old Mill Counter Model 850-3 seed counter (International Marketing and Design Corporation, San Antonio, Texas). Kernel number represents the kernel number per unit of area (hectare). Kernel production efficiency is expressed as the number of kernels per unit biomass at anthesis (Fischer, 1979) (equation 2-1).

$$\text{Kernel production efficiency} = \frac{\text{kernel number/hectare}}{\text{anthesis biomass weight/hectare}} \quad (2\text{-}1)$$

Kernel production efficiency was calculated for Carman and Libau (2020, 2021, 2022) and Oxbow (2021) only. Harvest index was calculated to determine how efficiently genotypes translocated dry matter into grain yield (equation 2-2).

$$\text{Harvest index}\ (\%) = \frac{\text{grain weight / hectare}}{\text{mature biomass weight/hectare}} \text{ x } 100 \quad (2\text{-}2)$$

Harvest index was calculated for Carman and Libau (2020, 2021, 2022) and Oxbow (2021) only.

2.3.5. Data Analyses

2.3.5.1. Analysis of Variance

Each environment was considered one unique environment, totaling 12 environments. Analysis of variance was conducted using PROC MIXED in SAS Software 9.4 (SAS, 2013a). Collected data was first analyzed combining environments together. Genotype was considered a fixed effect, and

block(environment), and environment were random effects. Normality of residuals produced by the model were tested with PROC UNIVARIATE, with Shapiro-Wilk values greater than 0.9 considered normally distributed data. If data was not normally distributed, data was transformed using the natural log function. Environments were separated when a genotype x environment interaction was detected. When environments were separated, genotype was a fixed effect and block was a random effect. Differences among genotypes and environments were tested using the protected Least Significant Difference (LSD) test and considered statistically significant at $P < 0.05$. Data was analyzed for linear correlations using the PROC CORR procedure (SAS, 2013a).

2.3.5.2. Genotype x year interactions and stability analyses

Yield stability was evaluated using Finlay-Wilkinson and Eberhart-Russel (Finlay and Wilkinson, 1963; Eberhart and Russell, 1966). Genotypic least-squares means (lsmeans) generated in the mixed model analysis for each environment were used. The genotypic lsmeans in each environment were plotted against the environment mean, and a regression line was then fit to each genotype's performance. An illustrative explanation is shown in Figure 2-1. The slope of the regression line (b_i) is then plotted against each genotypic mean across all 12 environments. Linear regression (b_i) coefficients were calculated using PROC GLM procedure with SAS program 9.4 (SAS, 2013a), with genotype as a fixed effect and environment as a random effect. Additionally, deviations from the regression line (S^2d_i) represents how far each genotype deviates from the regression line, which is useful to discern which genotypes' performance is more consistent than others (Eberhart and Russell, 1966). An illustrative explanation of the b_i and S^2d_i estimates are shown in Figure 2-1. Deviations from linear regression capture the 'spread' of data points away from the original regression line created, therefore, a stable genotype would have a $b_i = 1.0$, and $S^2d_i = 0$ (Eberhart and Russell, 1966).

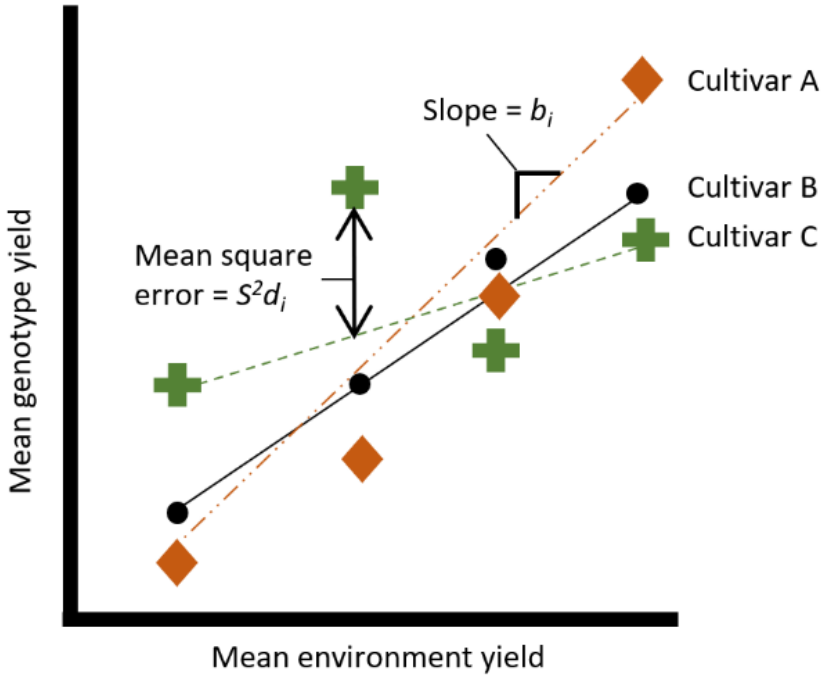


Figure 2-1. An illustrative explanation of the b_i and S^2d_i estimates for the Finlay-Wilkinson (1963) and Eberhart and Russell (1966) stability models.

Genotype x environment interactions were visually investigated using genotype plus genotype x environment (GGE) biplot analysis (Yan and Tinker, 2006). The genotypic lsmeans were generated using a restricted maximum likelihood (REML) approach within the SAS program developed by Dia et al. (2016) available at https://cucurbitbreeding.wordpress.ncsu.edu/publications/software-sas-r-project (accessed December 20, 2022). GGE biplots were created using the lsmeans from the SAS input into an R GGE package developed by Laffont et al. (2007) available at https://CRAN.R-project.org/package=gge (accessed December 20, 2022). To visually evaluate genotypic performance stability, the "which-won-where" view of the biplot was used (Yan et al., 2007). Which-won-where biplot views encapsulate crossover GE, mega-environment differentiation, and specific adaptation concepts (Yan and Tinker, 2006). An illustrative explanation of how to interpret the biplot is shown in Figure 2-2. The single line that runs horizontally through the biplot is called the AEC abscissa (AEA), and points to high mean yield across environments in accordance with Principle Component PC1, and stability parameter PC2. A polygon is

drawn around genotypes that performed the best and poorest across the environments, therefore furthest from the origin. The lines that connect genotypes via the polygon indicate the genotype performance rank in different environments. Lines are drawn within the polygon, and the winning genotype for each sector is the one located on the respective vertex (Yan and Tinker, 2006). The which-won-where view allows for environment separation, as environments are clustered together where the same genotypes performed the best. The discriminating power vs. representativeness view of the GGE biplot is environment-focused (Yan et al., 2007). An illustrative explanation of the discriminating power vs. representativeness biplot view is shown in Figure 2-3. The horizontal line is also referred to as AEA and represents the 'average-environment axis'. Test environments with longer vectors are more discriminating of genotypes, or more informative. For example, if the test environment falls closer to the origin, it provides little or no information about genotypic differences. On the other hand, if the environment is far away from the origin, this indicates that genotypes in that environment demonstrated genotypic differences. The cosine of the angle between any environment vector approximates the correlation coefficient between the genotype values in that environment and the genotype means across the environments. Therefore, if environments have small angles with the AEA, those environments may represent a 'mega-environment'. Yan et al. (2007) claim test environments should be classified into three categories; Type 1, short vectors, little to no genotypic information should not be used as test environments, Type 2, long vectors with small angles with the AEA represent the best test environments, and Type 3, long vectors with large angles from AEA, useful for culling unstable genotypes, but not selecting superior genotypes.

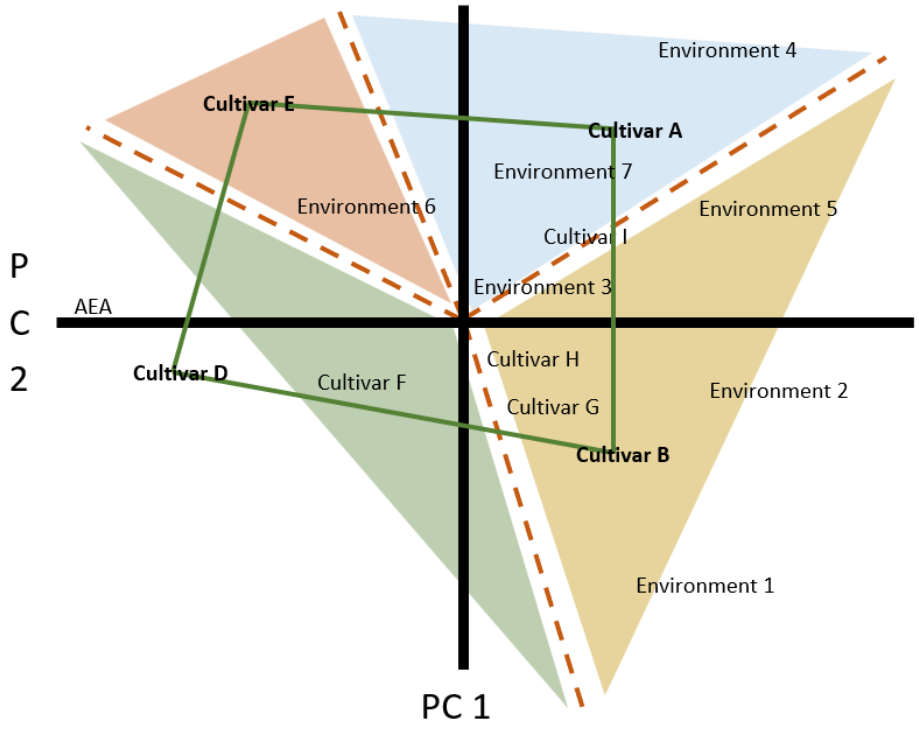
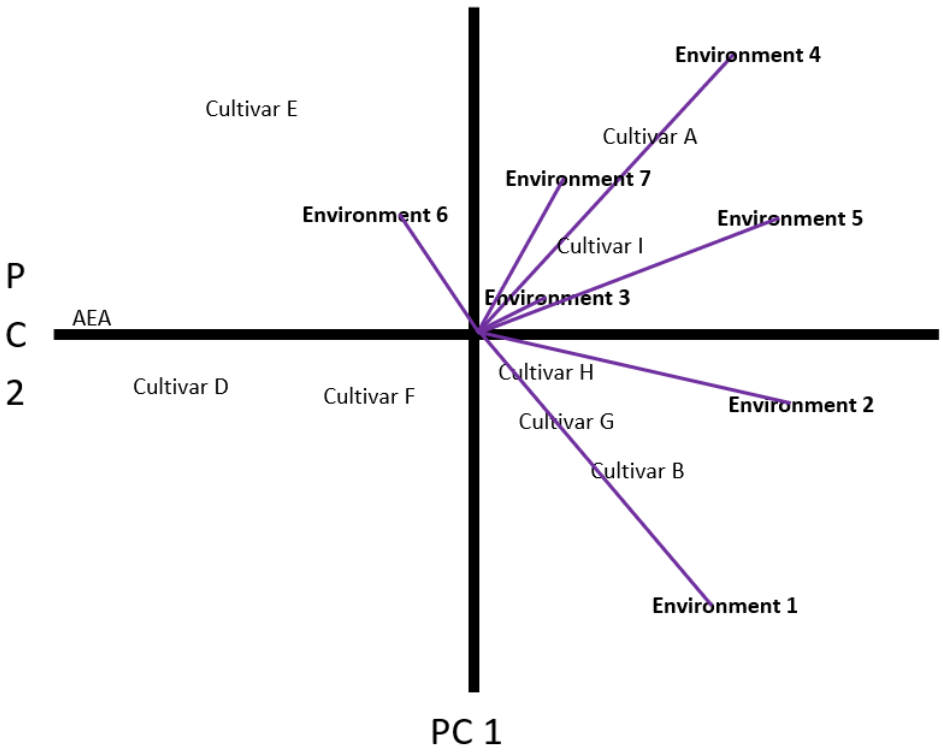


Figure 2-2. An illustrative interpretation guide of a "which-won-where" GGE biplot. A polygon is drawn around the best and poorest performing genotypes tested. The shaded areas represent sectors in the biplot where the cultivar at the apex of the polygon performed the best. For example, “Cultivar A” performed the best in environment 7 and environment 4. There are no environments in “Cultivar D” sector, and therefore is the poorest performing genotypes in all or some of the environments.
PC, principle component



1

Figure 2-3. An illustrative interpretation guide of the "representativeness vs. discriminatory" GGE biplot view. Test environments with longest vectors are the most discriminatory, ie. detect genotypic differences. For example, Environment 1 is more discriminatory than Environment 3. The closer an environment is to the AEA, the more representative the environment performance is of the average environment performance (for example, Environment 2). Environment vectors lines that have an acute angle with another environment vector indicate that the environments are similar to each other (for example, Environment 4 and 5), whereas obtuse able indicate dissimilarity (for example, Environment 2 and 6).
PC, principle component

2.4. Results

2.4.1. Crop Growth

Anthesis and mature biomass were collected in seven of 12 environments. There was a significant genotypic effect but no genotype x environment interaction indicating a stable genotypic response across environments (Table 2-7 and Table 2-8). Anthesis biomass ranged from 2988 (BJ11-KB) to 3955 (BL28-WM) kg ha^{-1}, slightly lower than Manitoba organic spring wheat data reported by Wiebe et al. (2017) and similar to data reported by Nicksy et al. (2022). Mature biomass ranged from 4975 (AAC Brandon) to 7468 (BL34-SW) kg ha^{-1}, which reflects organic spring wheat biomass reported in Manitoba (Wiebe et al., 2017; Nicksy et al., 2022). Post-anthesis biomass ranged from 1913 (AAC Brandon) to 4060 (BL34-SW) kg ha^{-1}. There were no overall genotypic differences detected, however, there was a genotypic x environment interaction (Table 2-7). When environments were analyzed separately, no genotypic differences were detected in any environment (Table 2-9).

Table 2-7. P-values resulting from Analysis of Variance for the effect of genotype and environment and their interaction on growth parameters, yield, yield physiology measurements for 25 farmer genotypes and 6 check cultivars grown in 12 organic environments in 2020, 2021, and 2022.

	Anthesis Biomass[Ŧ]	Mature Biomass[Ŧ]	Post-anthesis Biomass[Ŧ]	Plant Height	Yield	Kernel Mass	Grains m^{-2}	Harvest Index[Ŧ]	KNO:DMa *[Ŧ]
Parameter	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	cm	kg ha^{-1}	g 1000 $seeds^{-1}$	#	%	#
Genotype (G)	0.0124	0.0093	0.0566	<.0001	<.0001	<.0001	<.0001	0.048	0.0093
Environment (E)	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0275	<.0001
G x E	0.5407	0.0778	0.0372	<.0001	<.0001	<.0001	0.1492	0.1754	0.2006

*KNO:DMa, Kernel number efficiency, Kernel number per unit anthesis biomass

[Ŧ]Data collected from seven environments (Carman 2020, 2021, 2022; Libau 2020, 2021, 2022; Oxbow 2021)

Table 2-8. Lsmeans and analysis of variance comparing biomass accumulation of 25 spring wheat farmer genotypes and 6 registered checks averaged across seven organic environments grown in 2020, 2021, and 2022.

Genotype	Biomass at Anthesis	Biomass at Maturity
	kg ha^{-1}	kg ha^{-1}
BJ08A-CG	3277cdef	6107bcd
BJ08A-IG	3310cdef	5914cde
BJ10A-KB	3155ef	6482abcd
BJ10A-SC	3595abcde	6382bcd
BJ11A-CG	3302cdef	6100bcd
BJ11A-KB	2988f	6482abcd
BJ11A-SC	3260cdef	5952cd
BJ13-GW	3264cdef	5925cde
BJ13-HRE	3733abcd	6673abcd
BJ15-GW	3599abcde	6848abc
BJ15A-GM	3728abcd	6178bcd
BL22A-SW	3191def	5826de
BL23-AS	3717abcd	6178bcd
BL23-JM	3745abc	7021ab
BL28-JM	3384bcdef	7019ab
BL28-TM	3744abc	6619abcd
BL28-WM	3953a	6274bcd
BL34A-JM	3253cdef	6244bcd
BL34A-WM	3493abcdef	6678abcd
BL34-SW	3413abcdef	7468a
BL39A-WM	3240cdef	6620abcd
BL41A-AS	3892ab	5988cd
BL41A-MS	3751abc	6126bcd
BL43C-TM	3307cdef	6212bcd
PWA10B-LD	3208cdef	5933cde
AAC Brandon	3065ef	4975e
Vesper	3730abcd	6141bcd
AAC Tradition	3325cdef	5762de
Zealand	3383bcdef	5968cd
Jake	3592abcde	6477abcd
CDCKernen	3219cdef	5954cd
Genotype *P>F*	0.0124	0.0052
Coefficient of Variation (%)	38	36
Standard Error ±	210	394
Contrasts		
Farmer Genotype lsmeans	3457	5641
Check Cultivar lsmeans	3390	5298
Farmer Genotypes vs. Checks *P>F*	0.5669	0.0556

Table 2-9. Lsmeans and analysis of variance comparing post-anthesis biomass of 25 spring wheat farmer genotypes and 6 check cultivars grown in seven organic environments in 2020, 2021, and 2022.

	Genotype Mean	Carman 2020	Carman 2021	Carman 2022	Libau 2020	Libau 2021	Libau 2022	Oxbow 2021
Genotype				kg ha^{-1}				
BJ08A-CG	3105	4124	2247	3335	1757	2762	4429	3173
BJ08A-IG	2603	3290	1583	4430	1750	1352	4291	1528
BJ10A-KB	3326	2762	2851	4842	3015	1750	4894	3171
BJ10A-SC	2787	3026	1810	5842	1885	1475	2869	2596
BJ11A-CG	2798	2890	1520	6311	1603	1904	2135	3220
BJ11A-KB	3490	3190	1775	5814	2068	2345	7305	1974
BJ11A-SC	2691	1942	2167	5804	1519	1814	4567	1057
BJ13-GW	2660	3780	1974	4203	1750	2347	3059	1506
BJ13-HRE	2660	2790	1525	4463	1606	2128	5405	2658
BJ15-GW	2932	1884	2352	5231	1003	2015	5236	2845
BJ15A-GM	2449	2351	1011	4434	2235	1334	3652	2129
BL22A-SW	2631	2461	1567	6192	2129	583	4106	1423
BL23-AS	2461	1705	2650	4573	1087	1000	3568	2641
BL23-JM	3273	4030	1682	10669	802	1402	3195	1170
BL28-JM	3632	2804	3038	8087	2725	1447	4943	2434
BL28-TM	2877	2220	2050	7819	877	2033	3375	1823
BL28-WM	2320	1377	2188	4315	1039	2795	3693	836
BL34A-JM	2990	2818	1324	5973	2416	1074	4784	2533
BL34A-WM	3184	3050	2091	9129	2686	2837	1065	1429
BL34-SW	4060	3553	2110	11665	2686	3221	3360	1819
BL39A-WM	3379	2609	1875	7728	2732	1713	3390	3609
BL41A-AS	2095	2021	2110	3565	592	2025	3364	792
BL41A-MS	2375	3015	1668	4300	2023	567	2968	2084
BL43C-TM	2905	4831	1582	3732	1746	2065	5642	736
PWA10B-LD	2724	2198	1658	7544	1130	1776	3591	1171
AAC Brandon	1913	1964	860	1628	1169	1712	3709	2395
Vesper	2410	1952	659	4991	1441	1387	4669	1770
AAC Tradition	2436	2019	2796	2825	2269	1384	3438	1324
Zealand	2584	2127	2016	5090	1297	1287	3140	3131
Jake	2884	1465	1843	3194	1309	950	3394	2086
CDC Kernen	2739	3574	1845	4425	843	1880	4208	2328
Grand Mean	2867	2704	1885	5778	1716	1757	3916	2045
Genotype *P>F**	0.0566	0.6836	0.2146	0.1002	0.5836	0.2761	0.0747	0.3112
Coefficient of Variation (%)	87	58	53	67	69	80	48	73
Standard Error ±	402	433	376	377	435	376	376	376
Contrasts								
Farmer Genotype lsmeans	2941	2833	1936	6007	1794	1835	3954	2014
Check Cultivar lsmeans	2558	2183	1670	4876	1388	1433	3759	2173
Contrast *P>F*	0.0867	0.1038	0.2519	0.2136	0.1998	0.2134	0.6520	0.6436
Estimate	382	649	266	1130	406	401	195	-157

*Lsmeans within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.05$)

In general, farmer genotypes were between 4 to 12 cm taller than check cultivars across all environments except Roblin 2021, where no genotypic differences were observed (Table 2-10). A significant genotype x environment interaction for plant height indicated that genotypes responded differently to growing environments (Table 2-10). The environment with the shortest plant height was Roblin 2021 (44-59cm), with no main effect differences. Genotypes were the tallest in Carman 2022 (81-113cm). BJ08-IG was either the tallest or among the tallest in eight out of nine environments where significant main effects were detected. AAC Brandon, Vesper, and AAC Tradition were consistently the shortest genotypes in all environments. Plant height and yield were highly correlated (Table 2-11).

Table 2-10. Lsmeans and analysis of variance comparing plant height of 25 spring wheat farmer genotypes and 6 check cultivars grown in 12 organic environments in 2020, 2021, and 2022.

	Carman 2020	Carman 2021	Carman 2022	Edmonton 2021	Edmonton 2022	Libau 2020	Libau 2021	Libau 2022	Oxbow 2021	Oxbow 2022	Roblin 2021	Roblin 2022
Genotype	---- cm ----											
BJ08A-CG	86bcdefgh	65bcde	101cdef	75	88cdef	70abcdef	78bcdefghij	101ab	71ghij	85	48	83ghij
BJ08A-IG	91abcd	69abc	113a	72	97a	67abcdefg	81abcdef	95cdefgh	78ab	86	48	95ab
BJ10A-KB	92abc	69abc	111abc	78	93abcde	74a	85a	97abcdef	73cdefgh	83	52	94abc
BJ10A-SC	83efghij	67bcd	105abcde	72	87cdef	72abc	74ghijklm	94defgh	72efghij	82	53	87defghi
BJ11A-CG	96a	72abc	109abc	78	94abc	70abcdef	80abcdefgh	100abcd	74bcdefgh	83	48	96a
BJ11A-KB	88abcdefg	64cde	101cdef	73	85fg	65cdefg	77defghijkl	98abcde	72efghij	83	47	87cdefghi
BJ11A-SC	92ab	67abcd	113ab	73	96a	68abcdefg	85a	102a	78abc	84	46	90abcdefg
BJ13-GW	83defghij	69abc	103abcdef	68	87cdef	65cdefgh	77defghijk	93efghi	72efghij	80	44	88bcdefghi
BJ13-HRE	90abcde	70abc	111abc	73	93abcde	72abcd	84abc	100abc	76abcdef	86	45	93abcde
BJ15-GW	91abcd	69abc	107abcd	73	94abc	71abcde	85ab	99abcde	76abcde	82	47	94abcd
BJ15A-GM	92abc	67bcd	110abc	74	92abcdef	65cdefghi	80abcdefg	98abcde	77abcd	82	44	93abcde
BL22A-SW	81hijk	60de	105abcde	68	89cdef	62ghij	75efghijkl	88hij	71fghij	82	47	87efghi
BL23-AS	82fghijk	64cde	105abcde	72	88cdef	66bcdefg	73hijklm	88hij	71efghij	80	50	78jk
BL23-JM	82hijk	65bcde	106abcde	69	89bcdef	56jk	73hijklm	87ij	70ghij	83	52	83hij
BL28-JM	90abcde	75a	109abc	72	92abcdef	74ab	83abcd	101ab	79a	86	50	94abc
BL28-TM	87bcdefgh	66bcd	109abc	78	92abcdef	71abcdef	79abcdefghi	95bcdefg	73cdefgh	77	47	96a
BL28-WM	86cdefgh	70abc	101cdef	70	92abcdef	65cdefgh	84ab	97abcdef	74bcdefg	83	47	94abc
BL34A-JM	88bcdefg	70abc	107abcd	72	92abcdef	74ab	79abcdefghi	97abcdef	70ghij	88	46	91abcdef
BL34A-WM	89bcdef	68abc	109abc	68	97a	66cdefg	72jklmn	96bcdefg	69hijk	88	48	93abcde
BL34-SW	88bcdefg	68abcd	109abcd	81	91abcdef	64defghij	79abcdefghi	95bcdefg	72efghij	81	50	87defghi
BL39A-WM	88bcdefg	73ab	106abcde	73	89cdef	64efghij	76efghijkl	97abcdef	73cdefgh	80	51	93abcde
BL41A-AS	85cdefghi	65cde	101cdef	67	93abcd	66cdefg	77cdefghij	89ghij	74bcdefgh	83	45	84fghij
BL41A-MS	82ghijk	67abcd	107abcd	74	90abcdef	65cdefgh	76efghijkl	90ghij	78ab	85	59	84fghij
BL43C-TM	77jk	69abc	102bcdef	72	91abcdef	63fghij	78bcdefghij	92fghi	68ijk	87	47	91abcdefg
PWA10B-LD	87bcdefgh	68abcd	107abcd	75	86efg	69abcdef	82abcde	97abcdef	70ghij	78	48	81ijk
AAC Brandon	70l	52f	81g	65	71h	50k	76n	72l	66jk	80	45	69l
Vesper	82fghijk	57ef	102cdef	70	87cdef	58hijk	70lmn	87ij	67jk	82	43	82hij
AAC Tradition	75kl	65bcde	93f	62	79g	53k	68mn	79k	64k	81	43	75kl
Zealand	82fghijk	68abc	98def	69	96ab	62ghij	72ijklm	90ghij	74bcdefgh	83	44	89bcdefgh
Jake	78ijk	57ef	96ef	69	89cdef	57ijk	71klmn	84jk	69ghij	84	52	82hij
CDC Kernen	84defghi	66bcd	106abcde	72	87def	67bcdefg	75fghijklm	90ghij	72defghi	82	48	83ghij
Genotype *P>F**	<.0001	0.0001	<.0001	0.0834	<.0001	<.0001	<.0001	<.0001	<.0001	0.8697	0.255	<.0001
CV (%)[ŦF]	5.2	8	6	7	4	7	6	5	5	6	11	5
Standard Error ±	3	3	4	2	2.5	2.8	2.5	2.3	2	3.7	5.2	5
Contrasts												
Farmer Genotypes	87a	68a	106a	73a	99	67a	79a	95a	73a	83	48	89
Check Cultivars	78b	61b	102b	67b	98	58b	70b	84b	68b	82	45	80
Contrast *P>F*	<.0001	<.0001	0.0136	0.0014	0.2168	<.0001	<.0001	<.0001	<.0001	0.5108	0.0816	<.0001
Estimate	8.5	7	4	5	1	9	9	12	5	1	3	9

*Means within the same column followed by the same letter are not significantly different within the same groups of treatments by an analysis of variance (*P≤0.05*). [ŦF]Coefficient of Variation

Table 2-11. Spearman correlation values between mean yield and three growth efficiency measures in 25 sprign wheat farmer genotypes and 6 check cultivars in 12 organic environments in grown in 2020, 2021, and 2022.

	Anthesis Biomass[Ŧ]	Post-Anthesis Biomass	Maturity Biomass[Ŧ]	Plant Height	Kernel Size	Kernel Number	Harvest Index	KNO:DMa[Ŧz]
Yield	0.59***	0.46***	0.69***	0.7***	0.36***	0.94***	0.36***	0.21***

[Ŧ]Data from environments Carman (2020, 2021, 2022), Libau (2020, 2021, 2022), Oxbow (2021) only
[z]KNO:Dma, Kernel number per kg anthesis biomass
***Significant at the *P>F* <.0001 significance level)

2.4.2. Yield and Yield Components

Grain yield averaged between 2064 (PWA10B-LD) and 2541 (BL43C-TM) kg ha^{-1}, with an overall mean yield of 2343 kg ha^{-1} (Table 2-12) and reflected typical organic wheat grain yield in the region (Carkner et al., 2020). A significant genotype x environment interaction for grain yield was observed. Environment contributed 94% of yield variance to the ANOVA model, indicating that environment had a larger effect on grain yield than genotype (0.9%) and the interaction between genotype and environment (5%) (Table 2-14). Significant main effects in each environment were detected in Carman 2021, 2022, and Edmonton 2021, 2022. Farmer genotype BL43C-TM was one of the highest yielding genotypes in Carman 2021, Edmonton 2022, but not in Carman 2022 and Edmonton 2021. The lowest yielding genotypes were PWA10B-LD and Jake. Interestingly, AAC Brandon had overall lower yield, however, the highest yield in Edmonton 2022 (Table 2-12). When farmer genotypes were analyzed as a group, they did not yield significantly different from check cultivars except in Edmonton 2022 and Oxbow 2022. As a group, check cultivars yielded significantly greater than farmer genotypes in Edmonton 2022 by 119 kg ha^{-1} (Table 2-12). As a group, farmer genotypes yielded significantly greater than check cultivars by 178 kg ha^{-1} in Oxbow 2022 (Table 2-12). This supports other Canadian research where higher grain yield was observed for wheat genotypes selected under organic environments when compared to conventional checks (Kirk et al., 2012; Wiebe et al., 2017; Entz et al., 2018).

Kernel mass ranged from 28.6 (BL41A-MS) to 33.6 (AAC Tradition) g 1000 seeds^{-1} with an average kernel mass of 30.3 g 1000 seeds^{-1} (Table 2-13). A genotype x environment interaction indicated that kernel mass differed depending on the environment in which the genotype was grown (Table 2-7). Significant genotype main effects were detected in all environments except Roblin 2022. AAC Tradition had the greatest kernel mass across all environments, except at Carman 2022, where BJ13-HRE had the greatest kernel mass, and Oxbow 2022 where BJ13-GW had the greatest kernel mass. Genotypes with the lowest kernel mass were consistently in the bottom 20% of the kernel mass rankings in all environments. Jake had the lowest kernel mass at the greatest number of sites; Carman 2020, Edmonton 2022, Libau 2021, and Oxbow 2021. Although the genotype x environment interaction for kernel mass was highly significant, genotypic rankings between environments were similar to each other.

Yield efficiency parameters were assessed using kernel number, harvest index, and kernel production efficiency. Genotypic yield efficiency parameters did not differ depending on environment, because there was no genotype x environmental interaction (Table 2-7). Significant main effects in kernel number were detected, and kernel number ranged from 6556 (Check cultivar, AAC Tradition) to 8153 (Farmer genotype, BL23-SW) (Table 2-15). Kernel number values reflect what others have reported at higher yielding environments (ex. Edmonton 2022, 13999 kernels m^{-2}; Carman 2022, 10251 kernels m^{-2}) (Wiebe et al., 2017; Rivera-Amado et al., 2019). When analyzed as a group, farmer genotypes and check cultivars' kernel number did not significantly differ from each other.

Genotypes differed significantly from each other for harvest index. No significant genotype x environment interactions were detected, indicating that genotype response for harvest index was consistent across environments (Table 2-15). Harvest index ranged from 33% (BJ13-GW) to 43% (Vesper). As a group, the harvest index of farmer genotypes was significantly lower than that of the checks by 1% (Table 2-15). Historical increases in grain yield have been attributed to increased harvest indices (Thomas

and Graf, 2014). Check cultivars yielded significantly greater than farmer genotypes at the highest yielding environment, Edmonton 2022 (Table 2-12), this demonstrates that historical breeding efforts under conventional management may only be expressed at very high yielding growing conditions.

Kernel production efficiency (kernel number per unit anthesis dry matter) (Fischer, 1979) significantly differed among genotypes, ranging from 20731 (BL28-WM) to 28716 (BL34-SW) kernels kg^{-1} anthesis biomass. Environments ranged between 17660 (Libau 2020) and 30275 (Libau 2022) kernels kg^{-1} anthesis biomass. When Wiebe et al. (2017) tested genotypes under organic management, they reported values slightly lower than the present study (16652-22610 kernels kg^{-1} anthesis biomass).

Table 2-12. Lsmeans and analysis of variance comparing grain yield of 25 spring wheat farmer genotypes and 6 check cultivars grown in 12 organic environments in 2020, 2021, and 2022.

Genotype	Genotype Mean	Carman 2020	Carman 2021	Carman 2022	Edmonton 2021	Edmonton 2022	Libau 2020	Libau 2021	Libau 2022	Oxbow 2021	Oxbow 2022	Roblin 2021	Roblin 2022
						---- kg ha^{-1} ----							
BJ08A-CG	2303ef	2810	1619abcdefgh	2672defgh	2742efg	4933bcde	1385	2142	2458	2187	887	772	3053
BJ08A-IG	2387abcdef	3304	1260jk	3137abcdefgh	3042bcde	4862bcdefg	1574	1909	3072	2038	1219	636	2595
BJ10A-KB	2364bcdef	3059	1593bcdefghi	3222abcdefg	2755defg	4716defgh	1433	1986	3101	2174	907	703	2726
BJ10A-SC	2242fg	3210	1365hijk	3119abcdefgh	1979jk	4748cdefgh	1621	1686	2621	1636	1138	1010	2779
BJ11A-CG	2284ef	3304	1465defghijk	2845cdefgh	2483ghi	4745cdefgh	1471	1753	3127	1809	978	714	2758
BJ11A-KB	2344def	3208	1563bcdefghi	2329h	3490a	4916bcde	1327	2021	2626	2301	836	596	2899
BJ11A-SC	2353cdef	3041	1519cdefghi	2775cdefgh	2573fghi	5090abcd	1424	1954	3089	2138	953	609	3077
BJ13-GW	2497abcd	3383	1852a	3245abcdefg	2885bcdef	5081abcd	1198	1992	3127	2275	1249	440	3238
BJ13-HRE	2394abcdef	3388	1720abcd	2962bcdefgh	3081bc	4497fgh	1518	2134	2878	2128	1151	432	2839
BJ15-GW	2320ef	3178	1534cdefghi	2996bcdefgh	2762cdef	4719defgh	1347	2017	2919	1992	964	656	2758
BJ15A-GM	2359cdef	3622	1509cdefghij	3032bcdefgh	2865bcdef	4666efgh	1221	1787	2712	2151	1193	607	2945
BL22A-SW	2354cdef	2872	1603abcdefghi	3373abcd	2579fgh	5110abc	1442	1999	2571	2236	1093	738	2615
BL23-AS	2448abcde	3282	1455efghijk	3864a	2655fg	4721defgh	1556	1892	2838	2085	1483	866	2679
BL23-JM	2393abcdef	3598	1385ghijk	3585abc	2595fgh	4868bcdef	1272	1757	2703	1981	1219	697	3044
BL28-JM	2333def	3010	1651abcdef	3186abcdefg	2763cdef	4569efgh	1413	2057	2925	2166	1002	883	2357
BL28-TM	2406abcdef	3443	1488defghij	3752ab	2818cdef	4508fgh	1630	1945	3090	2061	1244	540	2360
BL28-WM	2268f	2869	1520cdefghi	2478gh	2434ghi	4709defgh	1508	2157	2913	2168	1137	866	2456
BL34A-JM	2274f	2916	1619abcdefgh	2568defgh	2683fg	4836bcdefg	1585	1896	3403	1690	856	547	2702
BL34A-WM	2363bcdef	3180	1641abcdefg	3203abcdefg	2689fg	4941bcde	1286	1875	2815	1773	1458	937	2560
BL34-SW	2521abc	3731	1684abcdef	3258abcdefg	2565fghi	4944bcde	1494	2116	3374	2118	1343	792	2829
BL39A-WM	2406abcdef	4035	1618abcdefgh	3189abcdefg	2657fg	4811bcdefg	1396	1828	3083	1590	1161	898	2611
BL41A-AS	2304ef	3201	1686abcde	3356abcde	2254ij	4501fgh	1290	1954	3033	1990	1026	781	2583
BL41A-MS	2303ef	3253	1576bcdefghi	3035bcdefgh	1862kl	4930bcde	1278	1755	3023	2370	1109	927	2524
BL43C-TM	2541a	3339	1717abcd	2998bcdefgh	3162b	5362a	1467	2160	3215	1969	1319	719	3067
PWA10B-LD	2064h	2622	1353ijk	2553efgh	2703fg	4371h	1297	1864	2445	1825	981	485	2269
AAC Brandon	2284ef	2668	1427fghijk	2285h	2793cdef	5371a	1226	1892	2728	2497	681	977	2860
Vesper	2529ab	3951	1666abcdef	3323abcdef	3070bcd	4919bcde	1435	1994	3230	2128	1260	668	2709
AAC Tradition	2276f	2950	1801ab	2618defgh	2321hi	5463a	1395	2086	2496	2145	961	663	2419
Zealand	2310ef	3065	1748abc	2515fgh	3038bcde	5153ab	1399	1893	2781	2272	848	292	2718
Jake	2098gh	2486	1213k	2748defgh	1588l	4478gh	1235	1721	2659	1927	1027	935	3162
CDC Kernen	2317ef	3106	1636abcdefg	2743defgh	3171ab	4882bcdef	1327	1892	2677	2051	852	803	2683
Grand Mean	2343	3176	1546	3014	2678	4852	1401	1939	2893	2056	1081	715	2742
Genotype *P>F**	<.0001	0.0681	0.0002	0.0189	<.0001	<.0001	0.4011	0.2807	0.333	0.3895	0.1179	0.1557	0.9607
CV (%)[ŦŦ]	48	16	12	30	7	5	15	16	18	22	25	68	20
Standard Error	76	263	97	464	118	146	125	159	266	225	160	284	330
Contrasts													
Farmer Genotypes	2353	3204	1560	3011	2681	4806b	1417	1945	2923	2034	1116a	714	2790
Check Cultivars	2306	3010	1582	2959	2664	5044a	1336	1913	2775	2170	938b	722	2570
Contrast *P>F*	0.5378	0.1432	0.6192	0.125	0.742	0.0007	0.1589	0.6569	0.1805	0.1699	0.0183	0.9454	0.8701
Estimate	46	193	-21	52	17	119	81	32	148	-135	178	-8	219

*Lsmeans within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.05$). [ŦŦ]Coefficient of Variation

Table 2-13. Lsmeans and analysis of variance comparing kernel mass of 25 farmer-selected wheat genotypes and 6 wheat check cultivars grown in 12 organic environments in 2020, 2021, and 2022.

Genotype	Genotype Mean	Carman 2020	Carman 2021	Carman 2022	Edmonton 2022	Libau 2020	Libau 2021	Libau 2022	Oxbow 2021	Oxbow 2022	Roblin 2021	Roblin 2022
						g 1000seeds^{-1}						
BJ08A-CG	30.1klm	34ghijk	25.5jklmn	28.5efghij	34.4cdef	30bcdefg	28.1ijk	27.1cdefghi	26.1cdefgh	33.2abcdefgh	29.9klmn	34.5
BJ08A-IG	31.3cde	36.8bc	27.7bcdefg	28.8defgh	36.1abc	29.6defghi	31.1bc	28.4abcde	27.3abcd	32.1cdefghijk	33.9ab	32.6
BJ10A-KB	31.1defg	36.8bc	26.9cdefghijkl	29.5bcdefgh	34.5cde	30.1bcdef	29.1efghi	28.5abcd	26.6bcdefg	32.6bcdefghij	32.8abcde	34.8
BJ10A-SC	30.8efghij	34.9efghi	27.4cdefghi	27.7ghij	34.6cde	30.4bcde	29.6defgh	28.4abcde	27.2bcd	33.1abcdefghi	33.1abcd	33.3
BJ11A-CG	30.8efghij	35.8cdef	28.1abcdef	29.1bcdefgh	34.8cde	30bcdefg	29efghi	27.5cdefghi	26cdefgh	30.5ijklm	32.6abcdef	36.2
BJ11A-KB	31.1def	35.1efgh	27.4bcdefgh	30.3abcdef	35.6bcd	31.3abc	29.8defg	28.4abcde	27.4bcd	30klm	32.1cdefg	34.8
BJ11A-SC	31.3cde	35.2defg	26.9cdefghijkl	29.9abcdefg	36bc	30.5bcde	29.6defgh	29.1abc	26.6bcdefg	33.3abcdefg	31.9cdefghi	36.1
BJ13-GW	31.2cde	36bcde	26.9defghijk	31.39abcd	34.6cde	30.9bcd	29.6defgh	26.8cdefghi	25.8cdefgh	35.6a	31.3efghijk	34.6
BJ13-HRE	32.3b	37.7ab	29.3ab	32.1a	36.2abc	32.6a	31.3b	28.9abc	27.4bcd	33.4abcdef	32.1cdefg	35.2
BJ15-GW	30.9efghi	35.4cdefg	27.1cdefghijk	28fghij	35.8bc	29.7defgh	31.2bcde	27.8bcdefgh	27.8abc	31.7defghijkl	31.5defghij	35.4
BJ15A-GM	30.9efghij	36.6bcd	26.4efghijkl	30abcdefg	34.8cde	29.8cdefgh	29.2efghi	30.1ab	26.9bcdef	30.4jklm	31.1fghijkl	34.9
BL22A-SW	31.8bc	36.6bc	28.2abcde	28.6efghi	37.1ab	30.6bcde	30.8bcd	26.2defghi	28.6ab	34.4abc	33.4abc	36
BL23-AS	29nop	33.3ijk	23.4o	29.2bcdefgh	32.4fg	27.8jk	28.1ijk	25.2i	24.9fghi	32.5cdefghijk	29.1no	36
BL23-JM	29.7mn	33.7hijk	25.9ghijklm	30.2abcdefg	33.6defg	27.8jk	28.2ij	26.1efghi	25.4defghi	32.4cdefghijk	29.2no	34.6
BL28-JM	31efgh	35.7cdef	28.8abc	31.5abc	34.6cde	29.8cdefgh	28.5ghi	28.8abc	26.6bcdefg	30.9fghijklm	32.5abcdefg	33
BL28-TM	30.8efghij	36.6bcd	26.8defghijkl	31abcde	33.3efg	29.8cdefgh	30.2bcde	27.8bcdefgh	27.1bcde	31.2efghijklm	31.3efghijk	33.6
BL28-WM	30.2jklm	34.4fghij	26.8defghijkl	28.6efghi	32.8efg	29.7defgh	28.8fghi	28.2bcdef	24.5ghi	31.2efghijklm	32.3cdefg	35.7
BL34A-JM	30.1lm	35.5cdef	25.6hijklm	28.9cdefgh	34.4cdef	29.7defgh	28ijk	28.1bcdefg	26.9bcdef	29.3lm	29.6lmn	34.4
BL34A-WM	30.4ghijkl	34.9efghi	26.3fghijklm	29.9abcdefg	37.6ab	28.6fghijk	28ijk	26.2defghi	27.3bcd	30.9fghijklm	31.9cdefgh	32.9
BL34-SW	30.4ijklm	36.7cdef	28.5abcd	30.2abcdefg	33.6defg	29.8cdefgh	28.5hi	25.6hi	24.5ghi	31.7defghijkl	31.9cdefghi	33.2
BL39A-WM	30.6fghijkl	36.4bcde	27.2cdefghij	27.7ghij	34.6cde	29.2efghij	29.6defgh	27.4cdefghi	25.8cdefgh	33.7abcde	30.9ghijklm	34.1
BL41A-AS	28.9opq	32.9jk	25.7hijklm	28.2fghij	32.4fg	27.6k	26.7l	27.1cdefghi	25.2hi	30.8ghijklm	27.7o	35.6
BL41A-MS	28.5q	32.9jk	25.1lmno	27.1hij	31.6g	27.3k	26.6l	26fghi	24.5ghi	29.1m	28.9no	34.5
BL43C-TM	30.7efghijk	35.9cdef	25.5ijklm	30.4abcdef	35.8bc	30.2bcde	30cdef	25.4i	25.5defghi	33.8abcd	30.1jklmn	36
PWA10B-LD	29.1nopq	32.6kl	25.3klmn	29.9abcdefg	33.2efg	27.7jk	27.1jkl	26.8cdefghi	25.7cdefghi	29.2lm	30.4hijklmn	32.2
AAC Brandon	30.3hijklm	34.8fgh	26ghijklm	25.7j	34.4cde	30.2bcde	28.8fghi	28bcdefg	26cdefgh	32.1cdefghijk	31.6defghij	36
Vesper	30.7efghijk	35.8cdef	26.2ghijklm	30.1abcdefg	34.5cde	28.5ghijk	30bcdef	25.3i	26cdefgh	33.6abcde	32cdefgh	36.2
AAC Tradition	33.6a	39.2a	29.9a	31.7ab	38.1a	32.8a	34a	30.6a	29.6a	35.2ab	34a	35.1
Zealand	29.3no	32.5kl	23.6no	28.2fghij	34.4cdef	28.1ijk	27kl	28.4abcde	25.1efghi	30.6hijklm	29mn	35.6
Jake	28.6pq	31.2l	24.5mno	26.1ij	32.3g	28.4hijk	26.1l	25.8ghi	23.6i	30klm	30.2ijklmn	36.2
CDC Kernen	31.7bcd	35.1defg	26.8defghijkl	30.1abcdefg	36.9ab	31.5ab	28.8fghi	27.9bcdefgh	27.6abc	35.6a	32.4bcdefg	36.5
Grand Mean	30.3	35.1	26.5	29.3	34.7	29.7	29	27	26.2	32	31.3	34.8
Genotype *P>F**	<.0001	<.0001	<.0001	0.0002	<.0001	<.0001	<.0001	0.0002	<.0001	<.0001	<.0001	0.8622
CV (%)[Ŧ]	12	3	5	8	4	3	3	6	6	6	3	8
Standard Error ±	0.3	0.7	0.7	1.2	0.7	0.6	0.4	0.9	0.8	1.2	0.6	1.5
Contrasts												
Farmer Genotypes	30.2	35.3	1560	29.4	34.5	29.6	29	27.4	26.2	31.8	31.3	34.6
Check Cultivars	30.4	34.9	1582	28.6	35.1	29.9	29.1	27.6	26.2	32.8	31.6	35.9
Contrast *P>F*	0.5534	0.1266	0.0712	0.1422	0.979	0.303	0.7717	0.5638	0.9836	0.0608	0.1534	0.067
Estimate	-0.2	0.42	-21	0.7	-0.5	-0.2	-0.05	-0.2	0	-0.9	-0.3	-1.3

*Lsmeans within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.05$). [Ŧ]Coefficient of Variation

Table 2-14. Genotype (G), environment (E), and genotype x environment (GxE) variance components of grain yield for 25 farmer-selected wheat genotypes and 6 check cultivars grown in 12 organic environments in 2020, 2021, and 2022.

Source	Degrees of Freedom	Sum of Squares	Portion of Total Sum of Squares (%)
G	30	13503489	0.9
E	11	1357685229	94
G x E	330	67549343	5
Total		1438738061	

Table 2-15. Lsmeans and analysis of variance comparing yield efficiency parameters of 25 farmer-selected wheat genotypes and 6 check cultivars averaged over 12 organic environments grown in 2020, 2021, and 2022.

Genotype	Kernels m^{-2}[₮]	Harvest Index[€]	KNO:DMa[¥€]
	#	%	#
BJ08A-CG	7366cdefgh	35cde	24421abcdefg
BJ08A-IG	7272defghi	38abcde	24484abcdefg
BJ10A-KB	7369cdefgh	34cde	26283abcd
BJ10A-SC	7169fghi	34de	22029defg
BJ11A-CG	7115ghij	37abcde	22442cdefg
BJ11A-KB	6967hij	36bcde	25253abcdef
BJ11A-SC	7319cdefgh	39abcd	23601bcdefg
BJ13-GW	7680abcdefg	38abcde	26181abcd
BJ13-HRE	7050hij	36bcde	21250fg
BJ15-GW	7177efghi	33e	22694bcdefg
BJ15A-GM	7207efghi	35cde	21213fg
BL22A-SW	7252defghi	39abc	25156abcdef
BL23-AS	8212a	37bcde	24685abcdefg
BL23-JM	7765abcde	37bcde	21997defg
BL28-JM	7222defghi	34de	24677abcdefg
BL28-TM	7489bcdefgh	38abcde	22292defg
BL28-WM	7341cdefgh	36bcde	20731g
BL34A-JM	7224defghi	37bcde	25063abcdef
BL34A-WM	7415cdefgh	35cde	25395abcdef
BL34-SW	8153a	35cde	28716a
BL39A-WM	7521bcdefgh	38abcd	26014abcd
BL41A-AS	7735abcdef	37bcde	22234defg
BL41A-MS	8035ab	38abcde	22769bcdefg
BL43C-TM	7804abcd	39abcd	26978ab
PWA10B-LD	6688ij	36bcde	23450bcdefg
AAC Brandon	7260defghi	41ab	25683abcde
Vesper	7901abc	43a	25223abcdef
AAC Tradition	6556j	37bcde	21469efg
Zealand	7389cdefgh	37bcde	26619abc
Jake	7369cdefgh	36cde	21238fg
CDC Kernen	6967hij	36bcde	24892abcdefg
Genotype *P>F**	<.0001	0.048	0.0093
Coefficient of Variation (%)	47	28	38
Standard Error ±	256	5	1634
Contrasts			
Farmer Genotypes	7537	37b	24358
Checks Cultivars	7366	38a	24496
F Slns vs. Checks *P>F*	0.1492	0.0468	0.2006

*Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.05$) [₮]Measurement from all environments except Edmonton 2021 [¥]KNO:DMa, Kernel number per unit anthesis biomass [€]Measurements represent data from Carman (2020, 2021, 2022), Libau (2020, 2021, 2022), and Oxbow 2022.

0

2.4.3. Yield Stability

Stability analysis using Finlay-Wilkinson model demonstrated that many genotypes tested were highly stable, falling within one standard error from the overall average regression coefficient (Figure 2-4). Because the farmer genotypes BL34-SW and BL23-AS yielded higher than the average yield standard error and still fell within one standard error of the average regression coefficient value, they would be considered well-suited to the organic environments. Genotypes BJ13-GW, BL43C-TM, and Vesper were the highest yielding on average and fell above the regression coefficient standard error line. These genotypes were therefore the most responsive to favourable conditions and would be considered well-suited for high yield potential organic environments. Check cultivars Jake, Zealand, AAC Brandon, AAC Tradition, and CDC Kernen were average yielding and considered to have average stability under organic management. PWA10B-LD and Jake had lower than average yield than other genotypes in all test environments and did not respond to more favourable environments. Finlay and Wilkinson (1963) suggest that genotypes that fall within the bottom left area of the graph would be adapted to poor environments.

According to the Eberhart-Russell deviation from regression coefficient (S^2d_i) model, an ideal genotype would have high yield and fall within one standard error of the mean deviation of regression coefficient. An S^2d_i value as close to 0 would be ideal (Eberhart and Russell, 1966). Using both Finlay-Wilkinson and Eberhart-Russell models in conjunction with each other provides a good estimate of performance stability (Crossa, 1990). The S^2d_i value represents the mean square error of the regression line through the data. While Jake and PWA10B-LD were grouped together on the Finlay-Wilkinson model, under the Eberhart-Russell model, PWA10B-LD exhibits greater stability than Jake (Figure 2-5). Check cultivars, AAC Tradition and AAC Brandon fall above the one standard error of the average S^2d_i value and are therefore not considered stable. A grouping of high performing genotypes (BJ13-GW, BL43C-TM, Vesper, BL34-SW) are in the bottom left corner of the graph, indicating good stability and high

performance. BL23-AS is still considered to be stable under the Eberhart-Russell model, however, it has a higher S^2d_i value than the high performing group, suggesting that the performance may be less stable.

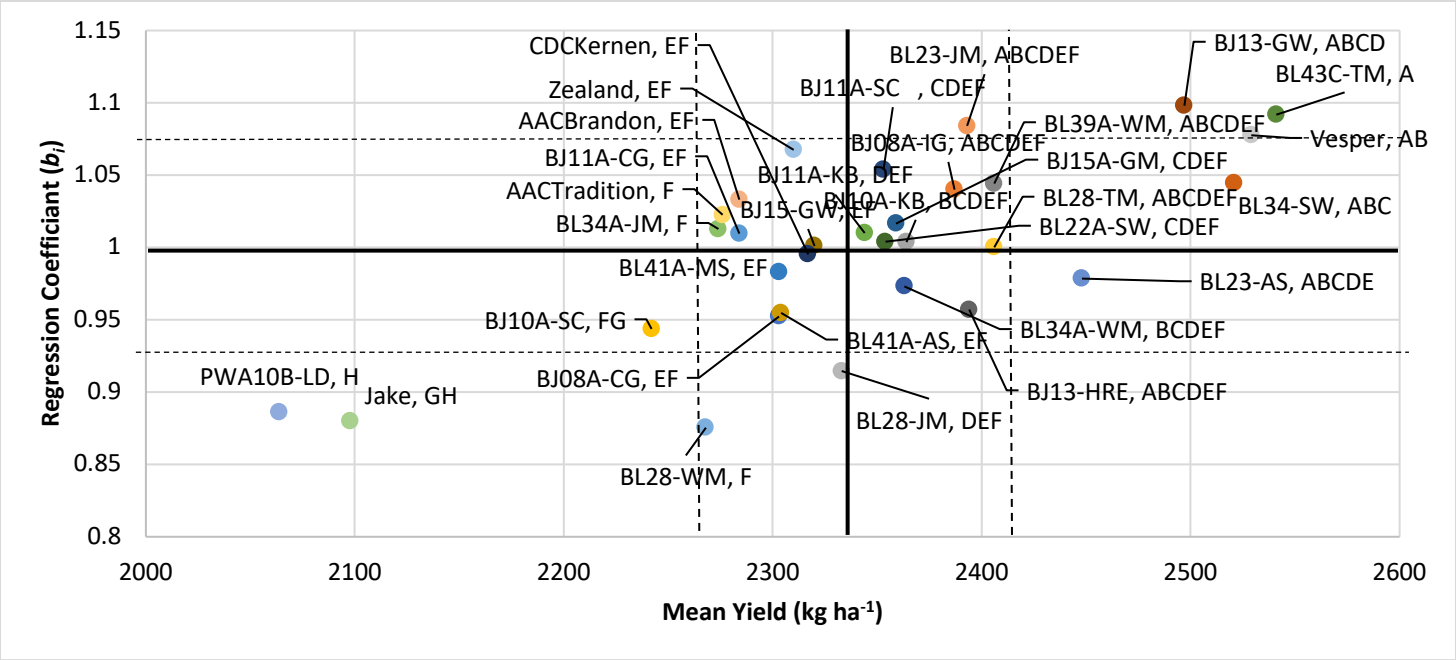
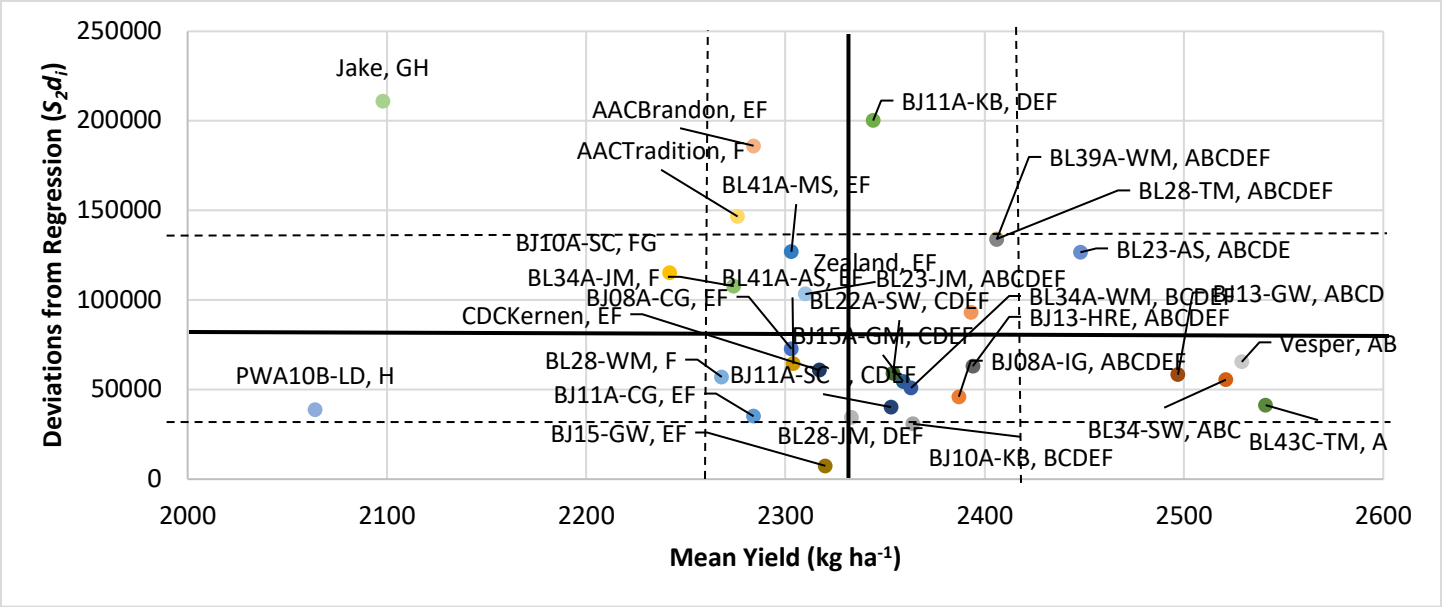


Figure 2-4. Finlay-Wilkinson yield stability model of 25 organic spring wheat farmer genotypes and 6 check cultivars grown in Alberta, Saskatchewan, and Manitoba under organic conditions in 2020, 2021, and 2022 resulting in 12 environments. The horizontal line represents the mean regression coefficient, and the vertical line represents the mean grain yield of the genotype across all 12 environments. The standard error (±SE) is included by the dotted lines. The letters after each genotype represent lsmeans grouping of yield data. When genotypes have different letters, the yield data is statistically different from one another at P≤0.5.

1



2

Figure 2-5. Eberhart-Russel yield stability model using deviation from regression value of 25 organic spring wheat farmer genotypes and 6 wheat check cultivars grown in Alberta, Saskatchewan, and Manitoba under organic conditions in 2020, 2021, and 2022 resulting in 12 environments. The horizontal line represents the mean deviation from regression value, and the vertical line represents the mean grain yield of the genotype across all 12 environments. The standard error (±SE) is included by the dotted lines. The letters after each genotype represent lsmeans grouping of yield data. When genotypes have different letters, the yield data is statistically different from one another at P≤0.5.

2.4.4. GGE Biplot Analysis

2.4.4.1. Genotypic-centered analysis

The GGE biplot (Figure 2-6) representing the "which-won-where" view shows the best performing genotypes for each test environment. It also groups similar environments together. GGE biplot analysis provides another perspective in addition to Finlay-Wilkinson and Eberhart-Russell models of which genotypes did the best in different environments as opposed to the overall performance with no environmental influence (Yan and Tinker, 2006). Here, the principal components of the GGE biplot explained approximately 57.2% of yield variability, which falls within typical GEI values reported elsewhere in the literature (Kaut et al., 2009; Bocci et al., 2020). The cultivars that form the polygon represent the best and poorest yield performers in most environments. The red dotted lines that run through the polygons create segments that contain the environments where those genotypes performed the best (Yan and Tinker, 2006). The best performing genotype within each segment is at the vertex of the segments. Using this interpretation, BL23-AS performed the best in Carman 2022, Oxbow 2022, and Libau 2020. Vesper performed the best in Carman 2020, and BJ11-KB performed the best in Roblin 2022, Libau 2021 and 2020, Oxbow 2021, and Edmonton 2022. Roblin 2021 fell within the Jake segment, indicating that Jake performed the best in Roblin 2021, only. Yan et al. (2007) states that genotypes with the lowest PC2 score and the highest PC1 scores are the best performing and are highly stable genotypes in the environments tested. Using Yan et al. (2007)'s criteria, BL23-AS and Vesper were the highest yielding and stable performers across all the environments tested. Although Jake performed the best in Roblin 2021, the biplot indicates that Jake and AAC Brandon had the lowest PC1 scores therefore were the poorest performers across most environments tested.

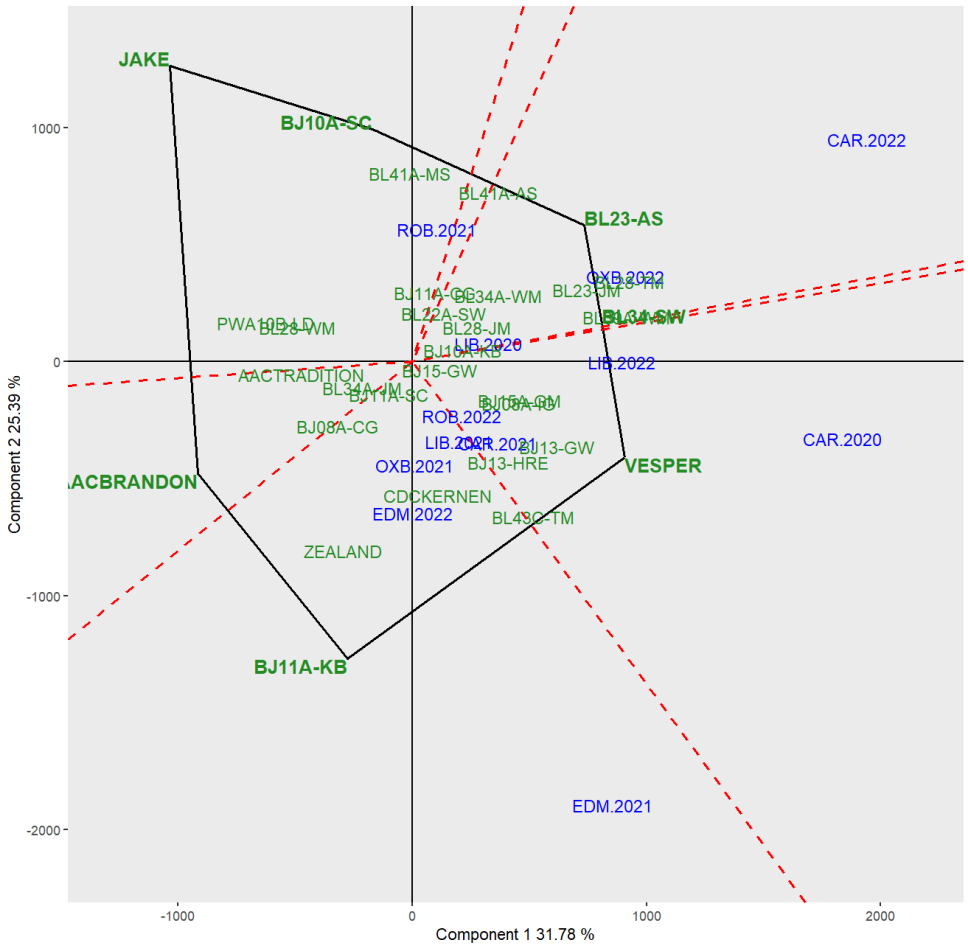


Figure 2-6. GGE Biplot 'which-won-where' visualization resulting from lsmeans of mean yield for 25 organic spring wheat farmer genotypes and 6 check cultivars in 12 organic environments grown in 2020, 2021, and 2022. Column metric preserving SVP and Tester-Centered G+GE with no scaling.

2.4.4.2. Environment-centred analysis

Figure 2-7 visualizes how environments relate to one another in response to genotypic performance. Interpretation of the GGE Biplot analysis often creates classification of mega-environments (Gauch and Zobel, 1997; Yan et al., 2023). However, given the extreme differences among locations and environmental conditions between years and lack of multi-year data, mega-environment interpretation is not appropriate. Instead, environments will be interpreted as what conditions facilitated genotypic discrimination and how representative some environments are of the average performance of all environments. Environments with the longest vectors from the origin are interpreted to be the most

discriminatory. Environments with vectors that have the smallest angle from the AEA are thought to be most representative of the average performance of all environments (Yan and Tinker, 2006). Using this criteria, Edmonton 2021 and Carman 2022 were the most discriminating environments indicated by the longest vector length. However, because the PC2 score is large, genotypic differences in these two environments may not reflect the average genotypic differences observed over all environments. Only the environments with vectors that have corresponding acute angles with the AEA are interpreted as the most representative of the average performance on the biplot. In other words, Carman 2020 was also highly discriminatory and would reflect the average genotypic performance observed across the other environments because the PC2 score was low, and the angle between AEA and the vector was small. Libau 2022 was more discriminatory than Edmonton 2022, Oxbow 2021, Libau 2020, Carman 2021, Roblin 2022, and Libau 2020. Additionally, Libau 2022's PC2 score was on the AEA therefore the environmental conditions in Libau 2022 were most reflective of the average performance of most environments. Environments Edmonton 2022, Oxbow 2021, Libau 2020, Libau 2021, and Roblin 2022 were very close to the origin and clustered together, indicating that these environments were not discriminatory, and genotypes performed similarly in those environments given the small vector angles between the environments. Roblin 2021 is situated far away from all environments, and the angle between Roblin 2021 and most environments is obtuse (except Carman 2022). The obtuse angle between Roblin 2021 and all other environments reveals that Roblin 2021 was an anomaly, and unlike any other environments (Yan and Tinker, 2006). In conclusion, the most discriminatory environments were Edmonton 2021, Carman 2020, and Carman 2022. The environments Carman 2020, Libau 2022, Oxbow 2022, Libau 2020, while Carman 2022 was most representative of the average performance across all environments. Similar genotypes performed well at Edmonton 2022, Oxbow 2021, Roblin 2022, Libau 2021, and Carman 2021, however, these environments were not discriminatory between genotypes. The genotypes that performed well at Roblin 2021 did not perform similarly in any other environment in the study.

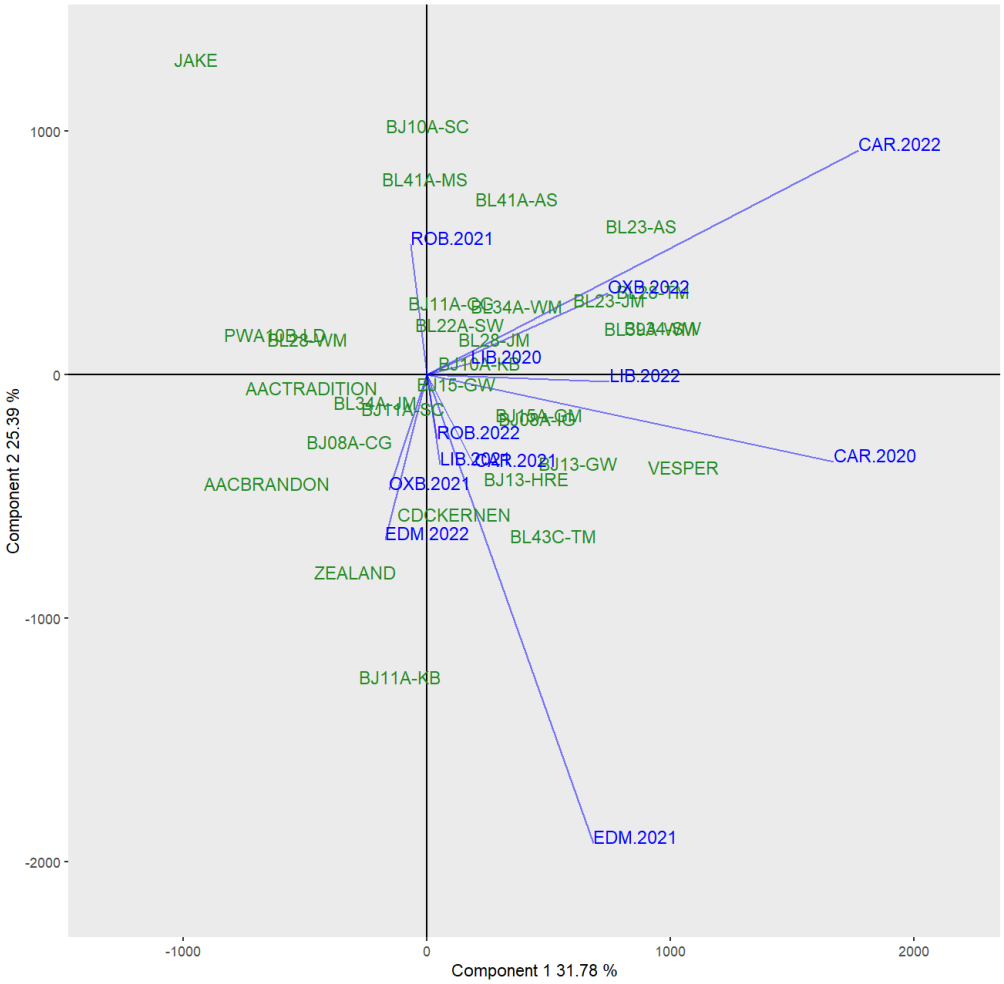


Figure 2-7. GGE Biplot resulting from lsmeans of mean yield for 25 organic spring wheat farmer genotypes and 6 check cultivars in 12 organic environments grown in 2020, 2021, and 2022. Column metric preserving SVP and Tester-Centered G+GE with no scaling**.**

2.5. Discussion

2.5.1. Benefits of farmer-selection on performance and wide adaptation under organic conditions

We evaluated 25 farmer genotypes and 6 check cultivars under 12 organically managed environments in Manitoba, Saskatchewan, and Alberta from 2020-2022. Farmer genotypes and check cultivars performed similar to other organic wheat trials conducted in western Canada (Kirk et al., 2012; Wiebe et al., 2017; Entz et al., 2018; Chen et al., 2019; Carkner et al., 2020). Sandro et al. (2022) also reported greater stability among farmer-selected hard red winter wheat genotypes compared to checks

in Midwestern United States. Differences in genotypes' yield, height, and kernel mass were dependent on growing environment. We fail to reject our first hypothesis that PPB genotypes will show significant yield variation across organic environments. Secondly, in some cases (ex. Oxbow 2022), PPB genotypes yielded significantly higher than most check cultivars, but not in all environments.

Three different stability models were used to evaluate the yield performance and stability of farmer genotypes and registered check cultivars. Verifying visual GGE Biplot results with the original dataset is crucial for proper interpretation (Yang et al., 2009). The GGE biplots concur with the original dataset, Vesper and BL23-AS yielded statistically similar to each other overall (Table 2-12) and is reflected graphically on the Which-Won-Where Biplot (Figure 2-6). Jake was the lowest yielding cultivar overall (Table 2-12) with high variability according to the Eberhart-Russell model, which was demonstrated on the Which-Won-Where GGE Biplot (Figure 2-6). Lastly, Roblin 2021 was extremely low yielding, with unique challenges such as heavy alfalfa competition, grasshopper infestation, and drought prior to anthesis. Roblin 2021 was classified as dissimilar to other environments in the location-focused GGE biplot analysis (Figure 2-7). Yang et al. (2009) suggests that biplot analysis should not be a used beyond a visual descriptive tool, because it does not involve statistical hypothesis testing tools. Consequently, we used multiple stability models and ANOVA in collaboration with the GGE Biplot analysis to make inferences on genotypic yield stability and genotype performance.

Generally, the different stability models showed similar genotypes as the best performers across all environments: BL23-AS, Vesper, BL43C-TM, and BJ13-GW. Under the GGE biplot analysis, BL23-AS and Vesper had the best performance in most environments tested (Figure 2-6). The poor performers captured in all three models were Jake, PWA10B-LD, and AAC Brandon. Entz et al. (2018) reported that Manitoba farmers were able to select genotypes that were similar or better than check cultivars when tested under organic conditions in Manitoba. The study similarly reported Vesper to be a very high yielding

cultivar across the organic environments tested (Entz et al., 2018). We have demonstrated that farmers from a wide geographic area selected genotypes that performed just as well and, in many cases, better than check cultivars under organic management. In addition, four of the five statistically highest yielding genotypes across most environments were PPB genotypes. We accept the hypothesis that farmer genotypes have greater yield performance and stability than most of the check cultivars. This adds support for the role of farmer selectors have to collaborate with plant breeders to increase genetic diversity and generate viable genotypes for organic agriculture in Canada. It is interesting that AAC Tradition, a cultivar bred under organic conditions by a formal breeding program, did not perform better or demonstrate greater stability than many farmer genotypes. Based on results from the Which-Won-Where analysis (Figure 2-6), BL34-SW and BL23-AS could be considered ideal genotypes for high moisture, higher weed pressure environments (Carman 2022 and Oxbow 2022) and Vesper, BL43C-TM and BJ13-GW would be considered better suited to drier, high fertility, low weed pressure environments (Edmonton 2021, Oxbow 2021, Edmonton 2022).

GGE biplot analysis specifically revealed that AAC Brandon was considered one the least adapted genotypes with the poorest performance across all environments. An exception was Edmonton 2022 (Figure 2-6) which was the highest yielding environment, with generous soil fertility (Table 2-4) and very little weed pressure. AAC Brandon was grown across 72-27% of all organic seeded wheat acres in Manitoba from 2019-2022, respectively (Manitoba Agricultural Services Corporation, 2019, 2020, 2021, 2022). The poor performance of AAC Brandon in the present study suggests that organic farmers in Manitoba are not growing the best performing cultivars and supports the need to test available cultivars under organic management. Additionally, this study demonstrated the need for farmers to diversify the cultivars grown in any one year to reduce the risks associated with environmental variability.

One major drawback of PPB genotype development is the ability for larger farms to market the grain (Storosko, 2022). The current wheat registration model requires that cultivars perform well under a wide range of conventional testing environments (Prairie Recommending Committee for Wheat Rye and Triticale, 2021). AAC Tradition was selected under organic conditions, however, for registration purposes, the cultivar needed to perform well under conventional conditions as well (Canadian Food Inspection Agency, 2017). Most large organic farmers in Canada sell to the export market that requires a named cultivar for marketing purposes. Consequently, without local market demand, Canadian organic farmers may always rely on conventionally bred cultivars. An example of genotypes from a PPB program integrating into the registration system is demonstrated in the PPB oat program. After three years of selection, the plant breeder has taken back the farmer genotypes and incorporated them into the ongoing breeding program for testing. We have demonstrated that cultivars from conventional-focused breeding programs can perform very well under diverse organic conditions (Vesper), however, more organic testing is required to determine these cultivars.

2.5.2. Usefulness of Multiple Stability Models for Contrasting Testing Environment Data

Increased yield is the outcome of three factors; genetic improvement, better crop management practices, and favorable growing conditions (Calderini and Slafer, 1998). Multiple yield stability models allowed for robust evaluation of genotype performance in contrast to using one model. Genetic stability using many models has been carried out by multiple studies (Kaut et al., 2009; Goyal et al., 2011; Subedi et al., 2021). The present study used test environments with multiple combinations of these three factors, and the goal of the Finlay-Wilkinson and Eberhart-Russell models is to attempt to separate genetic effects from environmental influences. However, the Finlay-Wilkinson and Eberhart-Russell models do not incorporate the unique features of each environment, and 'abnormal' years may result in conclusions that become biased against and towards certain growing environments. For example, AAC Brandon yielded the highest in Edmonton 2022, but performed poorly throughout most of the test environments. Due to

extremely high yields in Edmonton 2022, this environment's performance increased the average yield of AAC Brandon, such that it was seen as an 'average yielder' among cultivars in Finlay-Wilkinson and Eberhart-Russell models (Figure 2-4 and Figure 2-5). However, GGE Biplot analysis captured this inconsistency by visually representing the performance of AAC Brandon in relation how it performed in the majority of environments (Figure 2-6). Additionally, the GGE Biplot identified BL23-AS as one the most stable and highest yielding genotypes across the environments, however, the use of Finlay-Wilkinson and Eberhart-Russell showed that BL23-AS was not as responsive to favorable environments compared to other genotypes in the study (Finlay-Wilkinson model, Figure 2-4) and may in fact have greater variability (Eberhart-Russell model, Figure 2-5) than other genotypes. Multiple stability models are required and detailed knowledge of each environment would be useful for breeding and testing under organic environments given the heightened diversity of organic environments compared to conventional environments (Lammerts Van Bueren and Myers, 2012).

2.5.3. Evaluation of Test Environments

2.5.3.1. Stress vs. genetic expression

Genotype, Genotype by Environment Biplot analysis has the potential to help organic breeders evaluate the discriminatory potential of selection and test environments (Yan and Rajcan, 2002). Conventional breeding programs are assumed to service end-users (farmers) that have effective control over fertility, weeds, and diseases. Organic breeders have a unique challenge of delivering genotypes that can adapt to greater diversity of environmental factors, because organic farmers' weed control and fertility capabilities are more diverse. Cober and Morrison (2015) argue that breeders make slower genetic progress if using lower-yield potential environments to evaluate genotypes. They argue that test environments should be of high-yield potential to maximize genetic progress, even if it does not reflect farmer environments (Cober and Morrison, 2015). We did not observe similar patterns in this study, the test environments that allowed for greater genetic expression were not always the highest yielding (Table

2-12). Historically, genetic progress has not benefitted organic systems to the same degree as conventional systems, possibly because selection and evaluation environments were under conventional management (Pswarayi et al., 2014; Herrera et al., 2020). Test environment choice is a challenge for organic production systems because genotypic differences can be suppressed under stressful environments (Cober and Morrison, 2015), and test environments must reflect organic environments where reduced fertility and weed competition is common (Kirk et al., 2012). Genotype performance across multiple organic environments in the present study demonstrates the challenge of organically managed environments, eight out of 12 environments showed no genotypic main effects (Table 2-12). The eight environments that did not show genotypic main effects were not the lowest yielding, therefore genotypic expression was due to factors separate from pure yield performance (Table 2-12). Taken together, environment centered GGE Biplot analysis has the potential to visually aid plant breeders strategically choose test environments and find a balance between stress and genetic expression in organic environments. Therefore, GGE Biplot analysis should be used more extensively in organic breeding programs to identify the most suitable selection environments.

2.5.3.2. Utilizing GGE Biplot analysis to characterize test environments

Genotype, Genotype by Environment Biplot analysis can characterize environments and help visualize them in accordance to how representative some environments are of average performance observed throughout the trial. An ideal test environment would result in genotypic differences (discriminatory) and simultaneously reflect the average performance of other environments (representativeness) (Yan et al., 2007). A major challenge of organic G x E studies is the amount of influence environment carries as a portion of total variation in yield data. Environment contributed 94% of yield variability in our model (Table 2-14), more than others have reported under conventional evaluation (Robert, 2002; Yan and Rajcan, 2002; Mohammadi et al., 2010). Environmental variance can mask genotypic expression in multi-environmental trial data, especially when high yielding and low

yielding environments are weighted equally in stability models. For example, while Carman 2022 and Edmonton 2021 contributed to mean yield equally in the Finlay-Wilkinson and Eberhart-Russell models, GGE Biplot analysis revealed that the Carman 2022 and Edmonton 2021 environments were very discriminatory and similar to environments with acute angles with other environments, given the length of the vectors (Figure 2-7). In other words, plant breeders may be more interested in the genotypic rankings in Carman 2022 and Edmonton 2021 than other environments that were not discriminatory or representative (ex. Roblin 2021). Additionally, Edmonton 2021 and Carman 2022 may reflect two types of 'environmental clusters'. Edmonton 2021 and Carman 2022 vectors form an obtuse angle, therefore dissimilar genotypes performed well in those environments. Environments that are clustered close to Carman 2022 (Oxbow 2022, Libau 2022) had high rainfall and greater weed competition than other environments tested despite different soil fertility levels and soil types. Environments that clustered towards Edmonton 2021 (Oxbow 2021, Roblin 2022, Libau 2021, Carman 2021) had less than optimal precipitation, but were generally weed-free environments. GGE Biplot showed Roblin 2021 to have an obtuse angle from most other environments, indicating that Roblin 2021's performance was dissimilar to other environments. An organic plant breeder may choose to omit Roblin 2021 in further analysis and in the future, decide to choose against environments where stresses, such as heavy volunteer alfalfa competition, are present. Figure 2-7 also demonstrates that although Oxbow 2022 experienced heavy wild oat pressure, the genotype's yield performance was like many other test environments. Therefore, the species of weeds present in a test environment impacts genotypic discrimination, wild oat competition under organic conditions may not be detrimental to organic genotypic evaluation as nitrogen-fixing perennial competition, like alfalfa.

Organic plant breeders need to be cautious about making conclusions about a genotype's performance from biplot analysis without considering the data from ANOVA and other stability analyses but would benefit from using GGE Biplot analysis to evaluate test environments for genotypic evaluation.

Greater environmental influence over yield variation under low input conditions has prompted suggestions of a greater number of test environments to be included for stability detection (Herrera et al., 2020). However, more test environments are very expensive and like most breeding programs, organic breeding initiatives are constrained due to limited resources. Future research is needed to evaluate the impact of soil fertility, weed density, weed species, and environmental conditions to create more distinct 'clusters' of organic test environments for organic breeding programs. Such classification could enhance the efficiency and speed of genetic improvement of cultivars and cultivar testing under organic management.

2.5.4. Genotypic Drivers of Yield and Yield Stability

An attempt was made to understand the major contributions to yield variability in the study. The first was to use PLS regression, however, this analysis was deemed less useful since many factors that were significant were environmental according to the variable importance plot (Appendix A. Chapter 2. Figure A-1). Significant factors did not contribute to understanding genotypic differences beyond what correlation analysis could provide. It was observed that grain yield was significantly positively correlated with anthesis biomass, mature biomass, post-anthesis biomass, plant height, kernel size, kernel number, harvest index, and kernel production efficiency (Table 2-11). A correlation coefficient cut off >0.5 was used. Kernel size, post-anthesis biomass, harvest index, and kernel production efficiency are important, but only weakly associated with yield (ie. Less than 0.5).

The positive relationship between crop biomass (anthesis, maturity, post anthesis) and yield may indicate better competition with weeds. Lemerle et al. (1996) reported that wheat genotypes with high photosynthetically active radiation and greater early biomass were most competitive with weeds and therefore high anthesis biomass is thought to contribute significantly to yield. Final wheat yield is determined between stem elongation and anthesis (Fischer, 2007), it is during this critical period that

plants set the sink size (kernel number per spike) (Fischer, 2007). Wiebe et al. (2017) argued that maximizing the number of seeds per unit of anthesis biomass may be a selection strategy for organic wheat breeding. However, KNO:DMa (kernel production efficiency at anthesis) wasn't as strongly associated with yield as anthesis biomass in the present study (Table 2-11). Therefore, higher KNO:DMa was not as important as pure biomass accumulation among genotypes in this study. The greater anthesis biomass for higher yield genotypes may have a two-fold benefit for organic systems, greater early weed competitiveness and greater seed set for yield.

Final harvest plant biomass accumulation has been associated with recent yield gains in wheat since harvest index has stayed the same since 1980s (Rivera-Amado et al., 2019). This indicates that plants are producing more biomass, but not partitioning proportionately more carbon into the grain. Fischer (2008) argued that higher mature biomass accumulation has been the result of breeding for greater kernel numbers and higher post-anthesis dry matter accumulation. Our results support this, for example, AAC Brandon and PWA10B-LD were among the lowest biomass accumulators at anthesis and maturity and yielded poorly (Table 2-8 and Table 2-12). However, Jake, another poor yield performer, had high anthesis biomass and low mature biomass (Table 2-8). Post-anthesis biomass accumulation may be specifically important to genotypes grown in organic environments, crops are continuously competing with weeds while also maintaining growth and carbon translocation into the grain post-anthesis. High yield genotypes in the present study were able to accumulate greater biomass post-anthesis, while simultaneously competing against weeds and translocating carbon into the seeds efficiently (Table 2-11). Further research examining the timing of biomass accumulation for wheat under organic growing environments would be valuable.

Plant height was strongly correlated with grain yield in this study (Corr *P>F* <.0001 Estimate: 0.7, Table 2-11). Under organic environments, plant height and greater weed competitiveness is linked; due

to the crops' ability to compete for light (Wicks et al., 2004; Mason et al., 2007c; Zerner et al., 2008). When others evaluated organic-selected genotypes, they were taller than check cultivars by up to 10cm (Brumlop et al., 2017; Wiebe et al., 2017; Entz et al., 2018). Historically, wheat breeders in Canada have continuously selected against plant height to increase lodging resistance (Iqbal et al., 2016). The observation that AAC Brandon was both shorter (by 20cm compared with farmer genotypes) and lower yielding support the need for tall genotypes in organic production. However, taller plants are not the only phenotypic quality that contributes to greater weed competitiveness and performance under organic conditions (Mason and Spaner, 2006). For example, Vesper was one the highest yielding cultivars (Table 2-12), but among shortest plants (Table 2-10). On the other hand, while BJ08-IG was consistently one of the tallest genotypes (Table 2-10), it achieved only an average yield compared to other genotypes (Table 2-12). The lower yield for BJ08-IG could not be attributed to lodging as conditions in the present study were not conducive for lodging observations. Entz et al. (2018) reported higher lodging susceptibility among taller farmer genotypes when tested under more lodging conducive conditions. Therefore, it is important to recognize that although plant height is advantageous for organic conditions, a balance between competitiveness ability and lodging potential needs to be made.

As a sink-limited crop plant, wheat yield is typically connected with kernel production per unit area of land (Fischer, 2008). In the present study yield and kernel number were strongly correlated (Corr *P>F* <.0001 Estimate: 0.94, Table 2-11). Number of kernels per unit area is determined between the time of the head initiation phase and shortly after anthesis (Slafer and Rawson, 1994). Increased assimilate availability during this phase increases potential grain yield through increased kernel set (Duggan and Fowler, 2006). Wiebe et al. (2017) found that organically bred genotypes had greater kernel number per unit anthesis biomass than check cultivars under organic conditions.

It has been hypothesized that kernel size and kernel number are negatively correlated (Slafer et al., 2022). Kernel number and kernel size were not negatively correlated and were not strongly related (Corr *P>F* 0.021, Estimate: 0.067). Jake, a poor yielder, demonstrated low kernel number in combination with low kernel size, even in Edmonton 2022, a high yield environment. AAC Tradition had the lowest kernel number and the highest kernel weight (Table 2-13 and Table 2-15) but was not a high yielder (Table 2-12). BL23-AS was a consistent high-yielder with the greatest kernel number, however, had small kernel size (Table 2-12). Our results agree with Slafer et al. (2014), who argued that kernel number is the main driver of yield, and kernel size, while important, may only make small contributions to final yield. While kernel number was the main yield driver, genotypes' ability to fill kernels also played a minor role. In other words, higher kernel numbers did not come at the expense of smaller kernels.

Vesper's high yield performance may be due to its orange wheat blossom midge-resistance (*Sitodiplosis mosellana* Géhin) trait containing the antibiosis resistance gene *Sm*1 (Thomas et al., 2013). The year 2022 had heavy wheat midge pressure, mainly in the eastern prairie environments (Oxbow, Roblin, Carman, and Libau) (Figure 2-8). Orange wheat blossom midge likely impacted yield performance at those environments. While midge-resistant cultivar releases have been attributed to rising historical yields among hard red spring wheat in western Canada (Vera et al., 2013; Thomas and Graf, 2014). Vesper is a parent of farmer genotypes BL23 (AS and JM) and BL22-SW (Table 2-2). Shaw, another midge-resistant cultivar (Fox et al., 2013), is parent of BL43C-TM and BL34 (JM, WM, and SW). Farmer genotypes with the parental genotypes of Vesper and Shaw (notably, BL23-AS, BL43C-TM, and BL34-SW) were also top performers with good stability (Table 2-12, Figure 2-4 and Figure 2-5). Thus, this research has demonstrated the importance of incorporating midge-resistant genes into an organic breeding program on the Prairies.

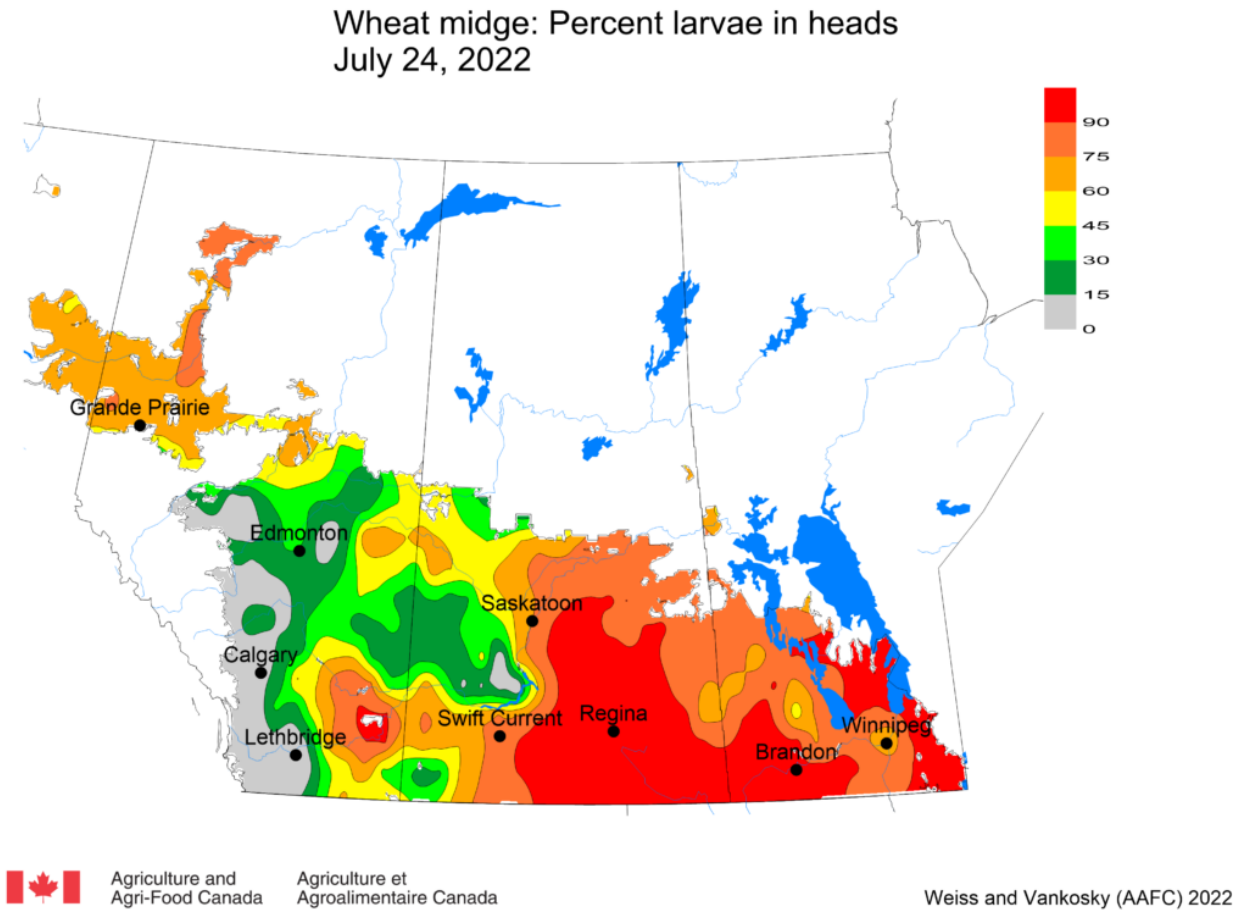


Figure 2-8. Per cent wheat midge population *(Sitodiplosis mosellana* Géhin) that is in the larval stage (in wheat heads), across western Canada as of July 25, 2022. (Weiss and Vankosky, 2022)

2.6. Conclusion

This study set out to ask the question about whether farmer genotypes from an organic PPB program would perform as well as commercial cultivars on the bases of yield and yield stability. Three farmer genotypes and one check cultivar (BL43C-TM, BL34-SW, BJ13-GW, and Vesper) were found to be top performers based upon ANOVA analysis, Finlay-Wilkinson stability analysis, and GGE Biplot analysis. Another farmer selected genotype (BL23-AS) demonstrated high yield, but also superior stability according to the Finlay-Wilkinson test and GGE Biplot analysis. AAC Brandon, the most popular commercial wheat cultivar in Manitoba, was found to have low yield and less stability. Therefore, wheat genotypes selected under organic conditions by organic farmers show promise for the organic sector.

Better performance of farmer genotypes over AAC Tradition an organically selected cultivar by researchers, further demonstrates the value of engaging farmers directly in the early generation selection process.

Main yield drivers were biomass accumulation at anthesis and at the soft dough stage as well as kernel number per unit area. Kernel number was not created at the expense of kernel size, indicating that larger kernel seed mass as well as kernel number can be achieved simultaneously under organic management. Parental material containing midge-resistance would be extremely valuable for organic breeding programs. Future research examining more detailed yield physiology parameters and belowground dynamics would be useful for organic breeding programs.

Organic breeders would benefit from using GGE biplot visualization to assess test and selection environments for their discriminatory and representativeness qualities among genotypes. In the present study, environmental characteristics such as soil fertility, seasonal precipitation amounts, weed species present, and weed abundance impacted which environments clustered together, and which environments had discriminatory power. BL23-AS may be better suited to environments with high rainfall and greater weed competition and Vesper may be better suited for low precipitation, less weed competition, and greater soil fertility. Given the wide diversity of organic environments, some argue *more* test environments are required under organic management. However, identifying what environmental qualities (weed species and abundance, soil fertility) could enhance or dampen genetic expression and discriminate between genotypes could reduce the number of test environments required for organic breeding programs, and therefore, increase efficiency. Organic production systems have greater environmental complexity than conventional production systems, which may require more complex, holistic selection criteria to perform optimally.

This study demonstrates that participatory wheat breeding is an effective program to create high performing, robust wheat genotypes for organic production systems in Canada. Marketability challenges still exist for unregistered, PPB-derived genotypes, restricting use among large organic farmers. However, to overcome this obstacle, many PPB farmers are currently creating and finding markets for PPB grain at the local level (Jowett, 2023). Additionally, the PPB model has the potential to be successfully integrated into larger, established breeding programs to create genetic material for underserved markets.

CHAPTER 3.

AGRONOMIC COMPARISON OF CONTRASTING SPRING WHEAT (*TRITICUM AESTIVUM* L.) PARENTAL MATERIAL AND THEIR FARMER-SELECTED POPULATIONS

3.1. Abstract

Organic farmers are minimally benefitting from modern spring wheat breeding efforts, prompting breeders and researchers to investigate the suitability of landrace genotypes for organic production systems as well as the benefits of dedicated organic breeding programs. An important question in breeding for cultivars adapted to organic production regards parental choice. Our first objective was to evaluate the performance of a modern (5602HR) genotype and a landrace (Red Fife) genotype under organic conditions to use as parental material for an organic Participatory Plant Breeding (PPB) program. Using the modern and landrace genotype as parental material, our second and third objectives were to evaluate how farmer genotypes were similar or different from i) the divergent parents, and, ii) each other under diverse organic conditions. Red Fife was taller, later maturing, more susceptible to lodging, had greater kernel mass, and lower protein than 5602HR. Specifically, 5602HR protein was more responsive to added fertility, and Red Fife reached its protein concentration potential at 13%, a lower concentration than 5602HR by 3.6%. Farmer genotypes were similar to the modern parent (5602HR) regarding protein level and lodging severity, and similar to the landrace parent (Red Fife) in plant height and kernel mass. Farmer genotypes did not differ from each other in terms of biomass accumulation, plant height, days to maturity, yield, kernel mass, test weight, protein, and yield efficiency measurements. However, farmer genotypes differed from each other in terms of test weight and protein levels depending on environmental conditions. We demonstrate that farmers can select genotypes that possess beneficial traits from landrace and modern parental material to better serve modern organic farmers in Canada.

3.2. Introduction

During the last century, wheat breeding efforts have been focussed on improving yield performance and bread-making characteristics under high fertility, pesticide-reliant agricultural systems (Lammerts Van Bueren and Myers, 2012). Modern genotypes are selected under environments with a high level of control over soil fertility status and weed pressure. This selection environment is not reflective of organic production environments since organic production excludes the use of synthetic fertilizers, herbicides, fungicides, and insecticides. Therefore, it is argued that organic farmers are not benefitting from modern wheat breeding efforts, prompting breeders and researchers to investigate the suitability of landrace genotypes for organic production systems as well as the benefits of dedicated organic breeding programs (Lammerts van Bueren et al., 2002; Murphy et al., 2005; Reid et al., 2011; Migliorini et al., 2016). The two approaches used to develop "organic genotypes" include standard scientist-led breeding programs on research stations (Kirk et al., 2012), and a farmer participatory model where farmers conduct early generation selection on their own fields (Entz et al., 2018).

An important question in breeding for genotypes adapted to organic production regards parental choice. Newer genotypes, certainly in Canada, contain high quality breadmaking attributes and good resistance to disease. However, there is also interest in old genotypes since these may be better suited to low external input farming (Yahiaoui et al., 2014; Casañas et al., 2017). Landrace cultivars are characterized as, 'heterogenous crop genotypes developed over time through both farmer selection and evolutionary processes' (Murphy et al., 2005). Landrace cultivars have been hypothesized to better suit organic production systems, because they were adapted, selected, and successfully grown before the advent of industrialized agricultural systems (Yahiaoui et al., 2014; Migliorini et al., 2016). While landraces are valuable for organic production systems, some possess traits that are not desirable, such as lodging risk, disease susceptibility, late maturity, low yield, low protein, and poor test weight (Paulsen and Shroyer, 2008; Jones and Econopouly, 2018). Modern genotypes, on the other hand, possess the genetic

potential to reduce lodging risk, increase disease resistance, reach higher yield, and superior bread-making qualities (Thomas and Graf, 2014). Conventional breeding programs are not aligned with phenotypic traits valuable to organic. Phenotypic traits valuable to organic production systems include early plant vigour, increased height, greater kernel mass, greater biomass accumulation, increased kernel production efficiency, higher nutrient uptake from mineralized sources, increased adaptability to environmental heterogeneity, greater disease resistance, high yield, and high protein (Huel and Hucl, 1996; Mason and Spaner, 2006; Mason et al., 2007a; Wolfe et al., 2008; Kaut et al., 2009; Lammerts Van Bueren and Myers, 2012; Benaragama et al., 2014; Kokare et al., 2017; Wiebe et al., 2017; Herrera et al., 2020; van Frank et al., 2020). Canadian organic farmers are specifically concerned with protein due to marketability standards; the Canadian Grain Commission requires a minimum 10% protein for Class 1 wheat (Canadian Grain Commission, 2023), and in some cases, farmers receive a premium for even higher protein wheat (Mangin et al., 2022). Pre-anthesis dry matter is often constrained by weed competition and slow soil nutrient release (Cicek et al., 2014; Wiebe et al., 2017). Kernel production efficiency at anthesis (KNO DMa^{-1}), or, kernel production efficiency (Fischer, 2008), has been hypothesized as an important performance parameter in breeding genotypes targeted for organic environments (Wiebe et al., 2017). Taken together, we hypothesize that crossing a landrace with a modern genotype would benefit organic farmers by incorporating both parental traits to meet the needs of modern organic production systems. The first objective of this study was to compare the performance of the two parental genotypes used to generate genotypes for our organic breeding program. These included the landrace cultivar (Red Fife) and the modern cultivar (5602HR) in phosphorus limited P and P-amended regimes under organic conditions.

The importance of selection environment in breeding programs is well established, which has prompted organic breeding efforts across the world (Murphy et al., 2007; Wolfe et al., 2008; Reid et al., 2009; Kirk et al., 2012). Participatory plant breeding (PPB) takes this concept a step further, involving

farmers in the parental selection and selection testing process of a breeding program (Colley et al., 2021). Initial PPB projects have taken place in developing countries to create locally-adapted cultivars under low-input, stressful environments (Almekinders and Elings, 2001b; Bänziger and Cooper, 2001; Murphy et al., 2005; Ceccarelli and Grando, 2007); however, PPB initiatives have gained momentum to meet the needs of ecological and organic agriculture in North America and Europe (Colley et al., 2021). Organic breeding within a PPB program at the University of Manitoba in collaboration with Agriculture and Agri-Food Canada (AAFC) has taken place from 2011-2020. Detailed account of the administration for this PPB program is described in Entz et al. (2018). These sources of genetic diversity offer an unprecedented opportunity to better understand the response of organically selected wheat to organic production systems. We hypothesized that the selection environment and farmer-breeder actions influence the genotypes to exhibit traits that were similar to the landrace genotype parent and modern genotype parent in ways that serve modern organic farmer needs. The second objective of this study was to investigate how farmer genotypes differed from their modern and landrace parents.

There is strong evidence that the selection environment creates distinctly different genotypes, whether through conscious selection by breeders (Kirk et al., 2012; Wiebe et al., 2017) or farmers (Entz et al., 2018), or through natural selection (Allard and Hansche, 1967; Horneburg and Becker, 2008; Knapp et al., 2020). To our knowledge, only Entz et al. (2018) has tested different PPB genotypes selected by different farmers from the same cross in Canada, however, the geographic distance between farmers was relatively small. Despite this, the authors noted multiple distinct differences between genotypes (Entz et al., 2018). Little is known about how farmers from distinct geographic areas impact the phenotypic performance in Canadian organic spring wheat. Our last objective was to evaluate the impact geographically divergent farmers and their respective environments had on a full sibling derived genotypes between modern and landrace parents. We hypothesize the farmer genotypes will contain multiple phenotypic traits that are valuable to organic production systems in Canada.

3.3. Materials and Methods

3.3.1. Genetic material

Genotypes were sourced from the University of Manitoba PPB wheat program as described by Entz et al. (2018). The PPB program used genetic material from different sources, including AAFC crosses and others. In the present study, parental choice for crosses were made on recommendation from organic farmers in Western Canada. Family 1 farmer genotypes (derived from the cross, 5602HR and Red Fife) completed selection in 2015. Treatments in the experiment are organized into a 'family'. A 'family' consists of the initial crossed parents (5602HR and Red Fife), and two full sibling selected genotypes derived from different farmers selected over three years (Farmer genotypes designated; Farm1 and Farm2). During the initial crossing between 5602HR and Red Fife, all pollen was removed from the female plant, and pollen-removal was confirmed daily until the cross. The cultivar 5602HR is a medium-height, awned cultivar, and Red Fife is an awnless, tall cultivar. The F_2 plants were observed to possess a diversity of short, medium, and tall plants, and a mixture of different lengths of awns (Kirk, pers, comm.). Seeds were increased at the Organic Research Farm at the Ian N. Morrison Centre in Carman, Manitoba to the F_3 generation. Farmers were sent 400 to 600g of seeds of the F_3 generation (4000 seeds). Plots were seeded on farm by the farmer using a garden seeder (Farm1) or a plot-sized disk drill (Fabro Industries, Swift Current SK) (Farm2), in 20-m^2 plots. Positive and negative selection occurred throughout the growing season based on farmer's preferences. Final selection of approximately 300 spikes were made at harvest. The selected spikes were sent to the University of Manitoba for threshing and returned to farmers the following spring. This process was repeated for three consecutive years in 2013, 2014, and 2015. In 2015, the F_5 generation seed was saved until it was grown out in a common experiment in 2019 under organic conditions in at the Ian Morrison Research Farm in Carman, Manitoba and stored under similar conditions. Therefore, the seed used in the present experiment is sourced from the same growing environment stored under the same conditions.

3.3.1.1. Description of parental cultivars

The cultivar 5602HR was registered in 2005 (registration number: 2202) under conventional conditions (Canadian Food Inspection Agency, 2005) and is designated under the Canadian Western Red Spring class (Canadian Grain Commission, 2023). 5602HR is the selected genotype of AC Barrie/Norpro, and was produced in Borthoud, Colorado. It has high yield and protein potential under conventional testing trials (Canadian Food Inspection Agency, 2005), 5602HR is moderately resistant to Fusarium head blight (*Fusarium graminearium)*, and common bunt *(Tilletia caries*, *Tilletia foetida*), and is resistant to loose smut *(Ustilago tritici)*, stem rust *(Puccinia graminis* f. sp. *tritici*), and leaf rust *(Puccinia triticina*) (Canadian Food Inspection Agency, 2005). Previous research demonstrated that under organic conditions, 5602HR had high protein and yield (Wiebe et al., 2017), however did not suppress weeds in some environments compared to other cultivars tested (Pridham et al., 2007; Wiebe et al., 2017).

Red Fife is a 'landrace' variety and is no longer registered with the Canadian Grain Commission. It is known to be tall, and late maturing (Paulsen and Shroyer, 2008). Red Fife is often grown by organic farmers in Canada and is widely believed to have originated in Ukraine and was cultivated in 1842 in southern Ontario by D.A. Fife (Paulsen and Shroyer, 2008). It dominated spring wheat acres during the late 19th century for more than 40 years (Olmstead and Rhode, 2002). It fell out of favour 1912 when its offspring, Marquis, was introduced (Clark and Martin, 1922). From previous studies, Red Fife is tall (Carr et al., 2006; Iqbal et al., 2016), susceptible to leaf rust (Martens et al., 2014), late maturing (Iqbal et al., 2016), yields comparable to modern genotypes under organic conditions (Pridham et al., 2007; Kirk et al., 2012), and has low protein (Mason et al., 2007a; Kirk et al., 2012; Wiebe et al., 2017).

3.3.1.2. Description of farmer selector and selection environment

Both Farm1 and Farm2 have been organically farming for over 20 years when selection of genotypes were conducted on-farm.

Participatory plant breeding genotype, Farm1, was selected on a 94-acre organic farm in Agassiz, British Columbia (49°14'24.2"N 121°45'56.3"W). The area's average growing season temperature is 11.5°C (Environment Canada, 2022). Winters are mild, daily minimum and maximum temperatures range from 0.5°C and 9°C. Precipitation throughout the year is in the form of rainfall (96%), with the average yearly precipitation of 1754mm (Environment Canada, 2022). Precipitation follows a typical pattern of heavy winter rainfall and drier summers (Comar et al., 1962). The soil is an imperfectly drained Fairfield silty clay loam; Gleyed Mull Regosol (Comar et al., 1962). The farm has been farming organically since 1976, and certified organic since 2010. The farmer primarily grows grains (spring and winter-wheat, rye, and oats), with a white clover-grass green manure. Each grain crop receives dairy slurry in the spring from a neighbouring farm. The straw is exported each year for community gardens and the local horse industry as bedding. A representative soil test is shown in Table 3-1. The primary market for grain is through a Grain Community Supported Agriculture (CSA) model, as well as farmers markets, serving the nearby Vancouver urban population.

Participatory plant breeding genotype, Farm2, was selected by an experienced organic grain farmer on a 10-acre organic research section within the University of Manitoba Ian N. Morrison Research Farm, in Carman, Manitoba (49°29'45.4"N 98°02'11.6"W). The area's average growing season is 15°C (Environment Canada, 2022). Rainfall accounts for 80% of yearly precipitation, with the average yearly precipitation being 545mm (Environment Canada, 2022). Most precipitation occurs during the growing season (May-August), however, moisture at seeding is heavily reliant on snowfall. Winter daily minimum and maximum temperatures range from -20.5°C and -0.6°C. The soil is a fine sandy loam and classified as an Orthic Black Chernozem in the Canadian soil classification, belonging to the Hibsin Series. The experimental farm has been organically managed since 2004. Wheat is grown in a six-year rotation after green manure crop (green manure-wheat-soybean-green manure-flax-oat). The crop rotation includes

two green manure phases with flax, wheat, oats, and peas. No manure has been applied to the rotation since 2004. A representation of the soil nutrient status is shown in Table 3-1.

Table 3-1. Soil nutrient status of the target growing environments for two spring wheat participatory farmer genotypes.

Site	Nitrate-N [a]	Sulphate-S [b]	P	K [d]	Organic Matter [e]	pH [f]
	kg ha^{-1}	kg ha^{-1}	mg kg^{-1}	mg kg^{-1}	%	
Agassiz, BC	82	22	26*[g]	47	3.8	5.5
Carman, MB	47	16	15**[c]	336	5.6	6.1

*Modified Kelowna-P, **Olsen-P

[a] Extraction in 0.2 M KCl using Cd reduction determination method (Gelderman and Beegle, 2015). Conversion to mass-per-area was determined by the soil analysis lab based on assumptions of regional soil bulk density.

[b] Extraction in 1.0 M Nh_4OAc at pH 7.0 with atomic emission spectroscopy (Warncke and Brown, 2015).

[c] Olsen-P: Extraction of 0.5 M $NaHCO_3$ at pH 8.5 (Olsen et al., 1954) using the spectrophotometry determination (Frank et al., 2015)

[d] Extraction in 0.2 M KCl at room temperature with the turbidimetric determination method (Cihacek et al., 2015).Conversion to mass-per-area was determined by the soil analysis lab based on assumptions of typical regional bulk density.

[e] Total organic matter by loss of ignition (Combs and Nathan, 2015)

[f] Determined in 1:1 soil:water (Peters et al., 2015)

[g] Modified Kelowna-P: Extraction with 0.015 M ammonium fluoride, 0.25 M ammonium acetate, and 0.25M acetic acid for 15 min on a reciprocal shaker. The suspension was filtered, and the clear solution stored for P determination (Qian et al., 1994). Phosphorus was determined colorimetrically using the molybdate blue method as described by Murphy and Riley, (1962).

3.3.1.3. Selection Criteria

The farmer who selected Farm2 reported that they were selecting for large heads, high number of seeds per spikelet, lodging resistance, seed chaff completely covering the seed, longer green flag leaf late in the season, and medium height. The farmer who selected Farm1 selected for lodging resistance, uniform and 'decent' spikes, rust resistance, and good plant growth when weeds are present.

3.3.2. Evaluation Experiment Description

The experiment took place in 2020, 2021, and 2022 in Libau, Manitoba (50°24'01" N, 96°72'95" W). The soil was a Gleyed Rego Black Chernozem soil belonging to the Dencross series and managed according to organic production standards since 2008. In all years, the experiment was preceded by a

green manure mixture which consisted of corn, sunflowers, oats, peas, and soybean which was incorporated into the soil with a heavy-duty field cultivator at the time of full flower (approximately mid-August). Soil tests revealed the fields to have very low soil available phosphorus. adequate in nitrogen, and high pH (Table 3-2). Very low soil P status was the result of continuous alfalfa hay harvest since 2006, and no additional nutrient application. Previous work has shown that continuous alfalfa hay removal with no nutrient replenishment substantially reduced soil test phosphorus on organically managed land (Welsh et al., 2009; Carkner et al., 2020).

Table 3-2. The soil nutrient status, organic matter content, and pH of experimental years in 2020, 2021, 2022.

Experimental sites	Depth	N [a]	S [b]	P [c]	K [d]	OM [e]	pH [f]
	cm	--kg ha^{-1}--		--ppm--		%	
2020	0-15	33.6	24	4	289	6.2	8.2
	15-60	94	73				8.3
2021	0-15	49	31	3	278	5.7	8.1
	15-60	94	53				8.3
2022	0-15	20	13	3	222	4.7	8.2
	15-60	78	40				8.4

[a] Extraction in 0.2 M KCl using Cd reduction determination method (Gelderman and Beegle, 2015). Conversion to mass-per-area was determined by the soil analysis lab based on assumptions of regional soil bulk density.
[b] Extraction in 1.0 M Nh_4OAc at pH 7.0 with atomic emission spectroscopy (Warncke and Brown, 2015).
[c] Olsen-P: Extraction of 0.5 M $NaHCO_3$ at pH 8.5 (Olsen et al., 1954) using the spectrophotometry determination (Frank et al., 2015)
[d] Extraction in 0.2 M KCl at room temperature with the turbidimetric determination method (Cihacek et al., 2015).Conversion to mass-per-area was determined by the soil analysis lab based on assumptions of typical regional bulk density.
[e] Total organic matter by loss of ignition (Combs and Nathan, 2015)
[f] Determined in 1:1 soil:water (Peters et al., 2015)

3.3.3. Experimental Design and Treatments

The experiment was a factorial randomized complete block design with two factors (genotype and nutrient status). Genotype treatments were 5602HR, Red Fife, farmer genotype 1 (Farm1), and farmer genotype 2 (Farm2). All seed was tested for germination rate to calculate seeding rate. The nutrient status

of composted manures (7.7 g kg^{-1} N, 6 g kg^{-1} P in 2020, and 29 g kg^{-1} N, 7 g kg^{-1} P in 2021 and 2022) were determined by Agvise Laboratories (North Dakota, USA). Composted manure was applied at a rate of 25 kg P ha^{-1}, assuming 50% P available in the first year (Eghball et al., 2002). Experimental units were 0.61m x 6m in 2020, and 1.5m x 6m in 2021 and 2022. All experimental plots had 15cm row spacing.

3.3.3.1. Field Management

The seedbed was prepared using a field cultivator, then was heavy diamond harrowed less than 24 hours prior to seeding to conserve moisture. Experiments were seeded into moist soil (2.5-cm to 3.8-cm depth depending on soil moisture level) using a disk drill (Fabro Industries, Swift Current, SK) at a target plant population of 350 live plants m^{-2}. Seeding dates were May 7, April 28, and May 24 in 2020, 2021, and 2022, respectively. Manure was surface broadcast on each experimental unit in the spring prior to or directly after seeding and spread by light raking. Hand weeding of Canadian thistle (*Cirsium arvense* L.*)*, wild mustard (*Sinapis arvensis* L.*)*, and sow thistle (*Sonchus arvensis* L.) from some experimental units was sparingly required in all years, as field experiments were generally weed free. Experimental units were harvested using a Hege plot combine 58 (Hege model 125, Hege Company, Waldenburg, Germany) and dried on forced air beds for 72 hours. Grain samples were cleaned using a Carter Day dockage tester (model 31624/W-3301). Harvest took place on August 19, August 11, and September 10 in 2020, 2021, and 2022, respectively.

3.3.3.2. Data Collection

Plant populations were measured at the three-leaf stage in two randomly selected 2m areas of each experimental unit. Aboveground biomass was sampled at three times in the growing season according to growth stages outlined by Zadoks et al. (1974): at stem elongation (Zadoks stage 30), at anthesis (Zadok stage 64), and at the hard dough stage (Zadok stage 87). Wheat biomass at the stem elongation and hard dough stage will be referred to as early and maturity biomass, respectively. At all

aboveground biomass samplings, plants were cut at ground level. Due to limited plot size in 2020, 0.075m^2 sample was taken at stem elongation (early) and anthesis, and 0.15m^2 sample was taken at the hard dough stage (maturity). In 2021 and 2022, 0.31m^2 sample was taken at stem elongation (early), and 0.15 m^2 sample was taken at anthesis and at maturity. Aboveground biomass was then dried at 70°C for 36 hours and weighed. Plant height measurements occurred at maturity by measuring the distance from the soil to the top of the spike at two randomly selected areas in each experimental unit. Lodging occurred in 2022 only, lodging measurements were taken at maturity on a 1-to-9 scale, which 1 representing upright rows and 9 representing plants lying flat on the ground. Days to maturity was determined when at least 50% of the plants in the experimental unit were at Zadoks stage 92.

Kernel mass was determined by counting 250 seeds with Old Mill Counter Model 850-3 (International Marketing and Design Corporation, San Antonio, Texas) seed counter, then weighing the sample and multiplying by four to find the weight of 1000 seeds. Protein and test weight were measures using a Foss Infratec Grain Analyzer (Foss Industries, Hillerød, Denmark). Protein was analyzed using Near Infrared Spectrometry. Kernel number represents the kernel number per unit of area (hectare) based on kernel mass and weight per unit area (yield). Kernel production efficiency is expressed as the number of kernels per unit biomass at anthesis and calculated by dividing the kernel number per hectare by the amount of anthesis biomass per hectare. Harvest index was calculated as the dry weight of yield divided by the dry weight of mature biomass multiplied by 100 to achieve a percentage value.

3.3.4. Data analyses

In-field measurements and calculations were analyzed with years combined to detect genotype x manure, genotype x year, year x manure, or year x genotype x manure interactions. In cases where an interaction was observed, years were analyzed separately. Datasets were analyzed using the PROC Mixed procedure with Statistical Analysis Software program 9.4 (SAS, 2013a). Manure, genotype, and manure x

genotype were fixed effects, and replicate (year) and year were random effects. Tests for normal distribution of residuals were carried out using PROC Univariate with Shapiro-Wilks values. Values greater than 0.9 were assumed to be normally distributed. Differences among genotypes and manure phosphorus levels were tested using the Least Significant Difference (LSD) test and considered significant at $p < 0.05$. Data shown in tables represents the Least Squares Means (lsmeans). To compare the farmer genotypes with the parents, treatments were combined and analyzed into three groups: Farmer genotypes contrasted with both parents, farmer genotypes contrasted with Red Fife, and farmer genotypes contrasted with 5602HR. Contrasts were carried out using PROC GLM procedure in SAS 9.4 (SAS, 2013b).

3.3.5. Environmental Conditions

Seasonal precipitation data was obtained through the Selkirk weather station monitored by Manitoba Agriculture, Food and Rural Development (MAFRD) (MAFRD, 2022). The weather station is approximately 8.3 km from the field site (50°17'71" N, 96°79'28" W). Monthly precipitation and growing degree day (GDD) data compared to long-term averages are shown in Table 3-3. Between May and August, the experiment received approximately 66%, 62%, and 125% of 30-yr average rainfall in 2020, 2021, and 2022, respectively. Seasonal temperatures were also unseasonably high and GDDs were above normal in almost every month in 2020 and 2021, particularly in June (114% of normal in 2020, and 119% of normal in 2021). Between May and August, growing season GDD were 108% of normal in 2020 and 109% of normal in 2021. Growing degree days in 2022 were equivalent to 30-yr average levels.

In 2020 and 2022, timely rains two weeks after seeding, at stem elongation (Zadoks 30), and anthesis (Zadoks 64), facilitated manure mineralization and assisted crop growth. However, in 2021, while precipitation occurred three weeks after seeding and a significant rainfall event took place at stem elongation, daily temperatures often reached above 30°C. Additionally, no precipitation from stem elongation to ripening resulted in poor growth and dampened manure response. Wet spring conditions

delayed seeding in 2022. Heavy rains immediately after seeding in 2022 lead to some crusting which hindered emergence in some experimental units.

Table 3-3. 2020, 2021, and 2022 monthly precipitation and growing degree day summaries and 30-yr averages (MAFRD, 2020, 2021) in nearby weather station in Selkirk, MB.

		Precipitation			**Heat**		
Month	Year	Precipitation	30-yr Average	% of 30-yr Average	Growing Degree Days	30-yr Average	% of 30-yr Average
			----mm----		--Growing Degree Days--		
May	2020	19	59	33	194	204	96
	2021	22		37	187		92
	2022	139		239	202		99
June	2020	58	85	68	407	357	114
	2021	45		53	427		119
	2022	55		65	369		103
July	2020	30	71	43	495	457	108
	2021	24		34	514		112
	2022	111		156	460		101
August	2020	85	74	114	448	413	109
	2021	88		119	439		106
	2022	60		81	410		99
May-August	2020	193	291	66	1548	1433	108
	2021	179		62	1568		109
	2022	366		125	1443		101

3.4. Results and Discussion

3.4.1. Manure and environmental main effects

Differences in manure response in all growth parameters across experimental years resulted in significant year main effects for early biomass, plant height, yield, kernel mass, test weight, kernel number per unit area, protein, and kernel per unit anthesis dry matter (Table 3-4). There was no year effect for plant population, anthesis biomass, mature biomass, and harvest index. The most productive year was 2022, followed by 2020, and 2021. For example, grain yields ranged between 1936-2359 kg ha^{-1} in 2020, 1450-1523 kg ha^{-1} in 2021, and 2679-2870 kg ha^{-1} in 2022 (Table 3-4). These yields are lower than other organic wheat yields reported in Manitoba (Wiebe et al., 2017; Carkner et al., 2020). Low yields were attributed to low soil-test P (STP) (Table 3-2) low precipitation (Table 3-2) and high temperature stress (Table 3-3). When STP was amended, and environmental conditions were optimal in the present study (P-amended treatments in 2022), yield ranged between 2994-3298 kg ha^{-1} (Table 3-7), higher than historical organic manure-amended wheat yields at the Glenlea long-term rotation in Manitoba (Carkner et al., 2020).

A significant year x manure interaction was detected in early biomass, anthesis biomass, yield, protein, and kernel number. The source of the interaction derives from the magnitude of manure response amongst the experimental years. For example, there was a highly significant difference between P-amended treatments ($P>F$ <.0001) for early biomass production in 2022, P-amended treatments had higher biomass than limited P treatments by 400 kg ha^{-1} (Table 3-7). However, in 2020 and 2021, there were no significant differences in biomass between manure treatments (Table 3-5 and Table 3-6). Dry spring conditions in 2020 and 2021 compared to 2022 (Table 3-4) most likely contributed to poor manure mineralization at early growth. A similar trend was observed for yield; yield did not significantly differ between manure treatments in 2021 (Table 3-6), however, P-amended treatments in 2020 and 2022 significantly increased yields, by 635 kg ha^{-1} in 2020 and 952 kg ha^{-1} in 2022 (Table 3-5 and Table 3-7). The

low manure response in 2021 compared with 2020 and 2022 was attributed to less cumulative precipitation in 2021 than in 2020 and 2022, resulting in poor manure mineralization and poor crop response to the added nutrients. The 2020/21 winter snowfall amounted to less than 30% average long-term snowfall (Environment Canada, 2021) and by anthesis, the experiment in 2021 received 67% of precipitation compared with 2020 (Table 3-3). Manure mineralization rates of N and P have been shown to be greatest at field capacity and decrease steadily as soil moisture declines (Grant et al., 2001; Whalen et al., 2001).

3.4.2. Plant Population

Genotypes ranged between 295-310 plants m^{-2} (Table 3-4). The plant populations in the present study were lower than the organic production target population of 350 plants m^{-2}, but within Manitoba's conventional target spring wheat stands of 248-302 plants m^{-2} (Kirk et al., 2018). There were no significant differences among environment, genotype, or manure additions. Although seed placed mineral P fertilizer has been shown to benefit speed of emergence (Grant et al., 2001), in the present experiment the manure was spring surface applied, therefore not immediately available to the seed at germination. The effect of composted manure to inhibit emergence in some crop species (Menalled et al., 2005) was not experienced in our study.

Table 3-4. Lsmeans and combined analysis of variance comparing agronomic parameters from three years of data (2020, 2021, 2022) collecting under organic conditions in Libau, Manitoba among two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) under limited soil test phosphorus (3ppm) and amended soil with composted manure at 25 kg P ha^{-1}

	Plant Density	Early Biomass	Anthesis Biomass	Maturity Biomass	Plant Height	Days to Maturity	Yield	Kernel Mass	Test Weight	Protein	Kernel # m^{-2}	HI	KNO:Dma[a]
Year (Y)	plants m^{-2}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	cm	days	kg ha^{-1}	g 1000 $seeds^{-1}$	kg hL^{-1}	%	#	%	# $x10^4$
2020	310	643b	3657	6943	81b	83b	2245b	33a	81a	12.5b	6591b	30	1.9b
2021	295	417c	2860	4869	81b	95a	1500c	29b	80.2b	15a	5029c	29	1.8b
2022	258	861a	3494	7544	101a	95a	2727a	30b	77c	15a	9987a	32	3.0a
Year *P>F**	0.2369	0.0012	0.0689	0.0734	<.0001	<.0001	<.0001	0.0001	<.0001	0.0002	<.0001	0.7192	0.0048
Genotype (-P,+P)[Ŧ] (G)													
Farm1	296	666	3631	6380	91a	92b	2205	31a	79.2	14b	6975	29	2.2
Farm2	279	619	3043	6596	91a	92b	2185	32a	79.6	14b	6755	30	2.4
Red Fife	292	621	3318	7068	92a	94a	2195	32a	79.4	12c	6812	31	2.4
5602HR	284	657	3357	5762	77b	89c	2045	30b	79.3	16a	6814	33	2.2
Genotype *P>F**	0.4879	0.7452	0.4414	0.1223	<.0001	<.0001	0.3539	<.0001	0.0719	<.0001	0.9141	0.2915	0.7195
Coefficient of Variation (%)	18	30	35	34	8	1.6	16	5	2	6	16	26	47
Standard Error ±	31	46	207	551	1.6	3	79	0.3	0.9	0.2	244	2	0.2
Manure (M)													
(+)P	290	709a	3960a	6871a	89a	91	2446a	32a	79.5	14b	7548a	31	2.1b
(-)P	286	572b	2713b	6031b	86b	92	1869b	30b	79.3	15a	6130b	31	2.5a
Manure *P>F*	0.6598	0.0007	<.0001	0.0324	0.009	0.3506	<.0001	<.0001	0.0882	<.0001	<.0001	0.8113	0.0218
Interactions *P>F*													
G x M	0.8075	0.1964	0.1617	0.6561	0.4173	0.5741	0.8869	0.4739	0.244	0.005	0.9216	0.0994	0.2337
G x Y	0.1723	0.9034	0.9716	0.1639	0.7095	0.9289	0.3506	0.0065	<.0001	0.0264	0.6396	0.8989	0.968
M x Y	0.9162	<.0001	0.0446	0.143	0.844	0.2368	<.0001	0.1398	0.3564	0.0188	0.0003	0.5261	0.2143
G x M x Y	0.7804	0.606	0.9901	0.3931	0.5597	0.585	0.6964	0.9429	0.0324	0.0971	0.7769	0.6732	0.6274
Farmer Genotypes Lsmeans	286	641	3298	6400	92a	92.7	2187	32	79.2	14.3	6908	30	2.3
Parental Cultivars Lsmeans	285	639	3317	6413	86b	92	2116	31	79.3	14.2	6841	32	2.3
Contrasts													
Farmer genotypes v. Parents													
P>F	0.9491	0.9806	0.9488	0.9809	0.0273	0.5316	0.6479	0.0873	0.7854	0.6372	0.8852	0.1923	0.8491
Estimate	0.73	1.5	-18	-12	6	0.8	71	0.95	-0.11	0.15	67	-2.1	-0.045
Farmer genotypes v. 5602HR													
P>F	0.7821	0.8345	0.8763	0.3703	<.0001	0.0419	0.4787	0.006	0.8723	0.0009	0.444	0.1637	0.7757
Estimate	3.9	-16	-55	583	13	3.2	132	1.9	-0.08	-1.3	39	-2.7	0.082
Farmer genotypes v. Red Fife													
P>F	0.8631	0.8036	0.9596	0.35	0.6072	0.3011	0.9579	0.99	0.7758	<.0001	0.8677	0.4579	0.5515
Estimate	-2.4	19	17	-608	-1.6	-1.6	10	0.003	-0.13	1.6	94	-1.5	-0.18

*Lsmeans within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.05$); [a]KNO:Dma, Kernel number per unit anthesis biomass. [Ŧ]Limited P treatment, (-)P; P-amended treatment, (+)P

Table 3-5. Lsmeans and analysis of variance comparing agronomic parameters from 2020, collected under organic conditions in Libau, Manitoba among two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) under limited soil test phosphorus (3ppm) and amended soil with composted manure at 25 kg P ha^{-1}

	Plant Density	Early Biomass	Anthesis Biomass	Maturity Biomass	Plant Height	Days to Maturity	Yield	Kernel Mass	Test Weight	Protein	Kernel # m^{-2}	HI	KNO:Dma[a]
Genotype (G) (-P,+P combined)	plants m^{-2}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	cm	days	kg ha^{-1}	g 1000 $seeds^{-1}$	kg hL^{-1}	%	#	%	# $x10^4$
Farm1	338	699	3689	7726a	86a	84ab	2296a	34b	81b	12ab	6783	31	1.7
Farm2	320	609	2883	7091a	83ab	84b	2359a	36a	82a	13a	6669	33	2.1
Red Fife	356	609	3254	7718a	85a	85a	2396a	35a	81ab	11b	6794	32	1.9
5602HR	321	655	2920	5152b	70c	81c	1936b	31c	80c	13a	6212	38	1.9
Genotype *P > F**	0.2467	0.8471	0.3726	0.002	<.0001	0.0008	0.0281	<.0001	0.0051	0.0262	0.7301	0.4759	0.7467
Coefficient of Variation (%)	10.7	33	28.7	22.5	6.4	2	12	2.8	5	10.4	18	20	24
Standard Error ±	15	61	424	925	1.9	0.7	110	0.3	1.2	0.5	304	0.04	0.2
Manure (M)													
(+)P[T]	346	654	3945a	8203a	84a	84	2543a	34a	78	12	7408a	33	1.9
(-)P	327	568	2661b	5985b	79b	83	1907b	33b	81	13	6762b	32	1.7
Manure *P > F*	0.0691	0.2483	<.0001	<.0001	0.0002	0.0712	<.0001	<.0001	0.7803	0.0579	<.0001	0.7548	0.1955
G x M *P>F*	0.0613	0.5748	0.2951	0.4192	0.1904	0.3271	0.7023	0.0022	0.4554	0.077	0.8762	0.5435	0.9136
Farmer Genotype Lsmeans	305	654	3756	7549	84a	84	2360	35a	81.4a	12	6765	32	1.9
Parental Cultivars Lsmeans	315	632	3557	6435	77b	83	2166	33b	80.6b	12	6502	35	1.9
Contrasts													
Farmer genotypes v. Parents *P>F*	0.4729	0.805	0.6566	0.1127	0.0014	0.1928	0.3141	0.0001	0.0179	0.5481	0.6064	0.2462	0.8801
Estimate	-9.9	21.8	198	1014	7.4	0.75	192	1.7	0.66	0.32	267	-3.6	0.0180
Farmer genotypes v. 5602HR *P>F*	0.6292	0.992	0.4	0.018	<.0001	0.0002	0.0756	<.001	0.0039	0.3721	0.3589	0.1019	0.9014
Estimate	7.3	-1.1	366	2297	15	3.1	422	3.8	1.02	-0.67	558	-6.3	0.0299
Farmer genotypes v. Red Fife *P>F*	0.082	0.6826	0.9504	0.7662	0.9742	0.0308	0.8696	0.5003	0.3409	0.0897	0.9691	0.7907	0.9801
Estimate	-27	44	31	-268	0.1	-1.6	-37	-0.36	0.3	1.3	-23	-0.99	0.0060

*Lsmeans within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.05$);
[a]KNO:Dma, Kernel number per unit anthesis biomass
[T]Limited P treatment, (-)P; P-amended treatment, (+)P

Table 3-6. Lsmeans and analysis of variance comparing agronomic parameters from 2021, collected under organic conditions in Libau, Manitoba among two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) under limited soil test phosphorus (3ppm) and amended soil with composted manure at 25 kg P ha^{-1}

	Plant Density	Early Biomass	Anthesis Biomass	Maturity Biomass	Plant Height	Days to Maturity	Yield	Kernel Mass	Test Weight	Protein	Kernel # m^{-2}	HI	KNO:Dma[a]
Genotype (G) (-P,+P combined)	plants m^{-2}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	cm	days	kg ha^{-1}	g 1000 $seeds^{-1}$	kg hL^{-1}	%	#	%	# $x10^4$
Farm1	297	404	3061	4950	82a	96b	1449	30	79.8b	15b	4825	28.1	1.7
Farm2	273	451	2699	4876	85a	96b	1523	30	80.6a	14.7b	4974	28.7	1.9
Red Fife	305	397	2750	4830	86a	98a	1514	29	79.8b	13.2c	5164	30.3	1.9
5602HR	305	418	2931	4819	70b	92c	1515	29	80b	16.5a	5155	29.4	1.8
Genotype *P > F**	0.4206	0.9284	0.6311	0.9952	0.0156	<.0001	0.9607	0.5302	0.0185	<.0001	0.911	0.8	0.5404
Coefficient of Variation (%)	12	38	31	28	9.9	0.92	29	6	0.63	4.5	23	20	35
Standard Error ±	12	66	297	695	3.8	0.2	144	0.8	0.9	0.2	523	2.6	0.5
Manure (M)													
(+)P[Ŧ]	288	407	3066	5017	82	96	1564	29.8	80.2	14.8	5235	29.7	1.6b
(-)P	296	242	2559	4592	80	96	1459	29.1	80	15	4986	29.5	2.1a
Manure *P > F*	0.4186	0.6865	0.0532	0.2796	0.4328	0.0857	0.3752	0.2306	0.0598	0.4335	0.5287	0.9829	0.0436
G x M *P>F*	0.3101	0.4826	0.482	0.9283	0.8643	0.147	0.9166	0.4169	0.5376	0.9855	0.9434	0.9729	0.2924
Farmer Genotype Lsmeans	284	427.2	2879	4912	83	96a	1485	30.3	80.4	14.9	4899	28.3	1.7
Parental Cultivars Lsmeans	305	407.5	2840	4824	79	95b	1514	29.2	80	14.8	5159	39.8	1.9
Contrasts													
Farmer genotypes v. Parents *P>F*	0.1111	0.7189	0.9036	0.8806	0.2622	0.0007	0.8485	0.1752	0.066	0.8764	0.5868	0.5255	0.4111
Estimate	-20	19	38	88	3.9	1.1	-28	1.1	0.39	0.03	-259	-1.4	-0.0143
Farmer genotypes v. 5602HR *P>F*	0.1857	0.894	0.8959	0.8966	0.0217	<.0001	0.8735	0.2709	0.2254	<.0001	0.6622	0.7131	0.6306
Estimate	-20	9	-51	93	10.2	3.7	-29	1.1	0.31	-1.6	-255	-1.1	-0.0102
Farmer genotypes v. Red Fife *P>F*	0.1942	0.6596	0.7425	0.9082	0.5776	<.0001	0.8786	0.2607	0.0699	<.0001	0.6516	0.503	0.3883
Estimate	-19	30	129	83	-2.3	-1.6	-28	1.2	0.47	1.6	-264	-1.9	-0.0184

*Lsmeans within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test (P ≤ 0.05); [a]KNO:Dma, Kernel number per unit anthesis biomass

[Ŧ]Limited P treatment, (-)P; P-amended treatment, (+)P

Table 3-7. Lsmeans and analysis of variance comparing agronomic parameters from 2022, collected under organic conditions in Libau, Manitoba among two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) under limited soil test phosphorus (3ppm) and amended soil with composted manure at 25 kg P ha^{-1}

	Plant Density	Early Biomass	Anthesis Biomass	Maturity Biomass	Plant Height	Days to Maturity	Yield	Kernel Mass	Test Weight	Protein	Kernel #	HI	KNO:Dma[a]
Genotype (G) (-P,+P combined)	plants m^{-2}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	cm	days	kg ha^{-1}	g 1000 $seeds^{-1}$	kg hL^{-1}	%	m^{-2} #	%	#
Farm1	277	895	3672	6463	105a	96a	2870	31ab	76b	15.3b	9320	30	3.1
Farm2	269	795	3077	7739	105a	96a	2679	30.5b	76b	14.8c	8715	33	3.1
Red Fife	240	856	3478	8656	107a	98a	2675	32a	77.5a	13.1d	8479	33	3.4
5602HR	248	898	3749	7314	89b	93b	2683	29c	77.7a	16.6a	9075	33	2.8
Genotype *P > F**	0.3604	0.6115	0.8278	0.2609	<.0001	0.0041	0.4112	0.0008	0.0051	<.0001	0.1901	0.836	0.8384
Coefficient of Variation (%)	27	24.7	44	38	4	2	10	3	1	3	9	28	44
Standard Error ±	11	90	519	1124	1.6	0.2	105	0.6	0.4	0.18	372	4	0.6
Manure (M)													
(+)P[₸]	262	1062a	4537a	7614	103a	96.1	3203a	32a	77.1	14b	10079a	31	2.6
(-)P	254	660b	2450b	7472	99b	95.6	2251b	29b	76.7	16a	7716b	33	3.5
Manure *P > F*	0.666	<.0001	0.0011	0.8545	0.0027	0.5642	<.0001	<.0001	0.1702	<.0001	<.0001	0.5013	0.0608
G x M *P>F*	0.8333	0.3152	0.6021	0.2609	0.0092	0.7548	0.1843	0.6146	0.0277	0.0164	0.1818	0.2093	0.3099
Farmer Genotype Lsmeans	273	845	3374	7101	105a	96	2774	30.5	76.5b	15.1	9017	31	3.1
Parental Cultivars Lsmeans	243	877	3613	7985	98b	95.5	2679	30.4	77.6a	14.9	8777	33	3.1
Contrasts													
Farmer genotypes v. Parents *P>F*	0.2429	0.7657	0.7205	0.3826	0.0012	0.4451	0.6463	0.7721	0.0067	0.6376	0.6612	0.5936	0.9793
Estimate	29	-31	-239	-883	6.7	0.5	95	0.2	-1.1	0.14	239	-1.7	-0.0133
Farmer genotypes v. 5602HR *P>F*	0.4047	0.6882	0.6475	0.8628	<.0001	0.0045	0.7194	0.1057	0.014	0.0001	0.9307	0.6217	0.6493
Estimate	25	-52	-374	-212	15.6	2.7	91	1.2	-1.1	-1.6	-58	-1.9	0.2858
Farmer genotypes v. Red Fife *P>F*	0.2777	0.932	0.899	0.2131	0.3291	0.0784	0.6961	0.2424	0.0397	<.0001	0.4242	0.7049	0.6193
Estimate	33	-11	-103	-1555	-2.5	-1.6	99	-0.87	-0.9	1.9	538	-1.5	-0.3125

*Lsmeans within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.05$);
[a]KNO:Dma, Kernel number per unit anthesis biomass
[₸]Limited P treatment, (-)P; P-amended treatment, (+)P

3.4.3. Evaluation of Parental Differences

3.4.3.1. Biomass Accumulation

Despite large differences in early biomass between years, the parents did not differ for biomass at early (Zadoks 30), anthesis, and at maturity growth stages (Table 3-4). No genotype x manure, genotype x year, or genotype x manure x year interactions were observed. Therefore, biomass accumulation of Red Fife and 5602HR was consistent regardless of fertility or environmental conditions. Despite their divergent breeding histories, biomass accumulation under organic conditions at any developmental stage did not differ. While many studies have evaluated old and new wheat cultivars under organic and conventional systems for early vigour ratings and weed biomass (Pridham et al., 2007; Wolfe et al., 2008; Kamran et al., 2014; Entz et al., 2018), yield and quality (Carr et al., 2006; Pswarayi et al., 2014; Thomas and Graf, 2014; Iqbal et al., 2016), comparisons of crop biomass at the early developmental stage was not evaluated. Wiebe et al. (2017) noted no genotypic differences or year interactions among organically-selected genotypes and modern genotypes for anthesis or mature biomass.

3.4.3.2. Plant Height and Lodging

It is well established that Red Fife is taller than most modern wheat genotypes (Carr et al., 2006; Mason et al., 2007a; Pswarayi et al., 2014; Entz et al., 2018) and plant height has been proposed to be of benefit for organically managed systems to combat weed competition (Mason et al., 2007a; Kokare et al., 2017). Significant differences between Red Fife and 5602HR were observed across all fertility treatments and years, Red Fife was 15cm taller than 5602HR. There was a significant genotype x manure interaction in 2022 only for plant height (Figure 3-1). Lodging occurred only in 2022, Red Fife lodged significantly more than 5602HR under P-amended treatments (genotype x manure interaction, 2022 *P>F* 0.0247) (Figure 3-2). Therefore, Red Fife was taller and had greater lodging severity under the P-amended treatment than 5602HR (Figure 3-1 and Figure 3-2).

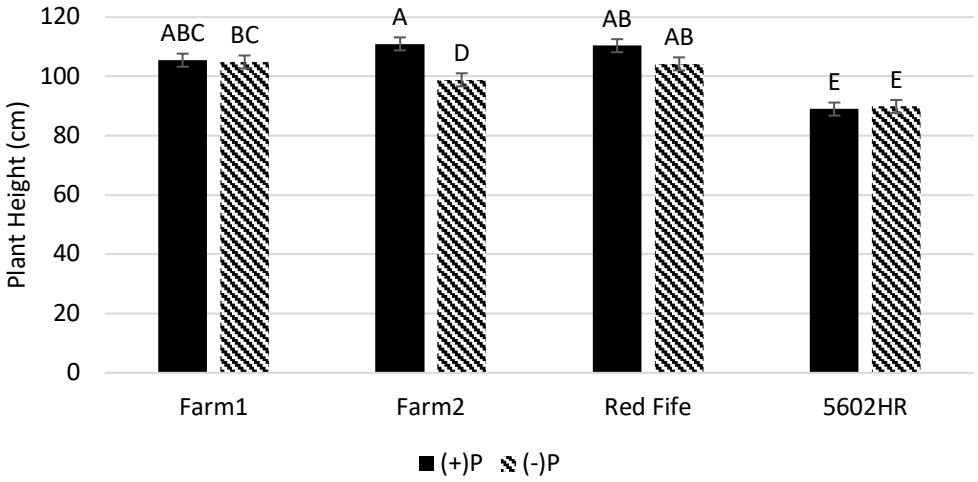


Figure 3-1. Plant height genotype x manure interaction effects conducted under organic conditions in Libau, Manitoba 2022 among two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) under limited soil test phosphorus (3ppm), (-)P; and amended soil with composted manure at 25 kg P ha^{-1}, (+)P.
Treatments with the same letter are not significantly different by an analysis of variance test ($P \leq 0.05$).

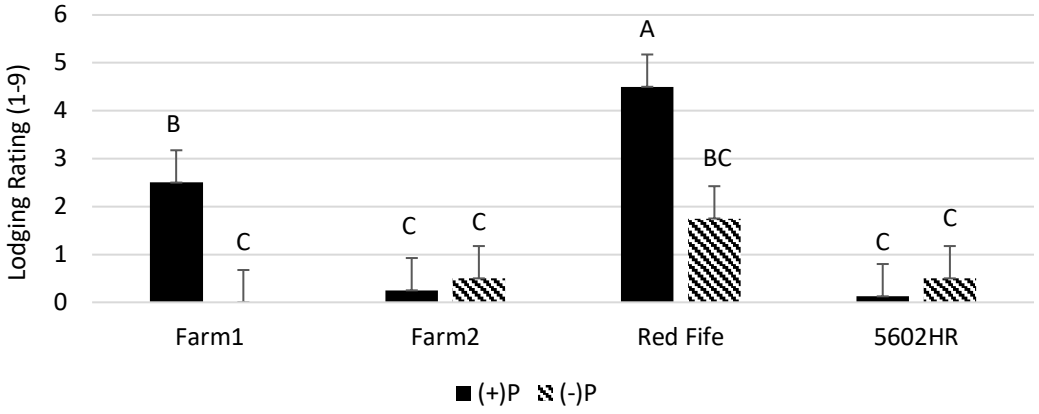


Figure 3-2. Lodging severity genotype x manure interaction effects conducted under organic conditions in Libau, Manitoba 2022 among two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) under limited soil test phosphorus (3ppm), (-)P; and amended soil with composted manure at 25 kg P ha^{-1}, (+)P.
Treatments with the same letter are not significantly different by an analysis of variance test ($P \leq 0.05$).

3.4.3.3. Days to Maturity

5602HR matured 6 days earlier than Red Fife (*P>0.05*). Red Fife is known to be a late maturing genotype, often suffering from frost damage when it was widely grown across the prairies in the late 1800s (McCallum and DePauw, 2008). The cultivar 5602HR, on the other hand, follows a maturity timeline more suited to the Canadian prairie environment (Iqbal et al., 2016). When Pswarayi et al. (2014) evaluated wheat cultivars registered between 1885 to 1999 under organic conditions, days to maturity significantly reduced with increasing registration year. Mason et al. (2007) reported a positive relationship between greater weed biomass and longer days to maturity and suggested that it may be desirable for organic wheat producers to use earlier maturing genotypes to reduce weed biomass. However, when Kamran et al. (2014) tested 32 wheat cultivars under organic management, they reported no yield advantage of early maturity. It may be more advantageous to select for maturity that coincides with geographic harvest needs for organic producers, rather than a broad target maturity timeline.

3.4.3.4. Yield and Yield Efficiency

Red Fife and 5602HR did not yield significantly different from each other, nor were genotype x year, genotype x manure, or genotype x manure x year interactions observed (Table 3-4). Pswarayi et al. (2014), observed that yield gains across registration years only increased under conventional conditions, not organic. They attributed the lack of genetic gain in organic to be due to poor growing conditions due to weeds and lower fertility resulting in lower yield potential test sites (Pswarayi et al., 2014). However, in the present study, even under high yield potential conditions (2022, P-amended treatment) (Table 3-7), no significant differences were observed between Red Fife and 5602HR. While breeding efforts over the past 138 years have significantly benefitted production in conventional systems (Thomas and Graf, 2014), similar gains in organic systems have not been realized through modern breeding efforts. Therefore, while under conventional production paradigms our two parents may have expressed differences in yield potential, this did not occur under organic production conditions here. The present study, along with

others (Murphy et al., 2007; Kirk et al., 2012; Kamran et al., 2014; Pswarayi et al., 2014; Wiebe et al., 2017) supports the importance of targeted breeding for organic production systems.

Kernel production efficiency, a trait used in the development of semi-dwarf wheat (Fischer, 2008), was not significantly different for Red Fife and 5602HR. While kernel production efficiency is recognized as useful for increasing yield in wheat (Entz and Fowler, 1990), it has not been investigated or selected for extensively (Rivera-Amado et al., 2019).

Harvest index was 31 and 33% for Red Fife and 5602HR, respectively (Table 3-4) and did not significantly differ from each other. The harvest indices measured here were slightly lower than other organic genotype trials, which ranged from 34 to 47% (Pswarayi et al., 2014; Wiebe et al., 2017). When Wang et al. (2003) compared harvest indices of new spring wheat cultivars with old cultivars, old cultivar harvest indices (Marquis and Neepawa; released 1960 and 1969, respectively) were 29 and 33.2%. New cultivars (released between 1994-1997) ranged between 32.4-35.9%. New cultivars were significantly different from only one of the older cultivars.

3.4.3.5. Seed Quality

There was a significant genotype x year interaction which showed Red Fife's kernel mass to be significantly higher than 5602HR in 2020, but not in 2021 or 2022. Increased seed mass is less important when breeding for higher yield; yield has been primarily associated with increased kernels per unit area (Rivera-Amado et al., 2019) and seed mass to a lesser degree (Slafer et al., 2014). Iqbal et al. (2016) reported that seed mass among cultivars within the Canadian Western Red Spring (CWRS) class increased from 1885-2012.

Red Fife and 5602HR test weights were not significantly different from one another, however, there was a significant genotype x manure x year interaction (Table 3-4, Figure 3-3). Red Fife and 5602HR did not significantly differ among each other or manure treatments in 2020 and 2021 (Table 3-5 and Table 3-6). In 2022, 5602HR test weight significantly reduced under limited P treatments, but Red Fife was not affected by a reduction in soil-P fertility (Table 3-7, Figure 3-3). This may indicate that Red Fife's ability to maintain grain density, and packing efficiency (shape, size) despite stressful growing conditions is superior to that of cultivar 5602HR. Test weight under conventional conditions has been reported to increase with breeding efforts within Canadian germplasm over time (Iqbal et al., 2016). We see an opposite result in the present study, where 5602HR had lower test weight than the landrace genotype under certain growing conditions.

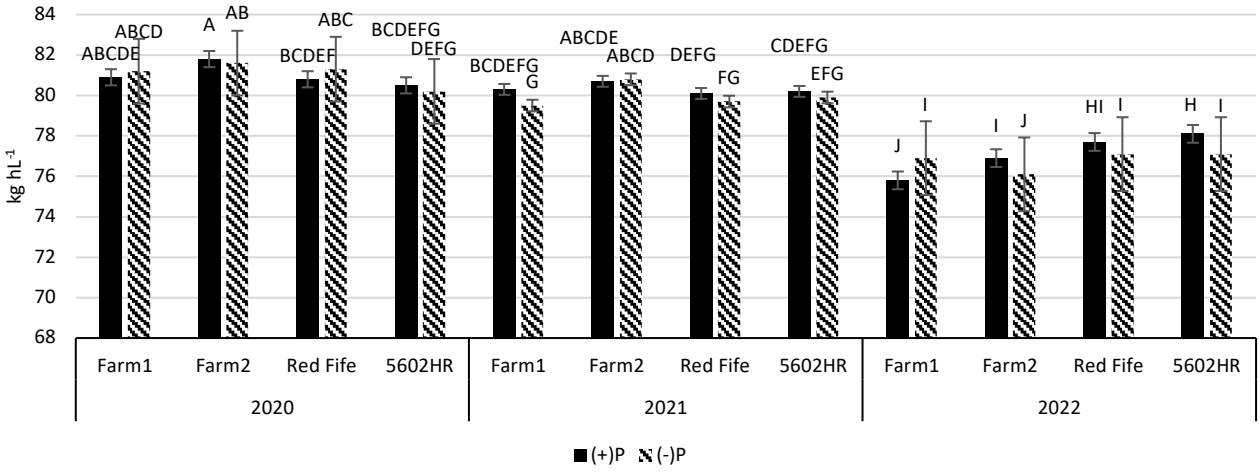


Figure 3-3. Test weight genotype x manure x year interactions conducted under organic conditions in Libau, Manitoba among two spring wheat cultivars and two spring wheat farmer genotypes under limited soil test phosphorus (3ppm), (-)P; and amended soil with composted manure at 25 kg P ha^{-1}, (+)P

Overall, 5602HR had higher grain protein (Table 3-4) and grain protein in this modern cultivar responded more to better growing conditions than Red Fife. For example, significant genotype x manure and genotype x year interactions (Figure 3-4A and Figure 3-4B) showed Red Fife was not responsive to increased fertility, but 5602HR was; the limited-P treatment significantly increased protein concentrations

for 5602HR above Red Fife (Figure 3-4A). The magnitude of increased protein concentration was different between 5602HR and Red Fife between years (Figure 3-4B). Another difference between parents was protein response to added moisture. Red Fife responded to added moisture (2022) to a smaller magnitude than 5602HR (Figure 3-4B). Higher protein in combination with higher yield (Ex., 2022 conditions) in the modern cultivar over the landrace is therefore not a surprise, given the specific importance of high protein and yield in Hard Red Spring wheat breeding programs in Canada. Red Fife had lower protein and high yield in 2022 (Table 3-7, Figure 3-4C), and 5602HR significantly increased grain protein while maintaining similar yield potential compared to Red Fife. It is well established that yield and protein have an inverse relationship, and modern breeding efforts have successfully increased grain protein and yield simultaneously (Nelson et al., 2011), while Red Fife may have reached an upper limit of protein genetic potential at 13% (Figure 3-4B).

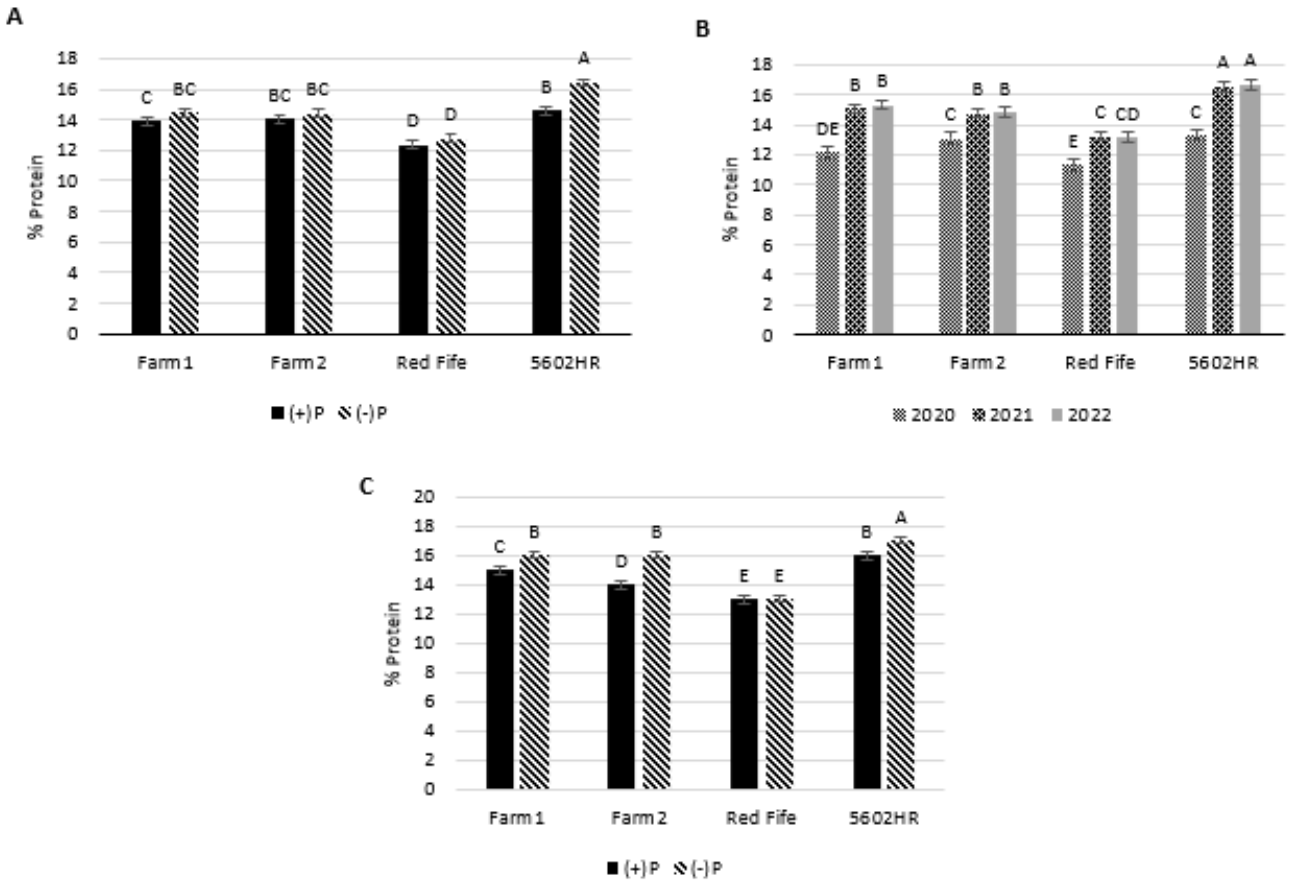


Figure 3-4. Per cent protein concentration representing genotype, manure, and year interactions collected under organic conditions in Libau, Manitoba among two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) under limited soil test phosphorus (3ppm), (-)P; and amended soil with composted manure at 25 kg P ha^{-1}, (+)P.
Genotype x manure interaction all years (2020, 2021, and 2022) combined, (A). Genotype x year interaction all fertility treatments combined ((-)P,(+)P), (B). Genotype x manure interaction in Libau 2022 only, (C). Treatments with the same letter are not significantly different by an analysis of variance test ($P \leq 0.05$).

3.4.4. Farmer genotype deviation from the parental cultivars

3.4.4.1. Biomass Accumulation

The farmer genotypes, Farm1 and Farm2, did not significantly differ from parents when contrasts were made comparing to parents as a group, or when parents were contrasted independently at the early, anthesis, or mature biomass developmental stages (Early Biomass: Farmer genotypes *P>F* 0.9806, Estimate 1.5 kg ha^{-1}; Farmer genotypes vs. 5602HR *P>F* 0.8345, Estimate: -16 kg ha^{-1}; Farmer genotypes vs. Red Fife *P>F* 0.8036, Estimate: 19 kg ha^{-1}) (Table 3-4). This suggests that biomass accumulation at

different developmental stages was kept consistent through the selection process from the parents. This observation is in contrast with Nicksy et al. (2022) who observed an organically selected, farmer-bred genotype from the same PPB program had greater biomass at stem elongation, anthesis, and maturity across multiple fertility treatments than a modern cultivar (AAC Brandon). However, Nicksy et al. (2022) was not comparing parental cultivars of the farmer genotype.

3.4.4.2. Plant Height and Lodging

Farmer genotypes were significantly taller than 5602HR by 16cm (Contrast farmer genotypes vs. 5602HR *P>F* 0.0009). Red Fife and the farmer genotypes did not differ in height (Contrast farmer genotypes vs. Red Fife *P>F* 0.6072, Estimate: -1.6cm) indicating that farmers were selecting for plants within the early-generation population that were taller the modern parent (5602HR), and similar to the landrace parent. Increased plant height has been widely reported to be beneficial to organic production systems for the ability to shade weed competitors for light (Lemerle et al., 1996; Wolfe et al., 2008; Kaut et al., 2009).

Patterns of height differences between parental cultivars and farmer genotypes were stable across growing environments as indicated by the lack of interaction between manure, and year. However, there was a significant genotype x manure interaction in 2022 (Figure 3-2). In 2022, 5602HR, Red Fife, and Farm1 were not responsive to nutrient addition, but Farm2's plant height increased by 13cm with added manure (Figure 3-2).

While the farmer genotypes had similar plant height to Red Fife, both farmer genotypes demonstrated better lodging resistance than Red Fife in 2022 (Figure 3-2). The parental cultivar, 5602HR had the lowest lodging severity. Despite similar height to Red Fife, Farm2 demonstrated similar lodging scores to 5602HR. Therefore, Farm2 possessed the beneficial traits of taller height for weed

competitiveness (Wolfe et al., 2008) similar the landrace cultivar, yet also had greater lodging resistance similar to modern cultivar parent.

3.4.4.3. Days to Maturity

Although days to maturity differed among years, farmer genotypes consistently matured between the two parent cultivars. This was consistent across all fertility treatments and years tested (Table 3-4, Table 3-5, Table 3-6, Table 3-7). As a group, farmer genotypes resembled Red Fife more-so than 5602HR, maturing 3.2 days later than 5602HR (Contrast *P>F* 0.0419), but not significantly different than Red Fife (Contrast *P>F* 0.3011). It is interesting that while current breeding efforts have resulted in fewer days to maturity, the farmers selected for a genotype that matured slightly longer than the modern genotype. Entz et al. (2018) also reported that farmer genotypes matured approximately 2 days later than conventional check cultivars. Mason et al. (2007) reported that yield and days to maturity were negatively correlated under organic production, however, in a more recent experiment under organic management, Kamran et al. (2014) found no clear yield advantage of early maturity. Therefore, it is unclear if longer days to maturity is advantageous for organic production.

3.4.4.4. Yield and Yield Efficiency

In 2020, both farmer genotypes yielded significantly higher than 5602HR (Table 3-5). This pattern was not replicated in 2021 or 2022, and it is not clear why 5602HR yielded significantly lower than farmer genotypes in 2020. When yield data was combined among all years, no genotypic differences in were detected in years, which demonstrates that under organic low-input systems, farmer genotypes of the cross performed similar to their parents. While not significant, it is interesting that across diverse environments and nutrient status, the farmer genotypes yielded between 140-160 kg ha^{-1} higher than 5602HR (Table 3-4). When Entz et al. (2018) tested farmer genotypes across 3 environments under organic

management, the yield of the farmer genotypes were significantly greater (149 kg ha^{-1}) than the modern checks when weed interference was high at one environment.

Genotypic differences in kernel production efficiency across all years and manure treatments did not differ, and there were no interactions detected (Table 3-4). This indicates that the parents and farmer genotypes' kernel production efficiency was stable among multiple years and manure treatments. This is opposite of what others have reported; Wiebe et al. (2017) found that spring wheat genotypes bred under organic conditions had greater kernel production efficiency, and a larger sink (kernels m^2) than conventional genotypes crediting better assimilate partitioning under organic genotypes than conventional cultivars.

No differences in harvest index were detected among farmer genotypes and either parent. Although not significant, 5602HR had the greater harvest index (33%) compared to farmer genotypes (Farm1; 29% and Farm2; 30%). Lower harvest index among farmer genotypes compared to modern cultivars was also observed by Entz et al. (2018). Higher harvest index may be connected to 5602HR's shorter height than the farmer genotypes. Greater harvest index has been associated with shorter height (Sharma and Smith, 1986; Addisu et al., 2010).

3.4.4.5. Seed Quality

Farmer genotypes' kernel mass was significantly greater than 5602HR (Contrast farmer genotypes vs. 5602HR *P>F* 0.006, Estimate: 1.9g $1000seeds^{-1}$) and resembled Red Fife (Contrast farmer genotypes vs. Red Fife *P>F* 0.99, Estimate: 0.003g $1000seeds^{-1}$). This demonstrated that farmers successfully moved the genotypes towards larger seed mass. The reasons for this trend are not clear. Neither farmer was specifically selecting for larger seed. There was a significant genotype x year interaction, farmer genotypes expressed larger seed in two of the three years compared to 5602HR (Table 3-5, Table 3-7), however, no differences were detected in 2021 (Table 3-6).

There was a genotype x year and genotype x manure x year interaction for test weights. Test weights ranged from 76 to 81 kg hL^{-1}, comparable to other studies examining hard red spring wheat on the Canadian prairies (Mason et al., 2007b; Kamran et al., 2014; Iqbal et al., 2016). Manure did not significantly impact test weights, except in 2022 (Figure 3-3). Red Fife and 5602HR's test weights were lower under the limited P treatment, and only Farm2's test weight was reduced in a similar pattern to the parents. However, test weight increased with reduced fertility for Farm1. Manure did not have a significant impact on test weight in 2020 and 2021, and there was no consistent pattern of increased or decreased test weight across all years tested. It is not clear what proportion of test weight variability of farmer genotypes was due to genetic differences or environmental influences. When Chen et al. (2016) evaluated 82 spring wheat cultivars (including Red Fife and 5602HR), they reported that test weight's heritability was moderately low (39%). Many others have reported that environment significantly impacts test weight, with no clear genotypic pattern why (Mason et al., 2007b; Pswarayi et al., 2014; Kissing Kucek et al., 2019). Despite this, farmers maintained test weight quality through the selection process like their parents, and at acceptable test weight marketability levels (>75 kg hL^{-1}) (Mason et al., 2007b).

Across all years and fertility treatments, the protein concentrations of farmer genotypes were significantly higher than Red Fife by 1.6% (Contrast farmer genotypes vs. Red Fife contrast, $P>F$ <.0001), and significantly lower than 5602HR by 1.6% (Contrast farmer genotypes vs. Red Fife contrast, $P>F$ <.0001) (Table 3-4). Therefore, farmers produced genotypes that had intermediate protein levels compared with parents. There were significant genotype x manure and genotype x year interactions (Figure 3-4A and Figure 3-4B). Across all experimental years, 5602HR's protein levels increased under limited P treatments, but Farm1 and Farm2 were not responsive (Figure 3-4A). Under P-amended treatments, 5602HR's protein levels increased to a greater degree than both Farm1 and Farm2 between 2020 and 2021 and 2022 (Figure 3-4B). In 2022, Farm1 and Farm2's protein levels significantly increased under limited P conditions similar to 5602HR, while Red Fife was not responsive.

Farmers increased protein levels above one of the parents, Red Fife, but also retained yield similar to both parents (Table 3-4). Protein was not evaluated and selected for throughout the selection process. Therefore, the farmers in the present study have coincidently successfully carried those traits forward under organic conditions. This indicates the benefit of including a high protein parent in any spring wheat participatory plant breeding scheme where protein content is not measured during the selection process.

3.4.5. Performance between farmer genotypes

Our last objective was to evaluate the impact of geographically divergent farmers and their respective environments on full sibling derived genotypes. Previous work investigating farmer wheat genotypes derived from the same parental material has been conducted in Canada and Italy (Rivière et al., 2013; Entz et al., 2018). However, comparing farmer genotypes from the same parental material from different geographic areas in Canada has not been investigated.

3.4.5.1. Biomass Accumulation

Averaged across three years of the experiments and two fertility treatments, farmer genotypes were not significantly different from each other for biomass accumulation at anthesis or at maturity developmental stages (Table 3-4). When Entz et al. (2018) contrasted 5 full combinations of farmer genotypes from the same parents, there were no differences in mature biomass in four out of five combinations.

3.4.5.2. Plant Height and Lodging

Under P-amended and limited P treatments in 2022, the farmer genotypes displayed different height responses. Farm1 was not responsive for height with manure addition while the height for Farm2 increased by 13cm (Figure 3-2). Additionally, under P-amended treatments in 2022, straw strength for Farm2 was higher than Farm1 as indicated by a lower lodging severity score (Figure 3-2). While both farmers selected against lodging and increased height, clearly one farmer selected for taller, stronger

plants over the other. This difference may be due to differences in wind severity between the two environments. Greater wind speed in Manitoba (maximum average wind speed in June-August 61 km h^{-1} in Glenlea, MB, 75km from Carman, (Environment Canada, 2022)), than British Columbia (maximum average wind speed in June-August 39 km h^{-1} in Hope, BC (Environment Canada, 2022)) may have selected for Farm2's greater lodging resistance . The difference was however, only observed at our highest yield potential year (2022, with manure) (Table 3-7).

3.4.5.3. Days to Maturity

When averaged across all years and manure treatments, farmer genotypes did not significantly differ in days to maturity. Farmer genotypes matured 12 days earlier in 2020 than in 2021 and 2022, however, farmer genotypes were not different from each other in any year.

3.4.5.4. Yield and Yield Efficiency

There were no significant differences in yield, or yield efficiency parameters between the farmer genotypes among years and fertility treatments (Table 3-4). Differences in yield performance between farmer genotypes under organic management is not consistent in the literature, as some have observed consistent yield differences (Goldringer et al., 2001; Bocci et al., 2020) and others have not (Ceccarelli et al., 2003; Entz et al., 2018; van Frank et al., 2020). Our results indicate that other than height, farmer genotypes had similar agronomic characteristics despite vastly different farming systems, soil types, and environmental conditions during selection. Working with barley in Syria, Ceccarelli et al., (2003) reported that the selection environment had a larger effect than the selector and specific agronomic traits (plant height, biomass, yield) did not differ among farmer genotypes.

3.4.5.5. Seed Quality

Differences between farmer genotypes for seed quality parameters were observed in specific cases. For example, kernel mass was on average no different between farmer genotypes when averaged

across years, but the Farm2 genotype had a significantly greater kernel mass than the Farm1, in 2020. (Table 3-5).

A significant genotype x manure x year interaction was observed for test weight (Figure 3-3). In 2022 Farm2 demonstrated test weight increases with manure, while test weights for Farm1 decreased. The reason for this difference was not clear, test weight is an indication of grain density as well as packing efficiency (seed shape and surface characteristics) (Lloyd et al., 1999). Therefore differences in seed shape may be the reason for the difference in test weights between the genotypes. Test weights are impacted by growing conditions during grain fill (Ozkan et al., 1998), harvest conditions and date (Lloyd et al., 1999; Dorrian et al., 2023) and management (Mason et al., 2007b; Kamran et al., 2014). Other studies have reported genotype x environment interactions for test weight and wheat under organic management (Carr et al., 2006; Kissing Kucek et al., 2019).

Farmer genotypes' grain protein content did not differ from each other under limited P and P-amended treatments (Figure 3-4A). Protein concentrations of both farmer genotypes were lowest in 2020 and increased similarly in 2021 and 2022 (Figure 3-4B). In 2022, under P-amended conditions, Farm1 had significantly higher protein levels than Farm2 by 1%, but similar protein levels under the limited P treatment (Figure 3-4C). Grain yield did not differ between farmer genotypes under P-amended treatments in 2022, indicating that Farm1 may have been able to avoid the yield-dilution effect (Calderini et al., 1995) better than Farm2.

3.5. Summary and Conclusions

The purpose of this study was to investigate the performance differences and similarities among divergent parental cultivars and farmer genotypes under a wide array of environmental conditions. To do this, we subjected the genotypes to different seasonal conditions, and under limited and P-amended treatments.

The first objective of this study was to investigate the suitability of combining a modern cultivar with a landrace cultivar as parental material for an organic participatory plant breeding program by comparing the performance under organic conditions. Under organic conditions, Red Fife (landrace cultivar), and 5602HR (modern cultivar), demonstrated similar biomass accumulation, yield, test weight, kernel efficiency, and harvest index. Red Fife was taller, later maturing, more susceptible to lodging, had greater kernel mass, and lower protein than 5602HR. Specifically, 5602HR protein was more responsive to added fertility, and Red Fife reached its protein level potential at 13%, a lower level than 5602HR (16.6%). Taken together, we accept the hypothesis that crossing a landrace with a modern cultivar should benefit organic farmers. Red Fife contained phenotypic traits useful for organic farmers such as tall plant height and greater kernel mass, while 5602HR more resistant to lodging high protein under high fertility conditions.

Our second objective was to then evaluate how farmer genotypes differed in their performance from their modern and landrace parents under a range of organic growing conditions. Farmer genotypes had similar biomass accumulation, yield, test weight, harvest index, and kernel production efficiency to both parents. Farmer genotypes resembled the landrace parent in height, kernel mass, and days to maturity, and therefore, were taller, longer maturing, had larger seed mass than the modern parent. Farmer genotypes were similar to the modern parent in protein levels and lodging resistance. We accept the hypothesis that farmer selection had a positive impact on the crossed genotypes. Crossing a landrace and a modern genotype is useful for organic production systems, the PPB program design facilitated the creation of genotypes that combined valuable traits of height and kernel mass from the landrace parent and lodging resistance and protein from the modern parent. Future crosses are recommended to incorporate newer tall cultivars for new crosses.

Our last objective was to evaluate the impact geographically divergent farmers and their respective environments had on full sibling derived genotypes. Farmer genotypes differed from each other only in terms of test weight and protein levels. High fertility and high moisture conditions in 2022 enabled Farm1 to have a higher protein than Farm2 by 1% in combination with similar yield to Farm2. Additionally, Farm1 and Farm2 exhibited opposite responses to manure addition for test weight, which may be due to different seed shape characteristics. Consequently, we accept the hypothesis that farmer genotypes will result in multiple different phenotypic traits, a concept that is further explored with a second proof-of-concept family within appendix B (see attached Table B-3).

The present study has demonstrated that PPB programs and farmers can help create valuable genotypes for organic production systems in Canada. However, in some cases, farmers were unable to visit the selections plots often enough to make detailed notes on leafiness, early vigour, and possibly rogue out plants whose diseases were most apparent during the growing season (for example, Fusarium Head Blight). This led to some genotypes only being selected from at the very end of the season when all the plants were mature. Another challenge of selecting only when the crop is mature, is that sometimes this was after harvest of their main economically important crops. This may have unintentionally led to genotypes that mature later than desired. Participatory breeding programs should ensure farmers have the time to make mid-season visits and make selections in a timely manner.

PPB programs can be a more affordable alternative to traditional breeding programs, especially for underserved sectors of the agricultural community. A challenge of a PPB wheat program in Canada is the stringent registration and export marketing system that prevents large organic farmers from growing PPB genotypes for export markets (Colley et al., 2021). However, many organic farms in Canada have been successful marketing PPB grains to local markets (Jowett, 2023). Greater investment into such programs

have the potential to not only serve organic farmers, but also to boost genetic prosperity and diversity, into the future.

Table B-3. A comparison table of study objectives between two spring wheat 'PPB Families': Family 1 and Family 2. Family 1 parental cross was between a modern (5602HR) and a landrace (Red Fife) cultivars. Family 2 parental cross was between two modern cultivars (AAC Scotia and Norwell). The experiment was conducted under organic conditions in Libau, Manitoba in 2020, 2021, and 2022 limited soil test phosphorus (3ppm); and amended soil with composted manure at 25 kg P ha^{-1}

Objective	Family 1: Red Fife x 5602HR	Family 2: AAC Scotia x Norwell
Suitability of combining wheat genotypes as parental material for an organic participatory plant breeding program by comparing the performance under organic conditions.	Red Fife was taller, greater lodging potential, larger kernel mass, and lower protein than 5602HR. Parental cultivars did not differ in yield.	AAC Scotia was taller, had greater lodging potential, higher yield, larger kernel mass, and lower protein than Norwell.
How farmer genotypes differed in their performance from their parents under a range of organic growing conditions.	Farmer genotypes did not differ from parents in biomass accumulation, and yield. Farmer genotypes resembled Red Fife in height and kernel mass. Farmer genotypes had similar grain protein and lodging resistance to 5602HR.	Farmer genotypes did not differ from the parents in biomass accumulation. FarmC was more similar to AAC Scotia in height, lodging severity, kernel mass, and grain protein. Farmer genotypes FarmA and FarmB were taller than Norwell, but shorter than AAC Scotia. FarmA and FarmB yields were lower than AAC Scotia and similar to Norwell. Farmer genotypes FarmA and FarmB fell between AAC Scotia and Norwell in height and protein.
Evaluate the impact geographically divergent farmers and their respective environments had on full sibling derived genotypes	Farmer genotypes did not differ in biomass accumulation, height, yield, and kernel mass. Farm2 had greater lodging resistance and lower protein levels than Farm1 under high fertility and high precipitation conditions.	Farmer genotypes did not differ in biomass accumulation and yield. FarmC was taller, had greater lodging severity, and lower grain protein than FarmA and FarmB. Under high fertility, high precipitation conditions, FarmC had significantly greater kernel mass than farmer genotypes FarmA and FarmB.

CHAPTER 4.

PHOSPHORUS UPTAKE, EFFICIENCY, AND PHYSIOLOGICAL COMPARISON OF CONTRASTING SPRING WHEAT (*TRITICUM AESTIVUM* L.) PARENTAL MATERIAL AND THEIR FARMER-SELECTED POPULATIONS

4.1. Abstract

Phosphorus, an essential macronutrient for plants, is stored and used in plants as inorganic phosphates. Maintaining yield under low soil test-P (STP) conditions is essential for continued sustainable production on organic farms on the Canadian prairies. Plant breeding is one proposed strategy to overcome this challenge. Using genotypes from a participatory plant breeding (PPB) wheat program, we investigated P uptake, partitioning, yield efficiency dynamics as well as biological belowground traits that are known to facilitate greater P uptake. The field experiment was conducted under limited P (3 ppm Olsen-P) and P-amended (25 kg P ha^{-1} composted manure) organic conditions across three years. The first objective was to evaluate the parental material used in the PPB program, a modern spring wheat cultivar, 5602HR, and a landrace cultivar, Red Fife. The second and third objectives of this study were to i) investigate how farmer selections differed from their parents, and 2) evaluate the impact geographically divergent farmers and their respective environments had on full sibling derived genotypes. The parental cultivar 5602HR was more responsive to P-amended treatments than Red Fife, however, Red Fife had greater phosphorus yield efficiency (kg grain yield per kg P uptake; PYE). Farm1 was more similar to 5602HR in responsiveness to P-amended treatments. Farm2 resembled Red Fife in phosphorus yield efficiency. Farm1 accumulated more total plant P and demonstrated greater phosphorus uptake efficiency (P taken up per available soil P; PUptE) than both parents under P-amended, and limited P conditions in 2022. Despite Farm1 taking up significantly greater amounts of P than Farm2, farmer genotypes yielded similarly, resulting in Farm2 having greater PYE than Farm1. Two different mechanisms may be occurring

in regards to the farmer selections. Farm1 may maximize P uptake but does not translate greater P uptake into greater yield, and Farm2 may take up less P, but more efficiently produces yield. We did not observe differences among farmer selections for the studied belowground dynamics, however, other traits significantly impact efficient P uptake. More research is needed to examine what underlying mechanisms contribute to greater P uptake and P yield efficiency among PPB wheat genotypes as well as the wider Canadian wheat germplasm. This research demonstrates that selection under organic conditions may facilitate greater P uptake traits, however, translating greater P uptake into greater yield efficiency remains a challenge.

4.2. Introduction

Wheat is an important crop for Canada and is sought after on the export market for its high quality. The Canadian prairie region (the provinces of Alberta, Saskatchewan, and Manitoba, and the Peace River region in British Columbia) grows 93% of Canada's organic wheat (Canada Organic Trade Association and Prairies Organic Development Fund, 2021). In 2020, Canada exported 237 000 metric tons of wheat worth $118 000 000 (Agriculture and Agrifood Canada, 2022). Wheat is often the 'cash crop' within organic crop rotations; recent premiums for organic grade wheat were valued at 200% of conventional grade (Organic Biz, 2023). While supplying nitrogen to organic wheat in the region is achieved through the inclusion of legume phases in the rotation, phosphorous (P) is more challenging owing to the lack of available manure sources (Entz et al., 2001).

Phosphorus, an essential macronutrient for plants, is taken up from the soil as orthophosphate anions ($H_2PO_4^-$ or HPO_4^{-2}) (Condron et al., 2005). Phosphorus is a key structural component in phospholipids, nucleic acids, sugar phosphates, and adenylates and is necessary for protein synthesis (Schachtman et al., 1998). Additionally, P is required for efficient photosynthesis, due to its role in phosphorylation and exchange of triose phosphate between the chloroplast and the cytosol (Plaxton and

Tran, 2011). Phosphorus fertility in the Canadian prairie region is most commonly assessed using sodium bicarbonate extraction, also known as Olsen-P (Olsen et al., 1954; Grant and Flaten, 2019), and referred to as soil test phosphorus (STP). Maintaining yield under low STP is essential for continued sustainable production on organic farms on the Canadian prairies. Plant breeding is one proposed strategy to overcome this challenge (Lynch and Brown, 2001; Ojeda-Rivera et al., 2022; Carkner et al., 2023).

Evaluating genetic material in terms of P be achieved in two ways; phosphorus uptake efficiency (PUptE; sometimes referred to as phosphorus acquisition efficiency) and phosphorus yield efficiency (PYE; sometimes referred to as phosphorus use efficiency, or phosphorus utilization efficiency) (Manske et al., 2001; Wang et al., 2010a; Mcdonald et al., 2015). Phosphorus uptake efficiency refers to the crops' ability to take up P from soils, and PYE refers to the ability to produce biomass or yield using the P that was taken up. The relative importance of these measures is debated and thought to depend on the type of environmental conditions the crop is grown in (Wang et al., 2010b; Vandamme et al., 2016; Lynch, 2019; Carkner et al., 2023). Lynch (2019) advises that greater uptake efficiency should be selected for under poor P fertility conditions, however, others have argued that a combination of uptake and yield efficiency are required (Wang et al., 2010b; Vandamme et al., 2016; Carkner et al., 2023). Currently, PUptE is often used to evaluate how responsive genotypes are to added P fertility (Manske et al., 2001; Osborne and Rengel, 2002; Mcdonald et al., 2015; Soumya et al., 2021; Thiessen Martens et al., 2021). This approach is appropriate for cropping systems in which additional fertilizer recovery is low, such as in Australia (Mcdonald et al., 2015) or testing new fertilizer P substrates (Osborne and Rengel, 2002; Thiessen Martens et al., 2021), but not necessarily appropriate for low-P organic production systems because the objective isn't to evaluate responsiveness to added fertility, rather, the objective is to evaluate a genotypes' ability to adapt to a low-P environment (Carkner et al., 2023).

Due to the immobile nature of P in soil, roots must move toward zones of available P to acquire it. When plant roots and microorganisms take up P, a 'depletion zone' is created in the soil system adjacent to the uptake site, therefore, greater P access is constantly required (Smith et al., 2003). Up to 95% of P uptake into root systems is through diffusion (Kovar and Claassen, 2005), which occurs when STP is lower in the soil than in the plant (ex. Under low STP). High-P affinity phosphate transporter proteins in the roots actively take up P against this concentration gradient (Heuer et al., 2017; Ojeda-Rivera et al., 2022).

Multiple studies have associated greater physical exploration of soil surface area and greater PUptE with wide basal and shallow seminal root angles, greater root hair density, and high branching potential (Lynch and Brown, 2001; Lynch, 2011; Haling et al., 2013). Multiple studies have demonstrated genotypic variation among wheat species under low-P supply (da Silva et al., 2016; Maccaferri et al., 2016; Fradgley et al., 2020; Pariyar et al., 2021).

An additional strategy plants can employ for greater exploration of soil surface area is through root colonization of arbuscular mycorrhizal fungi (AMF). Arbuscular mycorrhizal fungi infect plant roots and create a mutualistic relationship with the host plant using a bi-directional P and carbon transfer; the host plant receives P, and the fungi receives carbon substrates in return (Harrison, 2005). Greater soil surface area is explored through AMF by extending their hyphal network past the crops' roots (Pepe et al., 2018). Root colonization is affected by available-P supply, because if plant roots can access adequate P themselves, the carbon trade-off required for AMF colonization is not as advantageous for the plant (Kobae, 2019). For example, AMF colonization in flax was shown to be greater in low STP organic farming conditions compared to conventional conditions with higher STP in Canada (Entz et al., 2014). Genotypic variation in AMF colonization within wheat is well established (Kirk et al., 2011; Singh et al., 2012; De Vita et al., 2018; Nahar et al., 2020).

Past reports have indicated that when STP is low, the organic P pool may play a larger role in crop P supply (Oehl et al., 2002; Bünemann, 2015; Schneider et al., 2016). Mineralizing P bound in the organic pool is another way plant roots access available P (Condron et al., 2005). Plants can also exude organic acids to solubilize P from Fe and Al complexes in acidic soils and from Ca and Mg complexes in alkaline soils (Kovar and Claassen, 2005). Specific organic acids differ by plant species and even genotype (Jones and Darrah, 1994; Neumann and Römheld, 1999; Gaume et al., 2001; Nguyen et al., 2019; Richardson et al., 2022). Organic production systems have been shown to have higher microbial-P turnover and biological activity than conventional production systems (Oehl et al., 2001a; Braman et al., 2016; Schneider et al., 2016).

Various forms of P in the organic P pool are orthophosphate monoesters, inositol phosphates (e.g., phytic acid), phosphoproteins, mononucleotides, sugar phosphates, phospholipids, teichoic acid, aromatic compounds, phosphonates, and organic phosphate anhydrides (Condron et al., 2005). These are primarily made of stable ring structures, making them resistant to hydrolysis and not available to plants for uptake (Condron et al., 2005). Plant roots have been shown to exudate phosphatase enzymes to break ring structures within the organic P pool, and mineralize the organic P into orthophosphate anions for uptake (Juma and Tabatabai, 1988). Multiple studies have demonstrated genotypic variation in acid phosphatase activity in response to low-P conditions among wheat genotypes (Manske et al., 2000; Vance et al., 2003; Ciereszko et al., 2011; Wang et al., 2021).

Phosphorus yield efficiency (PYE) is mainly associated with efficient re-translocation and re-use of stored P_i in plants tissues, and is increased when plants are under deficient P environments (Wang et al., 2010b). Multiple genes, enzymes, and phosphate transport proteins are involved with the re-mobilization of P_i from old to new plant tissues. In some cases, plants can release stored P_i in vacuoles to maintain P_i homeostasis (Wang et al., 2010b). Additionally, many plants have the ability to adjust their metabolic

rates and use alternative glycolytic pathways in response to P_i starvation (Vance et al., 2003). Other strategies plants may use to conserve P_i include replacing membrane phospholipids with amphipathic galactolipids and sulfolipids, and P_i scavenging from other P_i compounds such as ribosomal RNA and organelle DNA (Dissanayaka et al., 2021). Significant genotypic differences in PYE have been demonstrated under controlled and field conditions in wheat (Korkmaz et al., 2009; Su et al., 2009; Mcdonald et al., 2015; Akhtar et al., 2016).

Genotypic variation in wheat for PUptE and PYE has been demonstrated in controlled environments (Fageria and Baligar, 1999; Wang et al., 2005, 2010a; Yuan et al., 2017; Bilal et al., 2018; Zhao et al., 2018) and field conditions (Batten et al., 1984; Batten and Khan, 1987; Elliot et al., 1998; Mcdonald et al., 2015; Zhao et al., 2018). However, many studies categorize 'P efficient genotypes' as genotypes that were more responsive to mineral fertilizer, or able to maintain yield under a range of mineral fertilizer rates. This is inappropriate for organic production systems on the Canadian prairies, where growing conditions are often low in available P because conventional soluble mineral fertilizers are prohibited. The wheat ideotype proposed by Carkner et al. (2023) minimizes P translocation from biomass into grain (Phosphorus harvest index; PHI). By lowering the PHI, less P is exported from the field, 'releasing' P back into the soil for subsequent soil fertility in the crop rotation (P return efficiency, PRE).

Modern wheat cultivars have been selected under high fertility environments, which may have led to genotypes that do not possess important traits to access soil-P bound within the organic P pool or physically inaccessible from the root zone (Veneklaas et al., 2012; McGrail et al., 2023). The selection environment in breeding programs can have a significant impact on final performance in cropping systems (Kirk et al., 2012; Wiebe et al., 2017). Using wheat landraces for low STP conditions has been proposed due to their ability to resist and tolerate abiotic and biotic stresses given that their selection environments took place prior to the use of heavy mineral fertilizer application (Wissuwa and Ae, 2001; Lin et al., 2020;

McGrail et al., 2023). Using genotypes from a Canadian organic participatory plant program (PPB) in wheat (Chapter 3, Entz et al., 2018), we evaluated the feasibility of the 'Low-P Ideotype' suggested by Carkner et al. (2023). Participatory plant breeding (PPB) genotypes used in the present study were a result of a cross between a modern spring wheat cultivar, 5602HR, and a landrace cultivar, Red Fife, and selected under organic management by two different farmers. The first objective of this study was to evaluate phosphorus uptake, yield efficiency, as well as belowground traits that facilitate P uptake of two parental wheat cultivars used to generate genotypes for the PPB organic breeding program. The second objective of this study was to investigate how farmer selections differed from their parents. The last objective was to evaluate the impact geographically divergent farmers and their respective environments had on full sibling derived genotypes between modern and landrace parental cultivars.

4.3. Materials and Methods

Information on genetic material, experiment design, field management, agronomic sampling procedures, and environmental descriptions are described in detail in Chapter 3.

4.3.1. Phosphorus determination

Dried anthesis and threshed mature biomass samples for total plant phosphorus analysis were finely ground (1mm) using a Wiley mill (Thomas Scientific, Pennsylvania USA). Grain samples were a composite year sample of harvested grain of each experimental unit and finely ground (1mm) using a coffee grinder. Ground subsamples of anthesis straw, mature straw, and grain for total P concentration were analyzed by Agvise Laboratories in North Dakota, USA, using inductively couple plasma-optical emission spectroscopy (ICP-OES; Optima 5300DV, Perkin Elmer, Waltham, MA) following digestion with HNO_3 and H_2O_2 at 150°C following the procedure by Havlin and Soltanpour (1980).

Total P accumulation in the total aboveground biomass at anthesis was calculated as a product of dried anthesis biomass and P concentration (kg P ha^{-1}). Total phosphorus accumulation in the straw at

maturity was calculated as a product of straw P concentration and mature straw biomass minus the grain (kg P ha^{-1}). Total phosphorus accumulation of grain P was calculated as a product of grain P concentration and grain yield (kg P ha^{-1}). Total plant P accumulation represents mature straw P accumulation and grain P accumulation combined. Post-anthesis P accumulation was calculated as total plant P accumulation minus anthesis straw P accumulation. Phosphorus uptake efficiency (PUptE) represents net total plant P uptake per P applied (indigenous and added) and was calculated using the formula in equation 4-1.

$$\text{PUptE } (\%) = \frac{Total\ plant\ P\ accumulation}{Total\ soil\ P\ (indigenous\ (STP)\ and\ added)}\ x\ 100 \quad (4\text{-}1)$$

P harvest index represents the amount of P translocated from the total whole plant into the grain and was calculated using the formula in equation 4-2.

$$\text{PHI } (\%) = \frac{Grain\ P\ accumulation}{Total\ plant\ P\ accumulation}\ x\ 100 \quad (4\text{-}2)$$

Phosphorus yield efficiency (PYE) represented as kg ha^{-1}, is the kg of grain yield produced per kg of P taken up in the plant. PYE was calculated using the formula in equation 4-3. (Moll et al., 1982; McDonald et al., 2015).

$$\text{PYE } = \frac{Grain\ yield}{Total\ plant\ P\ accmulation} \quad (4\text{-}3)$$

Phosphorus return efficiency (PRE) represented as kg ha^{-1}- is the kg of grain yield produced per kg of straw phosphorus accumulated and would theoretically be returned to the soil after harvest and is calculated using the formula in equation 4-4.

$$\text{PRE } = \frac{Grain\ yield}{Mature\ straw\ phosphorus\ accumulation} \quad (4\text{-}4)$$

Grain N:P ratio denotes the ratio of Grain N concentration (mg g^{-1}) and Grain P concentration (mg g^{-1}) in the final grain at harvest. Grain N:P ratio was calculated using the formula in equation 4-5.

$$\text{Grain N: P Ratio} = \frac{Grain\ N\ concentration}{Grain\ P\ concentration} \quad (4\text{-}5)$$

4.3.2. Acid phosphatase analysis

Wheat plants were excavated from one randomly selected area in each experimental unit in 2020, 2021, and 2022. Samples were collected at the anthesis stage (Zadok stage 64). A shovel (20-cm wide) was inserted 15-cm deep and 10cm away from the plant row. Approximately nine plants were excavated. Due to the soil's clay texture, loose soil was removed from the roots by carefully shaking the plants and gently breaking up clods of soil that had adhered to the larger pieces of soil away from the roots. The remaining soil that had strongly adhered to the roots was carefully removed by swishing the roots and soil in 100mL of reverse osmosis water for 1 minute. Two 15 mL subsamples of the rhizosphere-water solution were taken and stored in a screw-cap conical tube and placed in a freezer at -20°C until further processing.

Microplate fluorometric assays were used to determine the potential phosphatase activities of rhizosphere soils based on 4-methylumbelliferone (MUF) released by the enzymatic hydrolysis of MUF-labelled substrates incubated with soil at the optimal pH (Freeman et al., 1995; Deng et al., 2013). To create a calibration curve, MUF standards of 0, 5, 10, 20, 30, and 50 pmol were used. Frozen samples were thawed overnight in the refrigerator and brought up to room temperature (21°C). Samples were diluted to bring all samples to 40mL with Millipore water and were shaken in an agitator to homogenize for 30 minutes. Soil suspension samples were transferred from the conical tube into a 100-mL beaker with a magnetic stir bar set at 600 rpm to keep the soil suspended. The suspension was pipetted into Corning® 96-well black polystyrene microplates and mixed into solution with modified universal buffer at pH 6.5 (MUB) and MUF substrate (4-Methylumbelliferyl phosphate, Sigma Aldrich, St. Louis USA) with eight wells per sample. A standard curve was included for each sample analysed to account for any background differences, as well as two controls where the MUF substrate was not added to the control wells prior to

incubation. The solution was incubated in the dark at 37°C for exactly one hour. After incubation, the reaction was stopped with tris (hydroxymethyl) aminomethane (THAM; pH 10). MUF substrate was then added to the control wells. The microplate was read at 360nm excitation, and 460nm emission on a BioTek Synergy Neo2 Multimode Reader (Agilent, Santa Clara USA).

To calculate the standard curve, the average reading from the zero MUF standard was subtracted from all other standard readings, and the intercept of the calibration curve was forced through zero. The average autohydrolysis was calculated by subtracting the average relative fluorescence unit of the autohydrolysis wells incubated with the MUF substrate added after reactions termination from the average of the wells with substrate added before incubation (Equation 4-6).

$$\text{Corrected fluorescence } (F_{\text{corrected}}) = (F_{sample} - F_{avg\ control} - F_{avg\ autohydrolysis}) \quad (4\text{-}6)$$

The calculation used to determine the pmol of MUF released in each assay well is shown in Equation 4-7.

$$\text{pmol MUF released} = \frac{F_{corrected}}{\text{slope of the MUF calibration curve}} \quad (4\text{-}7)$$

Enzyme activity in the soil was determined by correcting the for soil weight and volume (Equation 4-8).

$$\mu\text{mole MUF kg}^{-1}\text{soil h}^{-1} = \frac{\text{pmol MUF released}}{100\ \mu\text{l moil suspension}} \text{ x } \frac{1000\ \mu\text{l}}{1\ \text{mL}} \text{ x } \frac{40\ \text{mL}}{1\ \text{g soil}} \text{ x } \frac{1\ \text{nmol}}{1000\ \text{pmol}} \text{ x } \frac{1}{1\text{h}} \quad (4\text{-}8)$$

4.3.3. Water extractable inorganic phosphorus

After the soil suspension was taken for phosphatase analysis, samples were returned to the original conical tube and centrifuged at 4000 rpm for 2 minutes. Centrifuged water was subsampled into smaller conical tubes and stored at 4°C for phosphate analysis. Excess water was carefully vacuumed out, and soil was dried for 72 hours at 60°C. The dried soil was then weighed and recorded.

The inorganic phosphate analysis methods were conducted following D'Angelo et al. (2001). Standards were made using stock phosphate solution (20 ppm tribasic sodium phosphate) with 0, 0.01, 0.05, 0.1, 0.25, and 0.5 ppm P to create a calibration curve. To check the accuracy of the standard curve, two check standards with the concentrations of 0.1 ppm-P and 0.25 ppm-P were used. Experimental samples were added to wells in triplicate. Standards, check standards, and experimental samples were added to clear microplate wells with 30 µl of ammonium para-molybdate and sulfuric acid solution as well as 30 µl malachite green solution. After 30 minutes, the microplate was read at 630nm.

Phosphate concentration (mg P L^{-1}) was calculated using equation 4-9.

$$\text{Phosphate released (mg P L}^{-1}) = (\text{sample [ ]})\ (\text{b} - \text{intercept of calibration curve}) \quad (4\text{-}9)$$

Inorganic phosphate concentration per unit of soil was calculated using equation 4-10.

$$\text{Mg P kg}^{-1}\text{soil} = \frac{(\text{phosphate released})(0.04)}{\frac{\text{soil weight}}{1000}} \quad (4\text{-}10)$$

4.3.4. Arbuscular mycorrhizal colonization

After rhizosphere soil was removed from the roots, fine roots were sampled and stored in 70% (v/v) ethyl alcohol solution at 4°C until further processing.

Prior to processing, roots were placed in bio-cassettes and gently rinse with reverse osmosis water to remove ethanol residue, before transferring to 100 mL glass beakers. A 10% potassium hydroxide (KOH) solution was added to the beakers to just cover the cassettes. The roots were autoclaved for 12 minutes to clear them of their internal structures. After clearing, the roots were rinsed with reverse osmosis water, followed by acidified water several times, and transferred back to glass beakers. The cassettes were covered with 5% (v/v) ink-vinegar (Sheaffer Liquid Skrip Black Ink; catalogue number SHF94231; Sheaffer Pen & Art Supply Co., Shelton, Connecticut USA) solution following the methods of Vierheilig et al. (1998) and brought to a rolling boil for 3 minutes. The ink-vinegar solution was discarded, and the roots were

rinsed several times with reverse osmosis water and acidified water several times. Glycerol was then added to de-stain the roots for 24 hours.

Stained roots were prepared for viewing by cutting roots into 1-cm sections and mounting 25 sections (5 sections by 5 sections) on a glass slide and covering with a glass cover slip. Slides were viewed under a light microscope set at 250x magnification using an ocular lens with a crosshair. Arbuscular and hyphae presence was scored using the method of McGonigle et al. (1990). Beginning with the root sample at the top left, the ocular lens was moved down, across, and up at regular intervals. Where the vertical cross hair in the ocular lens and a root intersected, the intersection was scored as a root, hyphae, or arbuscule. The intersection was scored as arbuscule when one more arbuscules were present, or as hyphae when the crosshair intersected with one or more hyphae. When both hyphae and arbuscle interacted with the crosshair on the same root, only arbuscule presence was recorded. Each root section was counted in regular intervals 4 times, resulting in 100 scores. Percent total colonization (the sum of arbuscular and hyphal colonization) and percent arbuscular colonization was determined by dividing the number of positive arbuscule/hyphae scores by the total counts and multiplied by 100.

4.3.5. Data analyses

Phosphorus concentrations and calculations were analyzed with years combined to detect genotype x manure, genotype x year, year x manure, or year x genotype x manure interactions. Given that year-to-year variability was high, data was separated by year. Significant genotype x year, genotype x manure, and genotype x manure x year interactions were further explored using bar graphs to observe relevant patterns, if required. Data was analyzed using PROC Mixed procedure with Statistical Analysis Software program 9.4 (SAS Institute, 2013). Manure, genotype, and manure x genotype were fixed effects, and replicate (year) and year were random effects. Tests for normal distribution of data were carried out using PROC Univariate with the Shapiro-Wilks statistic. Values greater than 0.9 were assumed to be

normally distributed. If the data were not normally distributed, the data were transformed. Differences among genotypes and manure phosphorus levels were tested using the Least Significant Difference (LSD) test and considered significant at *P<F 0.05* for phosphorus parameters and *P<F 0.10* for belowground microbial activity. Data shown in tables represents the Least Squares Means (lsmeans) and back-transformed if required. To compare the farmer genotypes with the parents, treatments were combined and analyzed into three groups: Farmer genotypes contrasted with both parents, farmer genotypes contrasted with Red Fife, and farmer genotypes contrasted with 5602HR. Contrasts were carried out using the PROC GLM procedure in SAS 9.4 (SAS Institute, 2013).

4.4. Results and Discussion

4.4.1. Manure and environmental main effects

Seasonal differences between years resulted in a significant year effect for all P parameters and calculations (Table 4-1). There was a significant manure x year interaction effect for straw and grain P concentration, straw and grain P accumulation, total plant P accumulation, and PUptE. Genotypes had the greatest P concentration and accumulation in 2022, followed by 2020, and lastly 2021. For example, total plant P accumulation ranged between 6.3-6.7 kg ha^{-1} in 2020, 2.9-3.1 kg ha^{-1} in 2021, and 10.6-12.4 kg ha^{-1} in 2022 (Table 4-1, Table 4-2, Table 4-3, Table 4-4).

Poor manure response for all P parameters and calculations in in 2021 compared with 2020 and 2022 was attributed to less cumulative precipitation in 2021 than in 2020 and 2022, resulting in poor manure mineralization and subsequent P uptake. The 2020/21 winter snowfall amounted to less than 30% of the average long-term snowfall (Environment Canada, 2021) and by anthesis, the experiment in 2021 received 67% of precipitation compared with 2020 (Chapter 3, Table 3-3). Manure mineralization rates of N and P have been shown to be greatest at field capacity and decrease steadily as soil moisture declines (Cassman and Munns, 1980; Whalen et al., 2001). Additionally, P moves into the plant primarily by mass

flow and diffusion, whereby soil moisture is the primary medium of P transport (Kovar and Claassen, 2005). Consequently, drought conditions significantly impacted the plant roots' ability to take up P, despite the presence of adequate available soil P content (He et al., 2002).

Phosphorus uptake efficiency values greater than 100% in 2022 under limited P treatments (Table 4-4) highlight the impact environmental conditions can have on phosphorus availability. Reports of adequate grain yield despite low soil available P tests (Martin et al., 2007) on organic farms has prompted work to understand less mobile forms of P becoming available throughout the season on organic farms (Schneider et al., 2016). Lack of understanding between the soil P forms and soil-root interactions has even called into question the reliance on soil P tests alone for predicting season-long P availability (Cooper et al., 2018; Schneider et al., 2019; Hallama et al., 2021). This study highlights the potential for genotypes to take up more P than previously perceived based on spring soil-tests. New breeding paradigms are emerging in nitrogen and phosphorus in an attempt to capture soil-plant interactions and nutrient use efficiency during screening in breeding programs (Ciampitti et al., 2022; Carkner et al., 2023).

Table 4-1. Lsmeans and combined analysis of variance comparing phosphorus parameters from three years of data (2020, 2021, 2022) collected under organic conditions in Libau, Manitoba from two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) under limited soil test Olsen-phosphorus (3ppm) and those amended with composted manure at 25 kg P ha^{-1}

	Anthesis Straw P[a] Conc[₮]	Anthesis Straw P Acc.[¥]	Straw P Conc.	Straw P Acc.	Grain P Conc.	Grain P Acc.	Total P Acc.	PUptE[b]	PHI[c]	PYE[d]	PRE[e]	GrainN:P Ratio[f]
Year (Y)	mg g^{-1}	kg ha^{-1}	mg g^{-1}	kg ha^{-1}	mg g^{-1}	kg ha^{-1}	kg ha^{-1}	%	%	kg ha^{-1}	kg ha^{-1}	
2020	1.4b	5.1b	0.25b	1.3b	2.6b	5.2b	6.6b	50b	79b	317b	.062b	4.2b
2021	0.8c	2.4c	0.15c	0.5c	1.8c	2.8c	3.3c	34c	84a	450a	0.035c	8.9a
2022	1.8a	6.5a	0.49a	3.2a	3.2a	8.1a	11.3a	77a	72c	230c	0.11a	3.3b
Year *P>F**	<.0001	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.001	<.0001	0.0001	<.0001
Genotype (G) (-P,+P)[β]												
Farm1	1.3	5.2	0.29	1.7	2.6ab	5.6	7.3	57	79	329ab	0.07	5.7a
Farm2	1.3	4.1	0.27	1.4	2.5bc	5.3	6.8	55	81	350a	0.061	5.3ab
Red Fife	1.4	4.8	0.31	1.6	2.4c	5.2	6.9	54	78	339a	0.07	4.6b
5602HR	1.3	4.6	0.32	1.7	2.7a	5.4	7.2	49	76	310b	0.08	6.2a
Genotype *P>F*	0.2102	0.2827	0.2171	0.4098	0.0002	0.7151	0.271	0.5343	0.2469	0.0142	0.1851	0.0116
Coefficient of Variation (%)	36	68	30	37	28	18	13	36	9	12	12	56
Standard Error ±	0.1	0.5	0.002	0.1	0.006	0.2	0.2	4.2	1.5	12	0.001	0.2
Manure (M)												
(+)P	1.5a	6.1a	0.33a	2.1a	2.7a	6.4a	8.5a	33b	78.0	314b	0.077a	4.8b
(-)P	1.2b	3.2b	0.26b	1.2b	2.3b	4.3b	5.5b	74a	79	351a	0.064b	6.1a
Manure *P>F*	<.0001	<.0001	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001	0.4112	<.0001	0.0442	<.0001
Interactions *P>F*												
G x M	0.4797	0.4096	0.5398	0.6599	0.0599	0.4091	0.4228	0.3543	0.5133	0.7008	0.719	0.7014
G x Y	0.7327	0.9186	0.4558	0.8907	0.1193	0.055	0.0064	0.2066	0.822	0.1188	0.6087	0.0334
M x Y	<.0001	<.0001	0.0007	<.0001	<.0001	<.0001	<.0001	<.0001	0.1737	0.0803	0.0064	0.0007
G x M x Y	0.1146	0.7627	0.0282	0.2701	0.0404	0.3592	0.2265	0.9942	0.0475	0.3862	0.3392	0.1304
Farmer Genotypes lsmeans	1.3	4.6	0.28	1.66	2.53	5.5	7.2	56.6	79.9	340	0.067	5.7
Parental Cultivars lsmeans	1.3	4.7	0.32	1.75	2.54	5.4	7.1	51.8	77.6	327	0.075	5.5
Contrasts												
Farmer Genotypes vs. Parents *P>F*	0.8612	0.8833	0.3966	0.7907	0.9523	0.8124	0.9422	0.5309	0.2277	0.5671	0.3926	0.7931
Estimate	-0.01	-0.1	-0.06	-0.08	-0.009	0.15	0.06	4.8	2.3	13	-0.008	0.2
Farmer Genotypes vs. 5602HR *P>F*	0.9431	0.958	0.4061	0.7258	0.3547	0.9252	0.9544	0.4197	0.2102	0.3621	0.2863	0.4873
Estimate	0.009	-0.04	-0.04	-0.13	-0.17	0.073	-0.04	7.6	2.9	25	-0.013	-0.6
Farmer Genotypes vs. Red Fife *P>F*	0.7212	0.8516	0.5793	0.9334	0.4073	0.7685	0.8604	0.8275	0.47	0.9811	0.7399	0.2624
Estimate	-0.4	-0.15	-0.02	-0.03	0.15	0.2	0.19	2.1	1.6	0.66	-0.004	0.9

*Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.05$); [β](-)P, limited P treatment; (+)P, amended P treatment; [a]P, phosphorus; [b]PUptE, Phosphorus Uptake Efficiency; [c]PHI, Phosphorus Harvest Index; [d]PUE, Phosphorus Utilization Efficiency; [e]PRE, phosphorus return efficiency; [f]GrainN:P Ratio, Ratio of Grain N Concentration to Grain P Concentration, [₮]Conc., concentration; [¥]Acc. accumulation

Table 4-2. Lsmeans and combined analysis of variance comparing phosphorus parameters from 2020 collected under organic conditions in Libau, Manitoba from two spring wheat cultivars (5602HR and Red Fife) and two spring wheat farmer genotypes (Farm1 and Farm2) under limited soil test Olsen-phosphorus (3ppm) and those amended with composted manure at 25 kg P ha^{-1}

	Anthesis Straw P[a] Conc[₮]	Anthesis Straw P Acc.[¥]	Straw P Conc.	Straw P Acc.	Grain P Conc.	Grain P Acc.	Total P Acc.	PUptE[b]	PHI[c]	PYE[d]	PRE[e]	GrainN:P Ratio[f]
Genotype (G)	mg g^{-1}	kg ha^{-1}	mg g^{-1}	kg ha^{-1}	mg g^{-1}	kg ha^{-1}	kg ha^{-1}	%	%	kg ha^{-1}	kg ha^{-1}	
Farm1	1.4	6	0.3	1.4	2.6	5.3	6.7	53	79	317ab	0.06	8.7
Farm2	1.3	4.3	0.2	1.1	2.4	5.3	6.4	46	82	351a	0.04	8.4
Red Fife	1.4	5.3	0.2	1.4	2.4	5.2	6.6	49	80	339a	0.05	9.1
5602HR	1.3	4.7	0.3	0.9	2.9	5.3	6.3	49	83	263b	0.08	7.5
Genotype *P > F*	0.5286	0.0881	0.2575	0.3871	0.0583	0.9786	0.8343	0.8171	0.2676	0.0275	0.0581	0.1619
Coeff. Variation (%)	17	39	22	39	16	29	29	26	7.4	15	38	15
Standard Error ±	0.01	0.2	0.002	0.2	0.02	0.3	0.4	0.04	2.1	23	0.001	0.3
Manure (M)[β]												
(+)P	1.5a	6.3a	0.3a	1.49	2.7a	6.3a	7.9a	24b	80	305	0.06	8
(-)P	1.2b	3.8b	0.2b	1.2	2.4b	4.2b	5.1b	75a	81	330	0.07	8.8
Manure *P > F*	<.0001	<.0001	<.0001	0.1835	0.0411	<.0001	<.0001	<.0001	0.2509	0.2051	0.5377	0.124
G x M *P>F*	0.0621	0.2585	0.6162	0.135	0.0551	0.0635	0.2204	0.7364	0.0201	0.8363	0.0684	0.1434
Farmer Genotypes lsmeans	1.3	5.2	0.24	1.27	2.5	5.3	6.6	48.1	80.6	330	0.054	8.5
Parental Cultivars lsmeans	1.3	5	0.25	1.45	2.6	5.2	6.7	49.2	77.7	301	0.071	8.3
Contrasts												
Farmer Genotypes vs. Parents *P>F*	0.9339	0.8658	0.5343	0.4372	0.3482	0.9234	0.869	0.9342	0.3691	0.1758	0.1529	0.7585
Estimate	-0.008	0.14	-0.01	-0.18	-0.17	0.06	-0.11	-1.1	2.8	29	-0.017	0.18
Farmer Genotypes vs. 5602HR *P>F*	0.8923	0.6736	0.2114	0.3102	0.0663	0.9603	0.7717	0.9307	0.1943	0.0146	0.0337	0.1838
Estimate	0.02	0.4	-0.04	-0.29	-0.43	0.038	-0.24	-1.3	5	67	-0.002	0.99
Farmer Genotypes vs. Red Fife *P>F*	0.7867	0.8838	0.7989	0.7949	0.7172	0.9136	0.9849	0.9609	0.8677	0.7141	0.8733	0.3974
Estimate	-0.03	-0.15	0.008	-0.07	0.08	0.08	0.015	-0.77	0.62	-9	-0.002	-0.62

*Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.05$); [β](-)P, limited P treatment; (+)P, amended P treatment; [a]P, phosphorus; [b]PUptE, Phosphorus Uptake Efficiency; [c]PHI, Phosphorus Harvest Index; [d]PUE, Phosphorus Utilization Efficiency; [e]PRE, phosphorus return efficiency; [f]GrainN:P Ratio, Ratio of Grain N Concentration to Grain P Concentration

[₮]Conc., concentration; [¥]Acc. accumulation

Table 4-3. Lsmeans and combined analysis of variance comparing phosphorus parameters from 2021 collected under organic conditions in Libau, Manitoba from two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) under limited soil test Olsen-phosphorus (3ppm) and those amended with composted manure at 25 kg P ha^{-1}

	Anthesis Straw P[a] Conc[₮]	Anthesis Straw P Acc.[¥]	Straw P Conc.	Straw P Acc.	Grain P Conc.	Grain P Acc.	Total P Acc.	PUptE[b]	PHI[c]	PYE[d]	PRE[e]	GrainN:P Ratio[f]
Genotype (G)	mg g^{-1}	kg ha^{-1}	mg g^{-1}	kg ha^{-1}	mg g^{-1}	kg ha^{-1}	kg ha^{-1}	%	%	kg ha^{-1}	kg ha^{-1}	
Farm1	0.8	2.5	0.13	0.51	1.8bc	2.4	2.9	30.9	83	458	0.029	10.5a
Farm2	0.78	2.3	0.15	0.49	1.9ab	2.6	3.1	47.9	83	448	0.034	8.2bc
Red Fife	0.88	2.6	0.17	0.54	1.7c	2.4	2.9	38.1	81	472	0.035	7.2c
5602HR	0.83	2.3	0.13	0.46	2a	2.7	3.2	21.8	85	437	0.043	9.8ab
Genotype *P > F*	0.1792	0.7986	0.8012	0.98	0.0087	0.7869	0.8932	0.3578	0.8924	0.7817	0.4564	0.0225
Coefficient of Variation (%)	11	27	39	50	6	25	24	79	8	10	53	23
Standard Error ±	0.003	0.276	0.02	0.08	0.006	0.3	0.3	0.5	2.6	17	0.002	0.2
Manure (M)[β]												
(+)P	0.83	2.6	0.15	0.51	1.90	2.6	3.2	31.7	84	453	0.037	9.2
(-)P	0.82	2.3	0.15	0.47	1.80	2.4	2.9	37.6	83	456	0.034	8.6
Manure *P > F*	0.854	0.1172	0.8137	0.59	0.5277	0.3821	0.3252	0.5724	0.8758	0.8581	0.5609	0.4849
G x M *P>F*	0.688	0.4469	0.8012	0.71	0.0531	0.9792	0.9728	0.8719	0.7317	0.9199	0.5984	0.4689
Farmer Genotypes lsmeans	0.79b	2.4	0.14	0.443	1.8	2.7	3.2	39	86.2	458	0.03	9.3
Parental Cultivars lsmeans	0.86a	2.5	0.15	0.575	1.8	2.9	3.5	29	83.5	441	0.04	8.5
Contrasts												
Farmer Genotypes vs. Parents *P>F*	0.0433	0.8559	0.5814	0.138	1	0.3852	0.2050	0.343	0.2462	0.3242	0.2335	0.2830
Estimate	-0.06	-0.04	0.01	-0.130	0	-0.2	-0.33	9.4	2.6	17	-0.007	0.8
Farmer Genotypes vs. 5602HR *P>F*	0.2807	0.7999	0.8215	0.060	0.0824	0.9062	0.5006	0.1541	0.1471	0.4496	0.1532	0.5942
Estimate	-0.04	0.075	0.006	-0.180	-0.110	-0.033	-0.213	17.6	4	16	-0.011	-0.5
Farmer Genotypes vs. Red Fife *P>F*	0.0256	0.5831	0.2645	0.447	0.0824	0.197	0.1619	0.913	0.6613	0.3907	0.6069	0.0275
Estimate	-0.093	-0.16	-0.0013	-0.080	0.110	-0.375	-0.45	1.3	1.2	18	-0.004	2.1

*Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test (P ≤ 0.05); [β](-)P, limited P treatment; (+)P, amended P treatment; [a]P, phosphorus; [b]PUptE, Phosphorus Uptake Efficiency; [c]PHI, Phosphorus Harvest Index; [d]PUE, Phosphorus Utilization Efficiency; [e]PRE, phosphorus return efficiency; [f]GrainN:P Ratio, Ratio of Grain N Concentration to Grain P Concentration

[₮]Conc., concentration; [¥]Acc. accumulation

Table 4-4. Lsmeans and combined analysis of variance comparing phosphorus parameters from 2022 collected under organic conditions in Libau, Manitoba from two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) under limited soil test Olsen-phosphorus (3ppm) and those amended with composted manure at 25 kg P ha^{-1}

	Anthesis Straw P[a] Conc[₮]	Anthesis Straw P Acc.[¥]	Straw P Conc.	Straw P Acc.	Grain P Conc.	Grain P Acc.	Total P Acc.	PUptE[b]	PHI[c]	PYE[d]	PRE[e]	GrainN:P Ratio[f]
Genotype (G)	mg g^{-1}	kg ha^{-1}	mg g^{-1}	kg ha^{-1}	mg g^{-1}	kg ha^{-1}	kg ha^{-1}	%	%	kg ha^{-1}	kg ha^{-1}	
Farm1	1.8	7.2	0.48	3.4	3.4a	9.1	12.4a	88a	72	216	0.12	2.9a
Farm2	1.8	5.6	0.45	2.9	3.1b	7.7	10.6b	71b	73	242	0.11	3.5ab
Red Fife	1.8	6.5	0.51	3.1	3.0b	7.4	10.6b	75b	70	238	0.12	3.1b
5602HR	1.7	6.8	0.55	3.2	3.3ab	8.2	11.4ab	76b	73	224	0.11	3.7a
Genotype *P>F*	0.6699	0.7189	0.1631	0.6405	0.047	0.1035	0.003	0.0013	0.8164	0.0919	0.8994	0.0382
Coefficient of Variation (%)	18	62	30	34	11	17	12	13	11	10	35	39
Standard Error ±	0.1	0.5	0.006	0.4	0.01	0.5	0.6	0.07	3.2	7.8	0.001	0.1
Manure (M)[β]												
(+)P	2.1a	9.3a	0.62	4.5a	3.6a	10.6a	15.2a	45b	71	198b	.13a	2.2b
(-)P	1.5b	3.7b	0.41	2.1b	2.9b	5.9b	8.1b	109a	73	262a	.09b	4.4a
Manure *P>F*	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.3514	<.0001	0.0069	<.0001
G x M *P>F*	0.9285	0.5708	0.0434	0.1259	0.792	0.8405	0.1852	0.0058	0.7355	0.7774	0.9032	0.1704
Farmer Genotypes lsmeans	1.8	6.3	0.46	3.15	3.26	8.4	11.6	79.5	73.3	229	0.11	3.2
Parental Cultivars lsmeans	1.79	6.7	0.53	3.16	3.15	7.8	11.1	75.5	71.6	231	0.12	3.4
Contrasts												
Farmer Genotypes vs. Parents *P>F*	0.832	0.8185	0.2302	0.9905	0.5528	0.5668	0.6781	0.7569	0.5483	0.8514	0.7912	0.6843
Estimate	0.02	-0.33	-0.06	0.006	0.11	0.572	0.56	3.9	1.7	-2.6	-0.004	-0.2
Farmer Genotypes vs. 5602HR *P>F*	0.6971	0.7724	0.2129	0.992	0.8638	0.8683	0.9111	0.8077	0.9165	0.8072	0.9271	0.3858
Estimate	0.05	-0.52	-0.08	-0.006	-0.037	0.202	0.18	3.7	0.36	4.2	-0.002	-0.5
Farmer Genotypes vs. Red Fife *P>F*	0.9654	0.932	0.4724	0.9766	0.2581	0.4425	0.5717	0.7877	0.3829	0.5833	0.7333	0.8351
Estimate	0	-0.15	-0.05	0.018	0.25	0.942	0.94	4.2	3.1	-9.6	-0.006	0.11

*Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.05$); [β](-)P, limited P treatment; (+)P, amended P treatment; [a]P, phosphorus; [b]PUptE, Phosphorus Uptake Efficiency; [c]PHI, Phosphorus Harvest Index; [d]PUE, Phosphorus Utilization Efficiency; [e]PRE, phosphorus return efficiency; [f]GrainN:P Ratio, Ratio of Grain N Concentration to Grain P Concentration

[₮]Conc., concentration; [¥]Acc. accumulation

4.4.2. Evaluation of parental differences

4.4.2.1. Straw P concentration and accumulation

There were no differences between parents for straw P concentration and accumulation at anthesis and maturity developmental stages (Table 4-1). No genotype x manure, or genotype x year interactions were observed. However, there was a significant genotype x manure x year interaction (Figure 4-1). Generally, straw P concentration increased under amended P treatment to the same degree for all cultivars. However, in 2022, when P was added, 5602HR's straw P concentration increased by 0.3 mg g^{-1}, but P concentration for Red Fife did not change (Figure 4-1). Therefore, 5602HR was significantly more responsive to added P than Red Fife under adequate moisture and P-amended treatments of 2022. McDonald et al. (2015) examined the relationship between year of wheat cultivar release and P uptake and reported a negative relationship between year of release and straw P concentration. We observed the opposite results, the modern genotype had greater P uptake with added P. McDonald et al. (2015) compared only semi-dwarf genotypes released between 1974 and 2002. Additionally, McGrail et al. (2023) compared winter wheat genotypes released between 1808 and 2002 and reported no year of release effect in straw P concentration.

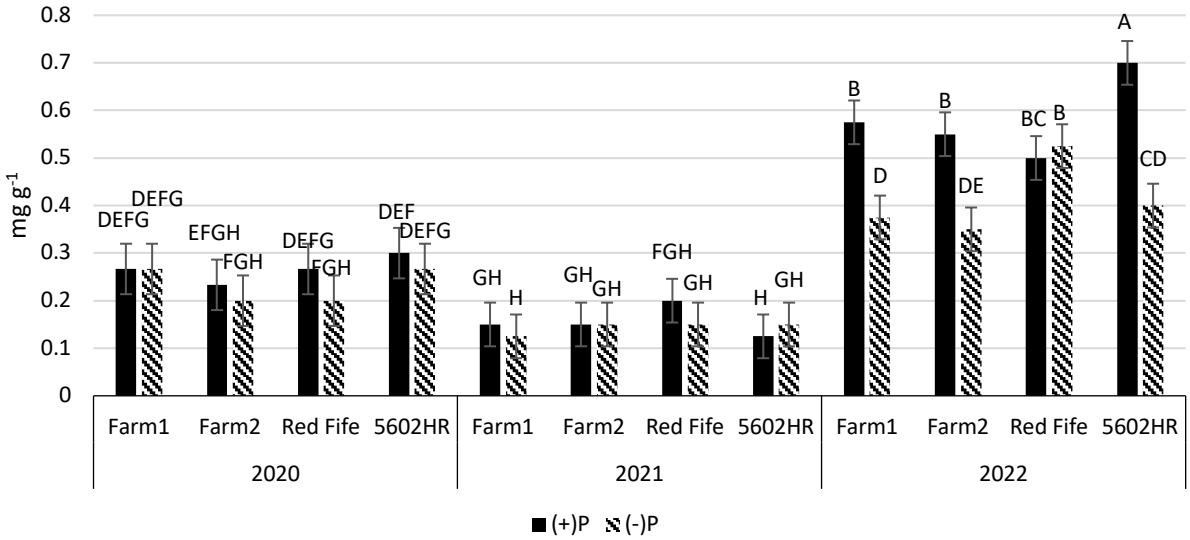


Figure 4-1. Straw phosphorus concentration genotype x manure interaction under organic conditions in Libau, Manitoba in 2022 among two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) under limited soil test phosphorus (3ppm), (-)P; and amended soil with composted manure at 25 kg ha^{-1}, (+)P.
Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.05$)

4.4.2.2. Grain P concentration and accumulation

The parental cultivar 5602HR had significantly greater (0.3 mg g^{-1}) grain P concentration than Red Fife when all years and manure treatments were combined (Table 4-1). There was a significant genotype x manure x year interaction (*P*>F 0.0404) (Figure 4-2). The source of the interaction was in 2020, 5602HR was the only cultivar that responded to manure addition. All other genotypes responded similarly to added fertility in 2021, and 2022. Under P limited treatments in 2022, Red Fife had significantly lower grain P concentration than 5602HR, however, when P was added, Red Fife and 5602R had similar grain P concentration.

In 2022, the P-amended treatment significantly increased grain P concentration in all genotypes to a similar concentration (between 13-15 mg g^{-1}). However, under limited P treatments, 5602HR had significantly greater P concentration than Red Fife by 0.425 mg g^{-1}.

A specific grain P concentration has not been an important breeding goal in Canadian wheat. Rather, simultaneously selecting for high yield and grain protein content have been two of the most important breeding priorities (McCallum and DePauw, 2008). While 5602HR did have significantly greater grain protein content than Red Fife by 4% (Chapter 3, Table 3-4), grain P concentration and protein content were not significantly correlated (*P>F* 0.5525; r = 0.099). Therefore, greater grain P concentration in 5602HR than Red Fife would have been coincidental. Grain P content has been linked to early vigour in crop plants (Bolland and Baker, 1988; White and Veneklaas, 2012), including wheat (De Marco, 1990). However, others have argued targeted, early exogenous P source could aid seeds to overcome low seed P reserves (Julia et al., 2018).

Red Fife and 5602HR did not differ from each other in grain P accumulation (Table 4-1). There were no genotype x manure, genotype x year, or genotype x manure x year interactions. There were no genotypic main effects of genotype x manure interactions detected in any years. No main effects or interactions indicates that 5602HR and Red Fife had similar grain P accumulation despite the wide range of environmental conditions between years and different fertility treatments.

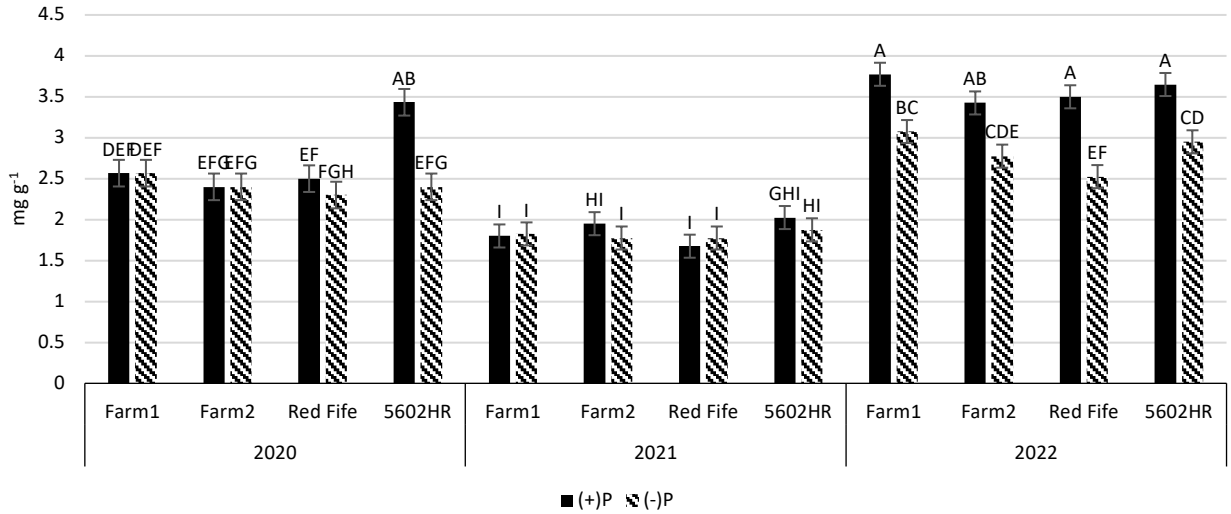


Figure 4-2. Grain phosphorus concentration genotype x manure x year interaction under organic conditions in Libau, Manitoba in 2020, 2021, 2022 among two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) under limited soil test phosphorus (3ppm), (-)P; and amended soil with composted manure at 25 kg ha^{-1}, (+)P. Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test (P ≤ 0.05).

4.4.2.3. Total plant P accumulation

Red Fife and 5602HR did not differ from each other for total aboveground plant P accumulation (Table 4-1), nor were there significant interactions between genotype x manure, or genotype x manure x year. There was a significant genotype x year interaction, however, the source of the interaction was not due to performance among either parent (Figure 4-3). Therefore, the overwhelming observation here is that total plant P uptake was similar between Red Fife and 5602HR among multiple years and fertility treatments.

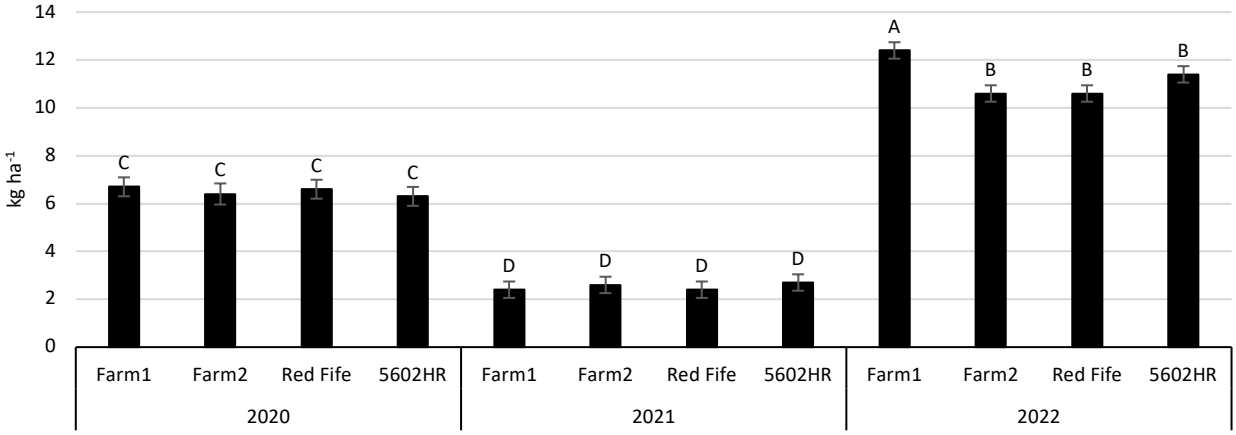


Figure 4-3. Total plant phosphorus accumulation genotype x year interaction under organic conditions in Libau, Manitoba in 2020, 2021, 2022 among two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) averaged among limited soil test phosphorus (3ppm), (-)P; and amended soil with composted manure at 25 kg ha^{-1}, (+)P.
Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.05$)

4.4.2.4. P Uptake and Partitioning Dynamics

4.4.2.4.1. Phosphorus uptake efficiency

Phosphorus uptake efficiency (PUptE) represents total plant P uptake as a percentage of indigenous and added P in the soil. While all genotypes had significant greater PUptE among fertility treatments, the parents did not differ from each other significantly (Table 4-1). No significant interactions between genotype x manure, genotype x year, and genotype x manure x year interactions were observed.

In 2022, there was significant genotypic effect and significant genotype x manure interaction (Table 4-4, Figure 4-4). However, the interaction derived from farmer genotypes' PUptE, and not parents. Differences between modern and landrace cultivar in PUptE are not consistent in the literature. For example, Wissuwa and Ae (2001) observed greater P uptake among landrace rice cultivars than modern rice cultivars under P-deficient conditions. However, working with wheat, Mcdonald et al., (2015) did not find differences in PUptE between modern and landrace cultivars under added-P and P-deficient environments. Greater PUptE has not been a historical breeding goal for Canadian spring wheat breeding programs.

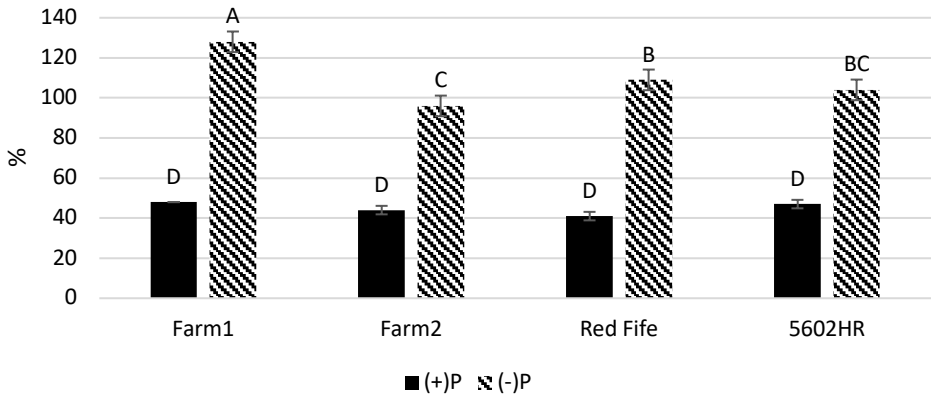


Figure 4-4. Phosphorus uptake efficiency genotype x manure interaction under organic conditions in Libau, Manitoba in 2022 among two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) under limited soil test phosphorus (3ppm), (-)P; and amended soil with composted manure at 25 kg ha^{-1}, (+)P.
Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.05$)

4.4.2.4.2. Phosphorus harvest index

No significant differences were observed among Red Fife and 5602HR for phosphorus harvest index (PHI) (Table 4-1). Phosphorus translocation values were similar to reported values in literature, Batten (1992) reported rainfed spring wheat PHI values ranging from 81-85% under a range of P application rates. No genotype x manure, genotype x year interactions were observed. There was a

significant genotype x manure x year interaction (*P>F* 0.0475) (Figure 4-5), deriving from 5602HR's inconsistent PHI among manure treatments between years.

While PHI differed significantly from year to year, 5602HR was the only genotype that differed among years and fertility treatments. In 2020, PHI was significantly greater under the P-amended treatment, however, in 2021, PHI was significantly greater under the limited P treatment. There were no differences between fertility treatments in 2022. It is unclear why opposite results were apparent in 2020 and 2021, and no difference was observed in 2022. Differences in PHI in response to P-supply is inconsistent; when comparing two durum wheat cultivars under controlled conditions using ^{32}P labeling under high and low P rates, El Mazlouzi et al. (2020b) found that PHI values were 30.6% and 79.1% under high and low P levels, respectively. McDonald et al. (2018) also reported a significant P fertilization effect when comparing wheat genotypes under diverse, rain-fed environments in Australia. However, Batten and Khan (1992) and Batten (1987) did not observe PHI impacted by fertilization rate when testing spring wheat genotypes.

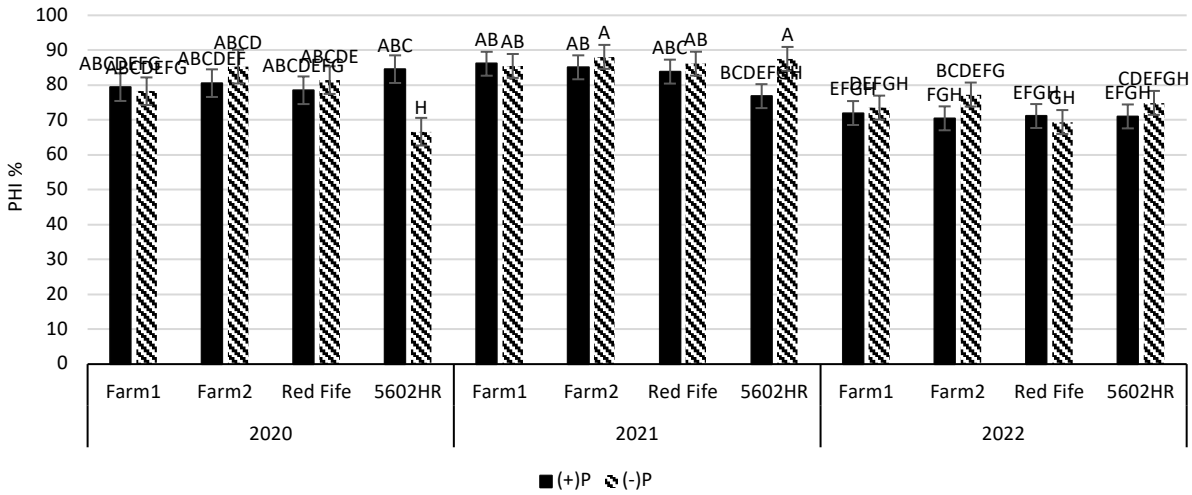


Figure 4-5. Phosphorus harvest index (PHI) genotype x manure x year interaction under organic conditions in Libau, Manitoba in 2020, 2021, and 2022 among two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) under limited soil test phosphorus (3ppm), (-)P; and amended soil with composted manure at 25 kg ha^{-1}, (+)P.
Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.05$)

4.4.2.4.3. Phosphorus yield efficiency

Phosphorus yield efficiency (PYE) represents the yield per unit total P accumulated. Red Fife exhibited significantly greater PYE than 5602HR by 29 kg ha^{-1} across all years and fertility treatments (Table 4-1). No significant interactions were detected for genotype x manure, genotype x year, or genotype x manure x year, indicating that PYE was consistent under a wide range of environmental conditions. Genotypic differences with similar grain PYE values have been reported by Wang et al. (2005), McDonald et al., (2005); Deng et al., (2018) when comparing spring wheat genotypes.

There were no significant differences between parents' grain yields when P-amended and limited P treatments were combined (Red Fife: 2195 kg ha^{-1}, 5602HR: 2045 kg ha^{-1}; see Chapter 3, Table 3-4). Additionally, there was no significant difference between the parents' total P accumulation (Table 4-4). However, Red Fife took up slightly less P (0.3 kg ha^{-1}) and yielded slightly more (150 kg ha^{-1}) than 5602HR. Therefore, this slight difference in both parameters may explain cultivar differences in PYE. Zhu et al.,

(2001) compared old (released 1860) and new (released 1996) spring wheat genotypes and found significant differences among genotypes in PYE, however, did not find a consistent pattern corresponding with year of release.

4.4.2.4.4. Phosphorus return efficiency

The P return efficiency (PRE) value represents the amount of P left in straw residue as a unit of yield. Therefore, a high PRE would be valuable, as more P is returned back into the soil system via residue for subsequent fertility with, hopefully, similar yield (Carkner et al., 2023). 5602HR and Red Fife did not significantly differ from one another (Table 4-1). There were no significant interactions among genotype x manure or genotype x year. Although not significant, 5602HR left more P behind in the residue per unit of grain yield than Red Fife. This was an artifact of lower yield and greater straw P accumulation by 5602HR (Yield, 5602HR; 2045 kg ha^{-1} vs. Red Fife; 2195 ka ha^{-1}). However, more P was exported off-farm by the modern genotype, 5602HR, in the form of grain yield (Grain P accumulation, 5602HR; 5.4 kg ha^{-1} vs. Red Fife; 5.2 kg ha^{-1}) due to significantly greater grain P concentration (Grain P concentration, 5602HR; 2.7 mg g^{-1} vs. 2.4 mg g^{-1}). These results are not surprising, greater P uptake resulted in more P in the biomass left behind, with no significant difference between landrace and modern cultivars. PRE has never been a breeding goal for spring wheat. However, it would be valuable for long-term sustainability of low-P organic farms (Carkner et al., 2023).

4.4.2.4.5. Grain N:P ratio

Decreasing grain P concentration has been proposed as a solution to reduce P export off farm therefore reducing supplement fertilizer application (Veneklaas et al., 2012; Bovill et al., 2013; Rose and Raymond, 2020; Carkner et al., 2023). However, the question remains if grain N translocation, ie., protein, would be sacrificed to reduce grain P (Veneklaas et al., 2012). Therefore, high Grain N:P ratios are of value to reduce grain P concentration but maintain protein quality for marketability. There were significant

differences among genotypes, 5602HR had significantly greater Grain N:P ratios than Red Fife by 1.6 (Table 4-1). There were no significant genotype x manure, or genotype x manure x year interactions. However, there was a significant genotype x year interaction (Figure 6).

Red Fife and 5602HR Seed N:P ratios were statistically similar to each other in 2020 and 2022. But during drought conditions in 2021, 5602HR had a significantly greater Seed N:P ratio than Red Fife by 2.6. Seed N:P ratios in 2021 were driving the combined genotypic effects (Table 4-1) since there were little differences between genotypes in 2020 and 2022 (Figure 4-6).

Protein levels and Grain N:P ratios did not follow a similar pattern. In 2020, 2021, and 2022, 5602HR had significantly higher protein levels by 2, 3.3, and 3.6% than Red Fife, respectively (Chapter 3; Table 3-5, Table 3-6, Table 3-7). The results demonstrate that grain nitrogen/protein have different uptake and accumulation patterns, and modern wheat cultivars, specifically, have the potential to maintain high protein while simultaneously possessing lower grain P.

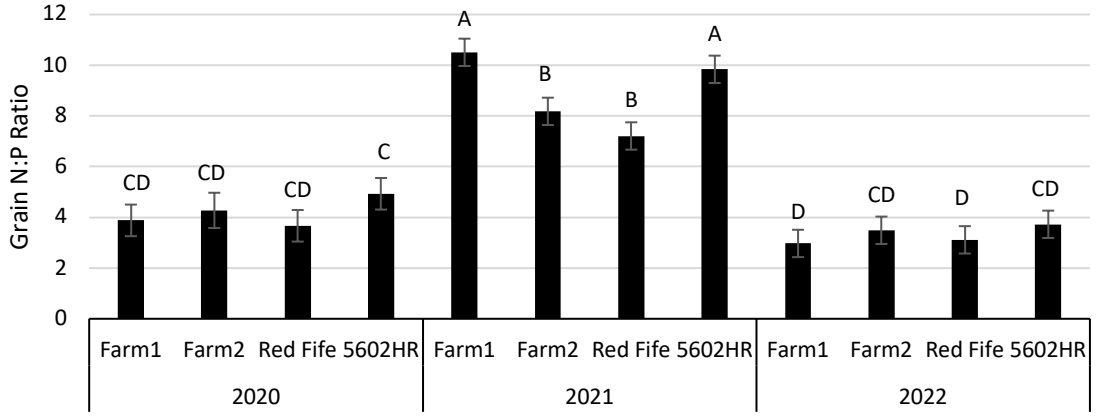


Figure 4-6. The genotype x manure x year interaction of Grain N:P ratio under organic conditions in Libau, Manitoba in 2020, 2021, and 2022 among two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) averaged among limited soil test phosphorus (3ppm), (-)P; and amended soil with composted manure at 25 kg ha^{-1}, (+)P.
Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.05$)

4.4.2.5. Belowground microbial activity

4.4.2.5.1. Rhizosphere phosphatase enzyme activity

There were no significant differences between 5602HR and Red Fife for acid phosphatase enzyme activity (APase) (Table 4-5), APase values were similar to other studies that sampled under field conditions (Lupwayi et al., 2023; Meier et al., 2023). No interactions between genotype x manure, or genotype x year were detected. However, in 2022, there was a significant genotype x manure interaction (*P>F* 0.0617) (Table 4-8, Figure 4-7).

The year 2022 was marked by favourable growing conditions with the highest grain yields among the three years the experiment took place (Chapter 3, Table 3-7). The parental cultivar, 5602HR's APase activity numerically declined but did not significantly differ between limited P and P-amended fertility treatments. However, Red Fife's APase activity was significantly higher than 5602HR under limited P conditions (Figure 4-7). There was a significant positive relationship between APase and rhizosphere water extractable phosphate (RhWEP), indicating that the presence of APase increased available P in the rhizosphere, which may have led to greater P uptake (Table 4-9). The results may demonstrate the potential for Red Fife to excrete APase under adequate moisture conditions.

Multiple studies have reported marked differences in rhizosphere assemblage and activity between landrace and modern cereal crops (Ahokas and Manninen, 2001; George et al., 2008; Bulgarelli et al., 2015; Cangioli et al., 2022; Gruet et al., 2022; McGrail et al., 2023), however, much of this work was done hydroponically. Genotypic difference may not have been apparent due to the sampling environment and variability. Working in wheat, George et al. (2008) reported that although APase activity impacted P uptake significantly under hydroponic conditions, similar results were not found when the same cultivars were grown in field conditions. Additionally, wheat cultivars have been shown to vary in their tendency

to use APase as opposed to other root exudate strategies (ex. Organic acids) (Deng et al., 2018; Nguyen et al., 2019; Cangioli et al., 2022).

Table 4-5. Lsmeans and combined analysis of variance comparing belowground microbial activity from three years of data (2020, 2021, 2022) collected under organic conditions in Libau, Manitoba among two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) under limited soil test Olsen-phosphorus (3ppm) and amended with composted manure at 25 kg P ha^{-1}

	Rhizosphere Acid Phosphatase	Rhizosphere WEP[Ŧα]	Arbuscule Infection	Hyphae	Arbuscules + Hyphae
Year (Y)	pmol MUF g^{-1} soil h^{-1}	mg P kg^{-1} soil	%	%	%
2020	882a	15	4c	4b	9b
2021	441b	2	10b	9a	19a
2022	361b	19	15a	6b	21a
Year *P>F**	0.0095	0.1747	0.0016	0.0039	0.001
Genotype (-P,+P)[β](G)					
Farm1	609	17	10	7	16
Farm1	666	11	10	7	16
Red Fife	539	12	9	7	15
5602HR	431	10	10	6	16
Genotype *P>F**	0.3327	0.5218	0.9121	0.9832	0.9669
Coefficient of Variation (%)	13	92	65	65	53
Standard Error ±	131	3.6	1.2	0.8	1.6
Manure (M)					
(+)P	605	14a	9	7	16.4
(-)P	517	9b	10	6	15.9
Manure *P>F*	0.9283	0.0771	0.7735	0.3445	0.7825
Interactions *P>F*					
G x M	0.3812	0.0964	0.7544	0.9497	0.8774
G x Y	0.86	0.6652	0.9695	0.0744	0.6013
M x Y	0.8156	0.3474	0.8584	0.8199	0.9975
G x M x Y	0.3936	0.4477	0.9102	0.6795	0.7525
Farmer Genotypes Lsmeans	614	14	10	7	17
Parental Cultivars Lsmeans	472	10	10	7	17
Contrasts					
Farmer Genotypes vs. Parents *P>F*	0.1052	0.669	0.811	0.6364	0.684
Estimate	141	3.43	0.33	0.45	0.78
Farmer Genotypes vs. 5602HR *P>F*	0.0554	0.4509	0.8729	0.6175	0.8958
Estimate	217	4.5	-0.27	0.58	0.31
Farmer Genotypes vs. Red Fife *P>F*	0.4689	0.9497	0.5853	0.7851	0.5938
Estimate	72	2.3	0.93	0.32	1.25

*Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.10$)

[Ŧ]WEP, water extractable phosphate; [α]data natural log-transformed; [β](-)P, Limited P treatment; (+)P, P-amended treatment

Table 4-6. Lsmeans and combined analysis of variance comparing belowground microbial activity in 2020 collected under organic conditions in Libau, Manitoba among two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) under limited soil test Olsen-phosphorus (3ppm) and amended with composted manure at 25 kg P ha^{-1}

	Rhizosphere Acid Phosphatase	Rhizosphere WEP[Ŧα]	Arbuscule Infection	Hyphae	Arbuscules + Hyphae
Genotype (-P,+P)[β](G)	pmol MUF g^{-1} soil h^{-1}	mg P kg^{-1} soil	%	%	%
Farm1	746	11	6	3	9
Farm2	1289	17	4	3	7
Red Fife	680	15	5	6	10
5602HR	812	13	4	5	9
Genotype *P>F**	0.3967	0.751	0.4154	0.1826	0.3563
Coefficient of Variation (%)	12	57	50	60	41
Standard Error ±	229	4.4	1.3	0.7	1.1
Manure (M)			0.66	0.76	1
(+)P	1007	12	5	4.3	9
(-)P	757	16	4	4.1	8
Manure *P>F*	0.7136	0.7383	0.7276	0.8147	0.6857
					8
Interactions *P>F*					
G x M	0.2593	0.1434	0.4749	0.6244	0.3208
Farmer Genotypes Lsmeans	1034	15	5	3b	8
Parental Cultivars Lsmeans	804	14	4	5a	10
Contrasts					
Farmer Genotypes vs. Parents *P>F*	0.5364	0.7169	0.5166	0.026	0.2069
Estimate	230	0.56	0.6	-2.47	-1.8
Farmer Genotypes vs. 5602HR *P>F*	0.801	0.6872	0.3796	0.1331	0.5361
Estimate	156	1.5	1	-1.9	-0.97
Farmer Genotypes vs. Red Fife *P>F*	0.4536	0.8497	0.8593	0.0286	0.1305
Estimate	305	-0.4	0.2	-2.9	-2.7

*Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test (P ≤ 0.10)

[Ŧ]WEP, water extractable phosphate; [α]data natural log-transformed; [β](-)P, Limited P treatment; (+)P, P-amended treatment

Table 4-7. Lsmeans and combined analysis of variance comparing belowground microbial activity in 2021 collected under organic conditions in Libau, Manitoba among two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) under limited soil test Olsen-phosphorus (3ppm) and amended with composted manure at 25 kg P ha^{-1}

	Rhizosphere Acid Phosphatase	Rhizosphere WEP[Ŧα]	Arbuscule Infection	Hyphae	Arbuscules + Hyphae
Genotype (-P,+P)[β](G)	pmol MUF g^{-1} soil h^{-1}	mg P kg^{-1} soil	%	%	%
Farm1	655	5	10	12	22
Farm2	323	0	10	9	19
Red Fife	564	4	9	10	18
5602HR	221	0.4	10	7	17
Genotype *P>F**	0.4138	0.2712	0.9485	0.2156	0.7761
Coefficient of Variation (%)	14.5	238	62	47	49
Standard Error ±	196	3.8	1.2	0.6	0.9
Manure (M)					
(+)P	480	3	10	10	19
(-)P	401	2	10	9	19
Manure *P>F*	0.8873	0.2122	0.9538	0.6967	0.8856
Interactions *P>F*					
G x M	0.3413	0.7088	0.8651	0.6324	0.7637
Farmer Genotypes Lsmeans	489	3	10	10	21
Parental Cultivars Lsmeans	392	2	9	8	18
Contrasts					
Farmer Genotypes vs. Parents *P>F*	0.6583	0.673	0.7326	0.1565	0.3712
Estimate	96	0.4	0.73	2.26	2.9
Farmer Genotypes vs. 5602HR *P>F*	0.3202	0.6501	0.9849	0.0511	0.3511
Estimate	268	2.6	-0.05	3.8	3.8
Farmer Genotypes vs. Red Fife *P>F*	0.78	0.2579	0.5648	0.7363	0.5963
Estimate	-74	-1.2	1.5	0.64	2.2

*Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test (P ≤ 0.10)

[Ŧ]WEP, water extractable phosphate; [α]data natural log-transformed; [β](-)P, Limited P treatment; (+)P, P-amended treatment

Table 4-8. Lsmeans and combined analysis of variance comparing belowground microbial activity in 2022 collected under organic conditions in Libau, Manitoba among two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) under limited soil test Olsen-phosphorus (3ppm) and amended with composted manure at 25 kg P ha^{-1}

	Rhizosphere Acid Phosphatase	Rhizosphere WEP[Ŧα]	Arbuscule Infection	Hyphae	Arbuscules + Hyphae
Genotype (-P,+P)[β](G)	pmol MUF g^{-1} soil h^{-1}	mg P kg^{-1} soil	%	%	%
Farm1	421	32	14	6	20
Farm2	386	14	15	8	23
Red Fife	375	15	14	4	17
5602HR	262	14	16	8	23
Genotype *P>F**	0.2148	0.2281	0.8275	0.2986	0.4676
Coeff. of Variation (%)	6.2	54	42	57	35
Standard Error ±	200	3.8	1.2	0.6	0.9
Manure (M)					
(+)P	330	28a	14	7	21
(-)P	392	10b	15	6	21
Manure *P>F*	0.4756	0.0213	0.5455	0.2693	0.9113
Interactions *P>F*					
G x M	0.0617	0.2829	0.6117	0.6673	0.635
Farmer Genotypes Lsmeans	410	23	14	7	21
Parental Cultivars Lsmeans	327	15	15	6	21
Contrasts					
Farmer Genotypes vs. Parents *P>F*	0.1052	0.1361	0.9027	0.5534	0.8317
Estimate	83	8	-0.26	0.83	0.56
Farmer Genotypes vs. 5602HR *P>F*	0.0554	0.1489	0.5898	0.646	0.4918
Estimate	156	8	-1.4	-0.8	-2.2
Farmer Genotypes vs. Red Fife *P>F*	0.489	0.3198	0.7334	0.1599	0.3041
Estimate	9	7	0.92	2.5	3.3

*Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test (P ≤ 0.10)

[Ŧ]WEP, water extractable phosphate; [α]data natural log-transformed; [β](-)P, Limited P treatment; (+)P, P-amended treatment

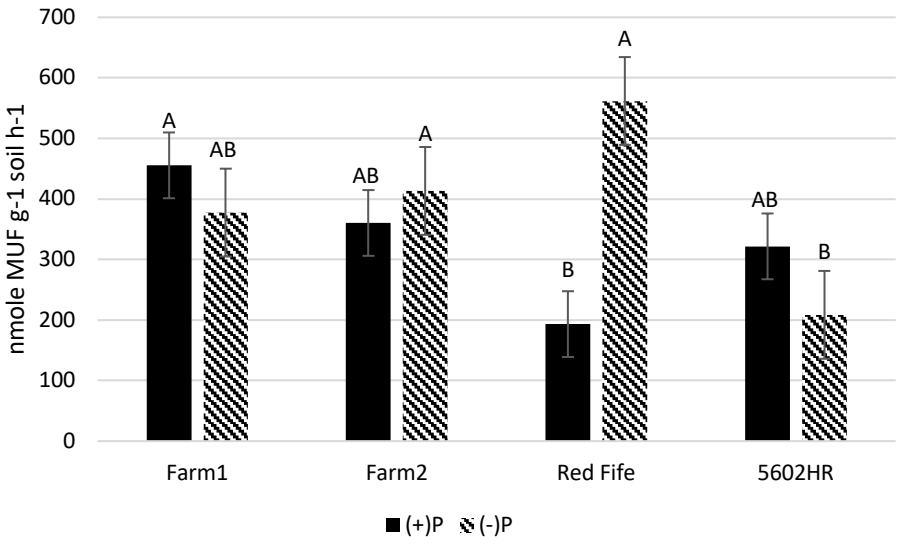


Figure 4-7. Rhizosphere acid phosphatase enzymatic activity genotype x manure interaction under organic conditions in Libau, Manitoba in 2022 among two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) under limited soil test phosphorus (3ppm), (-)P; and amended soil with composted manure at 25 kg ha^{-1}, (+)P.
Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.10$)

Table 4-9. Pearson's Correlation matrix of belowground microbial activity, yield, and phosphorus dynamics from three years (2020, 2021, 2022) conducted under organic conditions in Libau, Manitoba among two spring wheat cultivars and two spring wheat farmer genotypes under limited Olsen-P soil test status (3ppm)

(-)P	Yield	Total P Acc.	PUptE	Arbuscules + Hyphae	Rh PTase	Rh WEP
Yield	1	0.76495 <.0001	-0.2527 0.102	-0.022 0.5389	-0.10145 0.5389	0.2914 0.0579
Total P[a] Acc.[Ŧ]	0.76495 <.0001	1	-0.29 0.0547	0.102 0.5144	-0.067 0.6761	0.345 0.0234
PUptE[b]	-0.2527 0.102	-0.29 0.0547	1	-0.2241 0.1486	0.1348 0.4005	0.0787 0.6158
Arbuscules + Hyphae	-0.022 0.5389	0.102 0.5144	-0.2241 0.1486	1	-0.071 0.6639	0.07 0.6552
Rh PTase[c]	-0.10145 0.5389	-0.067 0.6761	0.1348 0.4005	-0.071 0.6639	1	0.469 0.0026
Rh WEP[d]	0.2914 0.0579	0.345 0.0234	0.0787 0.6158	0.07 0.6552	0.469 0.0026	1

[a]P, phosphorus; [Ŧ]Acc., accumulation; [b]PUptE, Phosphorus Uptake Efficiency; [c]Rh PTase; Rhizosphere Acid Phosphatase Activity; [d]Rhizosphere water-extractable phosphate.

Table 4-10. Pearson's Correlation matrix of belowground microbial activity, yield, and phosphorus dynamics from three years (2020, 2021, 2022) conducted under organic conditions in Libau, Manitoba among two spring wheat cultivars and two spring wheat farmer genotypes amended with composted manure at 25 kg P ha^{-1}

(+)P	Yield	Total P Acc.	PUptE	Arbuscules + Hyphae	Rh PTase	Rh WEP
Yield	1	0.8986 <.0001	-0.574 <.0001	0.007 0.961	0.081 0.6119	0.5530 <.0001
Total P[a] Acc.[Ŧ]	0.8986 <.0001	1	-0.6474 <.0001	0.1504 0.3296	-0.139 0.3841	0.582 <.0001
PUptE[b]	-0.0574 <.0001	-0.6474 <.0001	1	-0.3184 0.0351	-0.06 0.69	-0.402 0.0074
Arbuscules + Hyphae	0.007 0.961	0.1504 0.3296	-0.31844 0.0351	1	-0.161 0.3058	0.134 0.3854
Rh PTase[c]	0.081 0.6119	-0.139 0.3841	-0.06 0.69	-0.161 0.3058	1	0.131 0.4127
Rh WEP[d]	0.553 <.0001	0.582 <.0001	-0.402 0.0074	0.134 0.3854	0.131 0.4127	1

[a]P, phosphorus; [Ŧ]Acc., accumulation; [b]PUptE, Phosphorus Uptake Efficiency; [c]Rh PTase; Rhizosphere Acid Phosphatase Activity; [d]Rhizosphere water-extractable phosphate.

4.4.2.5.2. Rhizosphere water-extractable phosphate

Rhizosphere water-extractable phosphate (RhWEP) did not differ between Red Fife and 5602HR. There was a significant genotype x manure interaction (*P>F* 0.0964) (Table 4-5, Figure 4-8), however, the interaction was due to farmer genotype differences and not parental cultivar differences. RhWEP was significantly positively correlated with Total P accumulation under both fertility treatments, however, was only significantly positively correlated with yield under the P-amended treatment (Table 4-9 and Table 4-10). Interestingly, PUptE was negatively related to RhWEP when manure was added (Table 4-10). This may indicate an increase in P uptake speed as the plant root creates depletion zones.

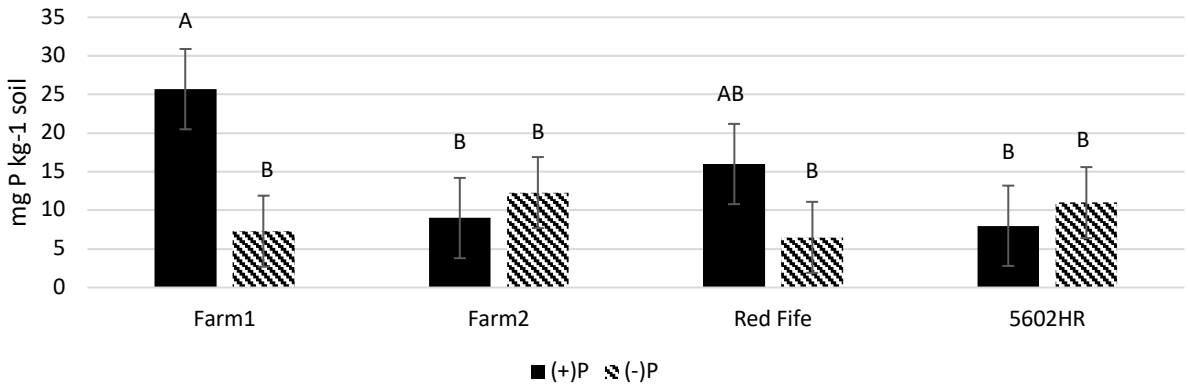


Figure 4-8. Rhizosphere water-extractable phosphate genotype x manure interaction under organic conditions in Libau, Manitoba in 2020, 2021, and 2022 among two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) under limited soil test phosphorus (3ppm), (-)P; and amended soil with composted manure at 25 kg ha^{-1}, (+)P.
Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.10$)

4.4.2.5.3. Arbuscular mycorrhizal colonization

Arbuscular mycorrhizal colonization rates ranged from 21% in 2022 to 9% in 2020 (Table 4-5). Colonization rates were similar to Singh et al. (2012), who reported Canadian durum wheat colonization rates ranging from 6-15% under medium fertility and 10-30% under low fertility in conventional management. Working under organic management, Kirk et al. (2011) compared historical and modern spring wheat cultivars and stated colonization rates that ranged between 9.9-18.2%. Red Fife and 5602HR did not significantly differ in arbuscule colonization, hyphae, or total colonization (Table 4-5). There was a significant genotype x year interaction (Table 4-5, Figure 4-9). However, the interaction did not derive from parental differences. Our results are in agreement with Kirk et al. (2011), who evaluated AMF colonization between Red Fife and 5602HR, and reported that 5602HR and Red Fife colonization were statistically similar. It should be noted that 5602HR had numerically higher colonization than Red Fife, and the author found that modern spring wheat cultivars had higher rates of infection than landrace cultivars under organic management (Kirk et al., 2011).

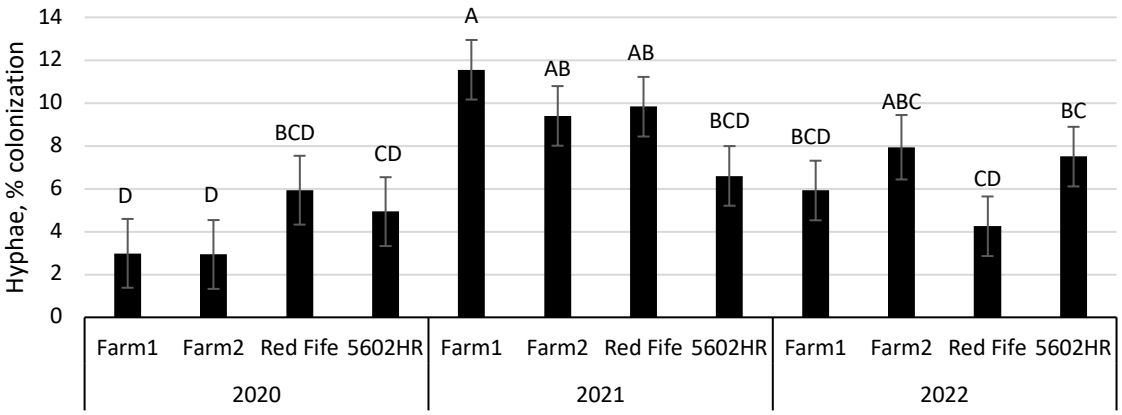


Figure 4-9. Hyphae percent colonization, genotype x year interaction under organic conditions in Libau, Manitoba in 2020, 2021, and 2022 among two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) under limited soil test phosphorus (3ppm), (-)P; and amended soil with composted manure at 25 kg ha^{-1}, (+)P.
Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test (P ≤ 0.10**)**

4.4.3. Farmer genotype deviation from parental material

4.4.3.1. Straw P concentration and accumulation

There were no differences in anthesis and mature P straw concentration and accumulation when the farmer genotypes (Farm1 and Farm2) were contrasted with the parental cultivars (Table 4-1). Nicksy et al. (2022) reported a PPB farmer genotype had higher anthesis P concentration and uptake compared to a popular modern wheat cultivar (AAC Brandon). No interactions among genotype x manure, genotype x year were shown, however, there was a significant genotype x manure x year interaction (Figure 4-1).

Farmer genotypes were more responsive to the P-amended treatment than Red Fife, but not as responsive as 5602HR (Figure 4-1). Under limited P conditions, farmer genotypes' straw P concentrations were lower than Red Fife, but similar to Red Fife under amended P treatments.

No genotypic differences were detected in straw biomass accumulation under limited P treatments (P>F 0.6897; Farm1: 5870 kg ha^{-1}; Farm2: 6173 kg ha $^{-1}$; Red Fife: 5836 kg ha^{-1}), nor did greater concentration result in greater plant P uptake (Table 4-1). Our results do not agree with Ozturk et al.

(2005) who reported straw dry weight and straw P concentration were negatively correlated. Generally, the farmer genotypes' straw P concentrations under two different fertility treatments were more similar to 5602HR, the modern cultivar, than the landrace cultivar, Red Fife.

4.4.3.2. Grain P concentration and accumulation

Across all years and fertility treatments, Farm1 was similar to 5602HR's grain P concentration, whereas Farm2 was similar to Red Fife's grain P concentration (Table 4-1). There was a significant genotype x manure x year interaction (Figure 4-2). The source of interaction was due to 5602HR's greater grain P concentration in 2020 than any other genotype. In 2022, under limited P conditions, Farm1 had significantly greater grain P concentration than Red Fife and was similar to 5602HR. Farm2 had similar grain P concentration to both Red Fife and 5602HR. Under P-amended conditions, farmer genotypes were similar to both parents.

Grain P concentration differences did not result in significant differences among farmer genotypes and the parental cultivars (Table 4-1). There were no genotype x manure, genotype x year, or genotype x manure x year interactions. Grain yield was positively correlated to grain P concentration under limited P (*P>F* <.0001; r = 0.6774) and P-amended (*P>F* <.0001; r = 0.7906) treatments. This positive correlation between grain P concentration and yield could indicate general P deficiency in the current study under both fertility treatments given how dry 2020 and 2021 were. However, when moisture was adequate and under P-amended treatments in 2022 (yield ranging from 2994-3298 kg ha^{-1}), there was a significant positive relationship between grain P concentration and yield (*P>F* 0.0344, r = 0.53079), indicating that greater grain P concentrations were positively correlated with yield in this study. Our results do not agree with Manske et al. (2001), who reported a negative relationship between grain yield and grain P concentration among wheat genotypes tested in Mexico under both amended and limited P conditions. Working under limited soil P organic conditions in wheat, Nicksy et al., (2022) reported a positive linear-

plateau relationship between grain yield and grain P concentration. The relationship between grain yield and grain P concentration plateaued at approximately 3500 kg ha^{-1} (a maximum of 3248 kg ha^{-1} was observed in the present study). Therefore, in the current experiment, farmer genotypes maintained parental cultivar characteristics of maintaining high P concentration and high yield simultaneously throughout the selection process, however, we did not observe high enough yield to reach a yield-grain P concentration relationship plateau. Ideally, organic cultivars would possess traits that are able to achieve high yields along with low grain P concentration in order to reduce off-farm P export as harvest (Rose et al., 2013; Carkner et al., 2023).

4.4.3.3. Total plant phosphorus accumulation

There were no significant differences between farmer genotypes and parents across all years and fertility treatments (Table 4-1). There were no genotype x manure or genotype x manure x year interactions. There was a significant genotype x year interaction. All genotypes had similar total P accumulation values in all years except in 2022 (Figure 4-3).

In 2022, Farm1 had significantly greater total P accumulation than Red Fife by 1.8 kg ha^{-1}, and numerically greater total plant P accumulation than 5602HR by 1 kg ha^{-1} (Table 4-4, Figure 4-3). Farm2 had similar total plant P accumulation to both parents. Total plant P accumulation values in 2022 were slightly greater than other reported studies under organic management in Canada (Thiessen Martens et al., 2021; Nicksy et al., 2022), but those studies were conducted in drier seasonal conditions. Greater total P accumulation by Farm1 demonstrates the potential for farmer selection to result in genotypes that possess traits that contribute to greater P accumulation under multiple fertility levels in favourable organic environmental conditions.

4.4.3.4. Phosphorus Uptake and Partitioning Dynamics

4.4.3.4.1. Phosphorus uptake efficiency

Farmer genotypes' PUptE ranged from 55% (Farm2) to 57% (Farm1) when data from limited P and P-amended fertility treatments were combined (Table 4-1). There were no differences observed when farmer genotypes were compared to parents as a group or when parents were contrasted individually. No genotype x manure, genotype x year, or genotype x manure x year interactions were detected.

In 2022, under P-amended treatments, genotypes did not significantly differ from each other and PUptE ranged between 41 to 48% (Figure 4-4). Phosphorus uptake efficiency values are greater than other studies conducted under field conditions (Manske et al., 2001), and similar to studies conducted hydroponically (Osborne and Rengel, 2002). However, under limited P conditions in 2022, PUptE ranged from 96 to 128% (Table 4-4). Farm2 had significantly lower PUptE than Red Fife (by 13%) but did not differ from 5602HR. Farm1 had significantly greater PUptE than both Red Fife and 5602HR by 19% and 24%, respectively. This study demonstrates the potential for: a) genotypic variation in PUptE under low-P organic conditions, and b) the ability for farmers to select a genotype for greater PUptE than both parents under high moisture, organic conditions. Given that greater PUptE has been examined as only a 'response' variable to added fertility, and many breeding programs value increased biomass/yield in response to greater fertility, it is not surprising that a farmer genotypes could have greater PUptE than Red Fife and 5602HR. Further research should focus on the crop traits that drive greater PUptE in organic production systems, and towards assessing the range of greater PUptE is possible under organic conditions.

4.4.3.4.2. Phosphorus harvest index

There is evidence that lowering PHI while maintaining yield is possible; as carbon assimilates and P were observed to move into the grain independently of one another (Peng and Li, 2005). Farmer genotypes were not significantly different from parents for PHI (Table 4-1). There were no genotype x

manure or genotype x year interactions. There was a genotype x manure x year interaction, however, the interaction derived from 5602HR's inconsistent PHI response to manure from year to year (Figure 4-5). This indicates that farmer selection did not influence changes to the PHI in this study. Multiple authors have proposed that a low PHI would be beneficial as a breeding goal for wheat in organic production systems, as less P would leave in the form of harvest, but greater P would be left behind in the biomass for subsequent crops (White and Veneklaas, 2012; Rose et al., 2013; Carkner et al., 2023).

Yield and PHI were negatively correlated under both fertility treatments (P-amended; Corr: -0.407, P>F 0.0061; Limited P; Corr: -0.344, P>F 0.0235), indicating that when genotypes had higher yield potential, the PHI reduced, however, lower PHI did not result in lower grain P concentration. In combination with differing PHIs under varying P availability, it appears that a pre-determined amount of P translocates from tissues into grain. The pre-determination may be set when yield is set; PHI did not change among P-amended and limited P conditions but did change among years. This agrees with El Mazlouzi et al. (2020b), who demonstrated that P allocation into grains was dependent on biomass P status, 72% and 56% of exogenous P post-anthesis uptake was translocated into the grain under deficient-P, and high-P biomass status, respectively. Despite potential for lower PHI, we were unable to achieve this through farmer selection on organic farms. This may indicate that *luxurious* P supply impacts PHI, El Mazlouzi et al. (2020b)'s work shows that plants only translocate a maximum amount of P into the seed, and after the maximum is reached, at some point stops translocation.

4.4.3.4.3. Phosphorus yield efficiency

Farm2 had significantly greater PYE than 5602HR by 40kg ha^{-1} when data was combined across all years and fertility treatments (Table 4-1). Farmer genotypes were not significantly different from Red Fife, however, Farm2 was numerically greater than Red Fife by 11 kg ha^{-1}. Farm1 was not significantly different

from Red Fife or 5602HR. There were no genotype x manure, genotype x year, or genotype x manure x interactions.

There were no significant differences between farmer genotypes and parents in grain yield (Chapter 3, Table 3-4), or total P accumulation (Table 4-1). However, Farm2 yielded numerically greater than all other genotypes, and accumulated slightly less total P. Additionally, in 2020, Farm2 was significantly more efficient than 5602HR by 88 kg ha^{-1} of grain per unit of P taken up (Table 4-2). No differences were observed in 2021 or 2022 (Table 4-3 and Table 4-4). In summary, Farm2 was significantly more efficient at translating P taken up into grain yield than the modern parent, 5602HR.

4.4.3.4.4. Phosphorus return efficiency

It would be valuable for a genotype in low-P organic conditions to take up plentiful P but leave it in the biomass resulting in a larger PRE (Carkner et al., 2023). No differences in PRE were observed between farmer genotypes and parents (Table 4-1). There were no genotype x manure, genotype x year, or genotype x manure x year interactions. Farm2 left behind numerically lower amount of P in the biomass per unit of grain. This may be because Farm2 also accumulated the least amount of total plant P (Table 4-1) and yielded marginally more than the other genotypes (Chapter 3, Table 3-4). Prior work has demonstrated that under low-P supply, wheat remobilizes a greater (81%) amount of stored P in the spikelets and leaves towards grain P than under high-P (65%) (El Mazlouzi et al., 2020a). Internal P remobilization is controlled by multiple families of phosphate transporter genes (Roch et al., 2019). Initiatives investigating the potential to reduce P remobilization into the grain under low-P supply would be extremely valuable for long-term sustainable P supply on organic farms. Less P would be exported off farm in the form of harvest and leave more P on farm in the form of unharvested straw.

4.4.3.4.5. Grain N:P ratio

Farm2's grain N:P ratios were statistically similar to both parents, falling in the middle (Farm2, 5.3; Red Fife, 4.6; 5602HR, 6.2). Farm1 had similar ratios to 5602HR, and greater (1.1) ratios than Red Fife. Farm1 had significantly greater protein and greater grain P concentration than Red Fife (Chapter 3, Table 3-4). There was a significantly genotype x year interaction (Figure 4-6). In the driest year, Libau 2021, both Farm1 and 5602HR maintained high grain N:P ratios to a greater degree than Farm2 and Red Fife.

An ideal genotype would have high protein, and low grain P concentration, and therefore, have a larger grain N:P ratio. High protein and low grain P concentration was not achieved in this study. Often times the genotypes with the larger ratios also possessed greater grain P concentration (e.g. 5602HR), however, it is interesting that Farm1 achieved greater values than Red Fife. This was probably due to Farm1's significantly greater protein concentration than Red Fife (Chapter 3, Table 3-4) and grain P concentration that were not proportionately higher than Red Fife (Table 4-1), which is positive in accordance to the low-P ideotype proposed in Chapter 1 (Carkner et al., 2023).

4.4.3.5. Belowground microbial activity

4.4.3.5.1. Rhizosphere acid phosphatase enzyme activity

Despite differences among years, no differences were detected between farmer genotypes and parental cultivars for APase activity. No genotype x manure, genotype x year, or genotype x manure x year interactions were observed (Table 4-5). When farmer genotypes were contrasted with 5602HR, farmer genotypes had significantly greater (*P>F* 0.0554) APase activity by 217 pmol MUF g^{-1} soil h^{-1}. Similar results were observed when farmer genotypes were contrasted with 5602HR in 2022. In 2022, where there was a significant genotype x manure interaction, but the source of the interaction was due to Red Fife (Figure 4-7). Farmer genotypes were more similar to Red Fife's phosphatase activity than 5602HR. Under favourable conditions, farmer genotypes demonstrated greater APase activity in the rhizosphere than the

modern parent. While genetic variation in APase among wheat has been observed (Meier et al., 2023), the heritability of APase is complex (Bovill et al., 2013). However, the discovery of a candidate gene *GMACP1* encoded an acid phosphatase in soybean (Zhang et al., 2014) and the identification of the purple acid phosphatase gene *TaPAP16* in wheat (Deng et al., 2018) demonstrate the potential to breed for greater APase activity.

4.4.3.5.2. Rhizosphere water-extractable phosphate

RhWEP between farmer genotypes and parental cultivars were not significantly different across all three years and fertility treatments. There were no genotype x year or genotype x manure x year interactions (Table 4-5). There was a significant genotype x manure interaction (Figure 4-8).

Farm1 had significantly greater RhWEP values under amended P treatments than 5602HR but was not significantly different than Red Fife (Figure 4-8). Greater RhWEP may indicate less rapid uptake of readily available P under amended P conditions than other genotypes, or, greater P mobilization occurring. Farm1 demonstrated greatest total P accumulation in 2022 that may indicate greater P mobilization ability under adequate moisture conditions (Table 4-4). However, multiple plant-induced and environmental factors contribute to greater RhWEP such as soil pH, anion/cation balance, gaseous exchanges, and organic acid exudation (not measured here) (Hinsinger, 2001).

4.4.3.5.3. Arbuscular mycorrhizal colonization

No genotypic differences were observed between farmer genotypes and parental cultivars for arbuscule, hyphae, or total colonization combined over three years and two fertility treatments (Table 4-5). No genotype x manure, genotype x year, or genotype x manure x year interactions were detected for arbuscules and total colonization. A significant genotype x year interaction for hyphae was observed (*P>F* 0.0744*)* (Table 4-5). There were no differences among all genotypes in 2020 and 2022 (Figure 4-9). However, Farm1 had significantly greater hyphal numbers than 5602HR in 2021. The genotypes

experienced drought in 2021 (Chapter 3, Table 3-2), there is evidence that AMF extraradical hyphal networks can aid the host crop to access moisture and nutrients during drought conditions (Abdalla et al., 2023). However, it should be noted that there were low colonization rates overall in 2021.

4.4.4. Performance between farmer genotypes

4.4.4.1. Straw phosphorus concentration and accumulation

There were no differences between farmer genotypes for straw P concentration and accumulation at anthesis and maturity developmental stages (Table 4-1). No genotype x manure, or genotype x year interactions were detected, but there was a significant genotype x manure x year interaction. However, the source of interaction was due to 5602HR, the farmer genotypes responded to manure addition similarly to each other in every year (Figure 4-1).

4.4.4.2. Grain phosphorus concentration and accumulation

No significant differences or interactions were detected among farmer genotypes for grain P accumulation (Table 4-1). There was a significant genotype x manure x year interaction, but the interaction was due to 5602HR's increased fertility response and not farmer genotype differences (Figure 4-2). Under adequate moisture conditions in 2022, Farm1 had significantly greater grain P concentration than Farm2 when manure treatments were combined (Table 4-4). But there was no interaction detected, indicating that Farm1 had greater grain P concentration under limited and P-amended treatments than Farm2.

Greater grain P concentration would have been coincidental in the selection process. Nicksy et al. (2022) postulated that wheat genotypes selected under low-P conditions may have led to greater allocation towards grain P concentration, due to its role in early seedling establishment (Derrick and Ryan, 1998; Yugandhar et al., 2022). Selection environment of Farm1 was not low in STP (Chapter 3, Table 3-1). Greater grain P concentration is not necessarily a positive trait, as more P may be exported off-farm

through harvest, that would deplete on-farm P on low-P organic farms (Carkner et al., 2023). Still, little is known how organic selection environments impact cultivar grain P allocation of cultivars. More research is needed to examine how the selection environment impacts grain P concentrations if organic breeding environments will be used.

4.4.4.3. Total plant phosphorus accumulation

A significant genotype x year interaction was observed, due to accumulation differences in 2022 (Figure 4-3). Farm1 accumulated significantly greater total plant P than Farm2 by 1.8 kg ha^{-1} in 2022 (Table 4-4). There was no genotype x manure interaction in 2022, indicating that the genotypes responded to the P-amended treatment to a similar degree. Farm1 and Farm2 were not significantly different from each other under P-amended conditions (*P>F* 0.1091; Farm1, 16 kg ha^{-1}; Farm2, 14 kg ha^{-1}). However, Farm1 took up significantly more P under limited P conditions than Farm2 (*P>F* 0.0105; Farm1: 9.2 kg ha^{-1}; Farm2: 6.9 kg ha^{-1}). It is not clear why Farm1 had greater P accumulation than Farm2. There were no significant differences between genotypes for rhizosphere phosphatase activity, AMF colonization or RhWEP (Table 4-8). Total P accumulation and RhWEP were significantly positively correlated (Table 9; *P>F* 0.0234, r = 0.345), which may indicate that Farm1 was able to cultivate greater RhWEP. Other belowground traits have been attributed to greater P accumulation such as organic acid and proton exudation, or cultivating P-solubilizing bacteria in the rhizosphere that we did not evaluate (Vance et al., 2003; Singh Gahoonia and Nielsen, 2004; Park et al., 2009; Nguyen et al., 2019; Yahya et al., 2021).

4.4.4.4. Phosphorus uptake and partitioning dynamics

4.4.4.4.1. Phosphorus uptake efficiency

The only significant genotypic difference or genotype x manure interaction was observed in 2022 (Table 4-1).

Farm1 and Farm2 did not differ from each other under P-amended treatments, 48% and 44%, respectively (Figure 4-4). Yet, under limited P conditions, Farm1 had greater PUptE than Farm2 by 32% (Figure 4-4). Therefore, our results demonstrate that genotypic variation exists for PUptE under limited P conditions, and this trait was indirectly selected for by one of the participating farmers.

4.4.4.4.2. Phosphorus harvest index

There were no genotypic differences between Farm1 and Farm2 for PHI when all years and fertility treatments were combined (Table 4-1). No genotype x manure or genotype x year interactions were observed. There was a significant genotype x manure x year interaction, but the interaction was the product of 5602HR's PHI, which decreased in 2020 under limited P conditions (Figure 4-5). Although multiple studies have reported genotypic variation in PHI (Yaseen and Malhi, 2009; Soumya et al., 2021), we did not observe similar results in our study among farmer genotypes.

4.4.4.4.3. Phosphorus yield efficiency

Although significant genotypic differences were observed for PYE when fertility treatments and years were combined, Farm1 and Farm2 did not differ from each other (Table 4-1). No genotype x manure, genotype x year, and genotype x manure x year interactions were shown. Farm1 and Farm2 did not significantly differ from each other in grain yield when data was combined (Chapter 3; Table 3-4), or when years were separately analyzed (Chapter 3; Table 3-5, Table 3-6, Table 3-7). Nonetheless, in 2022, Farm1 accumulated significantly greater total P than Farm2 (Table 4-4, Figure 4-4). PYE was not significantly different between farmer selections in 2022 either (Table 4-4). Regardless, it's worth noting that although Farm2 took up significantly less P, it yielded similarly to Farm1. It seems that farmer selections utilizing different pathways towards generating similar yield. Farm1 is accumulating excess P, and Farm2 is expressing greater phosphorus yield efficiency. Farm1 allocated a similar percentage of total plant P to the grain as Farm2, meaning that Farm1 also exported more P off the farm in the form of yield (Table 4-7).

4.4.4.4.4. Phosphorus return efficiency

Farm1 and Farm2 did not significantly differ from each other for PRE (Table 4-1). There were no genotype x manure, genotype x year, or genotype x manure x year interactions detected. Phosphorus return efficiency values represent the interplay between yield, P uptake, and PHI.

4.4.4.4.5. Grain N:P ratio

There were significant genotypic differences in Grain N:P ratio, however, Farm1 and Farm2 did not differ from each other (Table 4-1). There was a significant genotype x year interaction that derived from differences among genotypes in 2021 (Figure 4-6).

Under drought conditions in 2021, Farm1 had greater grain N:P ratio values than Farm2 by 2.3 (Figure 4-6). Farmer genotypes did not differ from each other in any other year. Therefore, under dry conditions, Farm1 had greater capacity to generate greater protein as a ratio of the grain P. Protein content was not a trait the farmers were actively selecting for; therefore, these results were coincidental.

4.4.4.5. Belowground microbial activity

4.4.4.5.1. Rhizosphere acid phosphatase enzyme activity

Farmer genotypes did not significantly differ from each other for rhizosphere APase when years and fertility treatments were combined (Table 4-5). No genotype x manure, genotype x year, or genotype x manure x year interactions were observed. Therefore, different selection environments and farmer selectors did not impact rhizosphere APase activity under a range of environmental conditions in this study.

4.4.4.5.2. Rhizosphere water-extractable phosphate

When years and fertility treatments were combined, no significant differences were detected between farmer genotypes for RhWEP (Table 4-5). No genotype x year, or genotype x manure x year interactions were observed. However, a significant genotype x manure interaction revealed Farm1 had a

significantly greater RhWEP under P-amended treatments than Farm2 (Figure 4-8). Farmer genotypes did not differ from each other under limited P treatments. Greater RhWEP under the P-amended treatment is intuitive, as more inorganic P is available for roots take up than under limited P supply. However, it is interesting that Farm2 had similar amounts of RhWEP under both manure treatments (Figure 4-8). It is not clear if lower RhWEP was due to more rapid uptake by Farm2, or due to greater mineralization potential by Farm1. Regardless, RhWEP was significantly positively associated with yield (*P>F* <.0001, r = 0.5530) (Table 4-10), indicating that greater RhWEP is a positive characteristic regarding productivity, although farmer genotypes did not yield significantly different from each other. Farmer selection seemed to have an impact on RhWEP, especially under adequate moisture conditions of 2022. Other studies have investigated the impact of phosphorus rhizosphere dynamics among historical and modern genotypes (Robertson-Albertyn et al., 2017; McGrail et al., 2023), but research exploring how participatory breeding genotypes differ is lacking.

4.4.4.5.3. Arbuscular Mycorrhizal Colonization

Farmer genotypes did not differ from each other in arbuscule, hyphae, or total colonization when all years and fertility treatments were combined (Table 4-5). No significant interactions were observed in genotype x manure or genotype x manure x year. There was a significant genotype x year interaction, however, the interaction was an artifact of parental differences with farmer selections (Figure 4-9).

4.5. Conclusion

Using genotypes from a Canadian organic wheat PPB program, we evaluated the feasibility of the Low-P Wheat Ideotype suggested by Carkner et al. (2023). The first objective of this study was to evaluate two parental cultivars used to generate genotypes for the PPB program. Given that greater production response to greater nutrient supply has been a prominent breeding goal, it is not surprising that the modern cultivar, 5602HR, was more responsive to added manure than Red Fife in total P accumulation.

However, despite taking up less P, Red Fife yielded similarly and demonstrated greater PYE. The parental cultivar, 5602HR, showed greater grain N:P ratios than Red Fife, providing evidence that greater grain protein in partnership with lower grain P is possible, especially among high protein modern hard red spring wheat cultivars. Results of this study indicate that a combination of greater total P accumulation (5602HR) and greater PYE (Red Fife) were acceptable parental choices for our study.

The second objective was to investigate how farmer genotypes differed from their parents. Farm1 resembled 5602HR more than Red Fife for PYE, and Farm2 reflected Red Fife. Farm1 accumulated more total plant P and revealed greater PUptE than both parental cultivars under favourable, limited P conditions in 2022. Multiple belowground traits contribute to greater PUptE, however, the biological parameters we investigated did not significantly contribute to greater PUptE. Additionally, we observed no genotypic differences in root biomass or architecture in our greenhouse trials (Appendix; Table C-6 and Table C-7). More research is needed to investigate what traits enabled Farm1 to achieve high P accumulation and PUptE values. This research illustrates that greater PUptE under organic limited P soil conditions can be facilitated through a participatory plant breeding model. Despite greater PUptE, this did not result in greater PYE for Farm1.

The last objective was to evaluate the impact geographically divergent farmers and their respective environments had on full sibling derived genotypes. Despite greater P uptake, farmer genotypes yielded similarly, resulting in Farm2 having greater PYE than Farm1. Two different mechanisms may be occurring in regards to the farmer genotypes. Farm1 may maximize P uptake, but is 'wasteful' with the P, and Farm2 may take up less P, but is more efficient. Greater P accumulation and PUptE under limited P conditions in 2022 may be the product of the selection environment, as Farm1's growing conditions experience significantly greater precipitation than Farm2. More research is needed to examine

what the underlying mechanisms contribute to greater P uptake and P yield efficiency among PPB wheat genotypes as well as the wider Canadian wheat germplasm.

Breeding for greater P uptake and yield efficiency and the associate traits has never been a breeding target in Canadian breeding programs. Our results provide evidence that strategies in response to limited P conditions differ among genotypes, especially under better growing conditions (high moisture conditions in our 2022 experiment). This research demonstrates that selection under organic conditions may facilitate greater P uptake traits, however, translating greater P uptake into greater yield efficiency remains a challenge, and further explored with a different PPB family in Appendix C. Given the nature of the PPB program and selection criteria, any genotypic differences we observed were coincidental. Coincidental results are not efficient enough for breeding programs, given the time and expense to run them. There is potential to investigate phosphate transporters that facilitate greater P uptake under low available P in combination with greater internal P economy to maintain yield (Bovill et al., 2013; López-Arredondo et al., 2014; Zhang et al., 2019). Using parents with differential organ-specific expression of P transporters, mycorrhizal-specific P transporters in combination with the PPB model may provide a wheat breeding stream that combines greater genetic diversity in combination with greater selection environment diversity that may unlock potential for low P organic production system.

CHAPTER 5. GENERAL DISCUSSION AND CONCLUSIONS

5.1. Organic and participatory plant breeding

The following discussion will categorize considerations learned from this body of work for organic plant breeders and breeding programs from the three essential components of a plant breeding program (Ceccarelli, 2015):

1. Creating genetic diversity
2. Selection and testing to identify superior recombinants
3. Release, distribution, and adoption of new cultivars

A conceptual illustration (Figure 5-1) synthesizes and outlines remaining questions and opportunities for organic and participatory wheat breeding schemes:

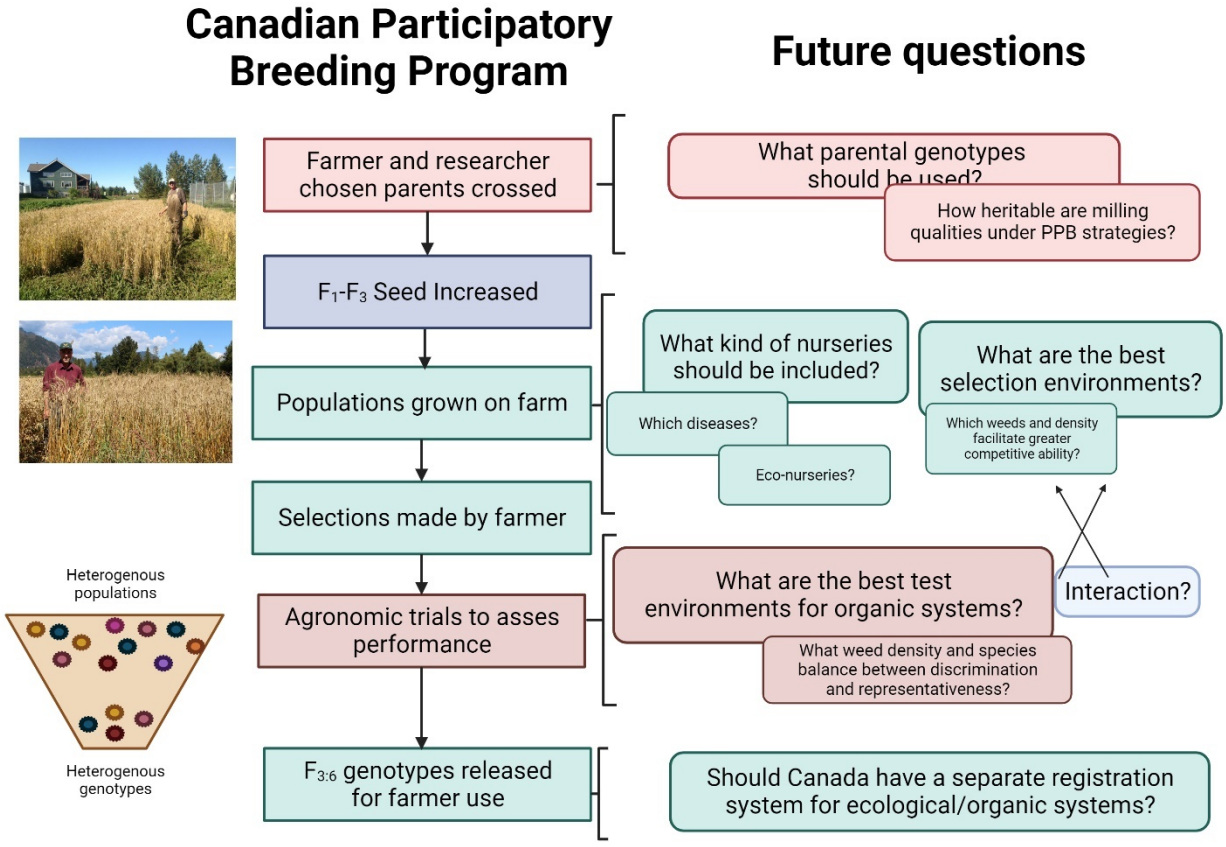


Figure 5-1. An illustrative guide for future research needs related to participatory and organic breeding programs. This image was created in BioRender. Photo credit: Michelle Carkner.

5.1.1. Creating genetic diversity

5.1.1.1. Parental Selection

Many parental cultivars (except landrace cultivars) used in the Canadian PPB program were within the Canadian Western Red Spring (CRWS) class. The CWRS class is known for its high yield, high protein, and excellent milling qualities. While centralized organic breeders who aren't selecting for the CWRS class may have more freedom combining parents with high- and low-quality attributes, this research has demonstrated that breeders interested in participatory breeding need to choose parental material where both parents are of moderate to high milling quality to ensure marketability, even if the goal is not for the CWRS class. Murphy et al. (2005) suggests using parental material that includes modern cultivars and breeding genotypes, material adapted to local environments or similar environments, landraces produced prior to the agricultural industrial revolution, and unadapted exotic germplasm that may contain traits or gene of interest. Other PPB programs have chosen a similar route, using modern breeding genotypes with superior disease resistance, and locally adapted landraces (Goldringer et al., 2020). Recently, a PPB program in midwestern USA specifically chose parents that were agronomically superior and had desirable artisanal (eg. Naturally leavened bread) baking qualities (Sandro et al., 2022). Canadian PPB programs need to consider the heritability of quality for both parents. Both farmer selection genotypes from Chapter 3 (Family 1) maintained protein of the modern parent and had even higher protein than the landrace. However, in Family 2, not all farmer genotypes reach protein levels comparable to the high protein parent, Norwell, and were not significantly higher than the lower protein parent, AAC Scotia (Appendix, Table B-1). Other breadmaking qualities such as falling number and gluten strength were not measured, therefore future research assessing how heritable other breadmaking qualities are in a PPB program would be valuable. Because farmers were selecting spikes in accordance with phenotypic traits they could observe, ensuring the background marketing qualities of a cross is essential.

Similar parental considerations are imperative for certain disease and insect resistance. Results from Chapter 2 clearly demonstrate the importance of incorporating insect resistance genes into cultivars for organic production systems. Most of the farmer genotypes and registered checks that demonstrated high yield and yield stability either possessed *Sm*1 gene, or its parental material possessed *Sm1* gene. Orange blossom wheat midge is a very small insect, and midge damage is very difficult to select against if farmers were not specifically scouting for it, or don't have the time to scout for it. Fusarium head blight (FHB), (*Fusarium graminearum*) is also of specific concern for Canadian organic wheat production (McCallum and DePauw, 2008). FHB has the potential to reduce wheat yields and produce mycotoxins rendering the grain toxic to humans. Mycotoxins impact local food supply as blending may not be accessible for direct-marketed grain. Centralized organic breeding programs and PPB programs on the Canadian prairies are recommended to incorporate wheat midge and FHB resistance into their genotypes, and specifically scout for orange wheat blossom midge.

5.1.1.2. Relevant genes for farming systems with low available P

Quantitative trait loci (QTL) studies in wheat have demonstrated that phosphorus uptake and yield efficiency is a complex, polygenic trait (Su et al., 2009). Phosphorus uptake and yield efficiency are associated with Pht genes that encode high-affinity phosphate transporters. Phosphate transporters belong to four families: Pht1, Pht2, Pht3, and Pht4. The majority of phosphate transporter research in wheat takes place within the Pht1 family, which are predominantly expressed in epidermal cells and the outer cortex of the root (Smith et al., 2003; Teng et al., 2017). The Pht1 gene family has been shown to be phosphate uptake mediators within the root-soil interface when available P is low. They are involved in the direct phosphate uptake pathway, taking up P against a concentration gradient (López-Arredondo et al., 2014). Pht1 genes have also been identified in leaves, stems, cotyledons, pollen grains, seeds, and flowers, indicating that the gene family is also involved in root-shoot distribution and remobilization.

Other gene families, Pht2, Pht3, and Pht4 are in the plastids, mitochondria, and endomembranes, respectively (Roch et al., 2019).

Multiple inducible Pht1 genes have been identified and classified for wheat. More specifically, TaPHT1 subgroups have been identified to correlate with greater P acquisition at low-P by changing the root morphology (Teng et al., 2017), and root exudates (Aziz et al., 2014), and inducible AMF colonization under low P supply (Zhang et al., 2019), and P-mobilization and uptake activated by *TaPHT1* genes in the aleurone tissues (Shukla et al., 2016).

Of interest to low-P ideotype breeding, Bhati et al. (2016) were able to reduce seed phytate by silencing the *ABCC13* transporters however, it should be noted that the spikes were developmentally defective due poor seed fill and number of spikelets. The authors report that although the seeds had delayed emergence, seedling viability was unaffected. The ability to reduce seed phytate genetically is encouraging, however, the authors did not note the *vigour* of the seedling, which is a valuable trait to organic farming systems. For example, Julia et al. (2018) argues that poor early growth vigour associated with low-P that is often observed (including my observations within the study in Appendix C3) is an artifact of deficient plants, and not due to the low phytate content in the seed. Taken together, there is potential for lowering the P Harvest Index while maintaining speedy emergence and high vigour.

Our knowledge of genomic connections with phenotypic traits that are valuable for organic production systems is growing every day (Semagn et al., 2022). Organic agricultural systems would benefit from the ability to alter specific genes to cope with current biotic and abiotic challenges. New gene editing tools such as CRISPR (Clustered Regularly Interspaced Repeats) are enabling greater affordability and editing specificity. However, there is a strong possibility that organic farmers and consumers of organic goods could identify gene-edited plants as Plants with Novel Traits and reject the technology.

Communication and transparency with farmers and the public is essential if genomic technologies are to be adopted in organic plant breeding.

5.1.2. Selection and testing to identify superior recombinants

5.1.2.1. Scouting and Rogueing

Scouting, tagging, and rogueing plants within a population was a challenge for farmers to set aside time for throughout the season in the Canadian PPB program. Depending on the trait, when scouting is required, it is often at the busiest time of year (eg. Early season vigour in the spring or selecting for early maturity around harvest). Dedicated plant breeders visit their breeding genotypes as many as once or twice a week during the growing season (Duncan, 2023; pers. comm.), which would be challenging for farmers to achieve. Skeptics of the PPB model may argue that farmers do not have the time for adequate scouting throughout the season. Although, despite this, farmer selection did result in genotypic differences in traits that were observable (eg. Height, yield; Chapter 2), and unobservable (eg. Phosphorus uptake efficiency, Chapter 4). Therefore, it is possible that the selection *environment* rather than the *selector* was the driving force behind genotypic differences in this study. Other work has reported differences among genotypes that were bred only through many years of natural selection with no direct selection in Italy and France (Rivière et al., 2013; Raggi et al., 2017). Mass selection from heavy weed pressure on-farm resulted in greater competitive ability by 11.48% compared to commercial checks in the Midwest United States (Kissing Kucek et al., 2021a). In conclusion, it is difficult to discern how much influence farmer selection vs. selection environment had on the final performance of a genotype, breeders and coordinators running PPB programs may benefit from more frequent visits to encourage and help farmers in their breeding efforts.

5.1.2.2. Selection environment in the participatory plant breeding program

Farmers in the Canadian PPB program were recommended to plant their early generation genotypes as close to their existing commercial wheat as possible and following the wheat in rotation each year. Therefore, the population would be grown in different areas on the farm in each of the three years. This suggestion was based on Murphy et al. (2005), who proposed a stratified bulk selection strategy. The genotypes could receive the same agricultural and environmental pressures (eg. different weed pressures, wind speeds, soil textures, and rainfall patterns) as the commercial crop and adapt accordingly. In some cases, the wheat was planted extremely close to a commercial field (Figure 5-2) and sometimes genotypes were planted closer to the house to maximize scouting ability throughout the season (Figure 5-3). This strategy was a hinderance for the phosphorus research in Chapters 3 and 4. Neither farm possessed deficient STP status. In hindsight, if we knew we were testing for greater phosphorus uptake and use efficiency, it would have been beneficial to carry out early generation selection under limited-P environments.

Figure 5-2. Participatory wheat breeding plots on-farm amongst commercial wheat field in Les Cedres, QC. Photo credit: Michelle Carkner

Figure 5-3. Participatory wheat breeding plots on-farm separated from the commercial wheat field in Winchester, Ontario. Photo credit: Michelle Carkner

Farmers were recommended to select in accordance with their own years of experience of growing wheat. If the farmer's goal is to breed for specific environments (eg. Low-P, or high weed competitiveness), the farmer should be advised to plant their genotypes in the target environment in each year. Strategic early generation planting into heavy weed competition has been shown to increase weed competitive ability in wheat (Kissing Kucek et al., 2021a). In that study, genotypes were selected based on vigour at the third to fifth leaf stage, or under intense weed competition from resident weeds. The paper did not describe the weed species the plants were in competition with at the early generation stage (Kissing Kucek et al., 2021a). More research examining which weeds would best facilitate greater weed competitive ability in the early generation selection process would be valuable. Another challenge for on-farm selection is disease resistance. Centralized breeding programs use dedicated disease nurseries and inoculate breeding material to identify resistant genotypes. This would be a challenge to identify on

organic farms because the environmental conditions required to identify susceptible genotypes is not consistent year to year. A recommendation to PPB programs would be to subject genotypes to a disease nursery of relevance at the F_3 generation, and then resistant genotypes are sent out to farmers for two years of selection.

5.1.2.3. Selection and test environments for organic breeding initiatives

Organic testing and selection environment choice was discussed in detail in Chapter 2. This research highlights the importance of maximizing the environmental testing and selection sites in organic plant breeding. Participatory plant breeding and collaboration with farmers may be the most efficient and economical way to incorporate diversity throughout the breeding process. Using existing genotype and environment characteristics data, breeders can use location-grouping GGE biplot analysis to identify repeatable genotype x environment interactions and examine how different characteristics influence the discriminatory power of that environment, and how environments are similar or dissimilar to each other. Environmental discriminatory knowledge is imperative for organic plant breeders, as the environmental characteristics become tools for knowledge rather than creating error and 'data noise' in traditional ANOVA analysis.

For example, it was a surprise that heavy wild oat pressure in Oxbow 2022 in Chapter 2 still enabled genotypes to express differences, but the alfalfa pressure in Roblin 2021 did not (Figure 5-4). It would be valuable to understand what species and weed density compete with wheat well enough to express competitive ability and mirror the target environment, but still allow genetic expression of other traits. While location-grouping GGE biplots have been used to identify mega-environments under conventional (Gauch and Zobel, 1997; Yan et al., 2023) and organic conditions (Kissing Kucek et al., 2019), to the authors' knowledge, the analysis has not been used for organic testing environments with the express use for choosing testing environments. Mega-environment identification is achieved using

multiple years of yield data at the same locations, with clear cluster patterns of the same genotypes performing well within a certain subset of environments. For example, using five years of oat variety data with ten locations (approximately 50 site-years), Yan et al. (2023) identified two mega-environments in eastern Canada, and one in western Canada. The research in chapter 2 tested 2-3 years of each location, each with different weed characteristics, which were not recorded in detail.

More preliminary research is needed to verify the concept I am proposing. One main mega-environment was identified in western Canada using oat variety trial data, but spring wheat data has not been analyzed (Yan et al., 2023). Mega-environment analysis needs to be first completed to ensure disease, insect, and climatic conditions that identify mega-environments are taken into consideration in further analysis. This analysis can be done using conventional spring wheat variety trial data already in existence. It is assumed that the majority of weeds, fertility, insect, and disease pressure are controlled in conventional data, therefore soil type, precipitation, wind, etc. are the pressures that are encapsulated in the first mega-environment analyses. Once the preliminary mega-environment analyses is completed, working within a specific mega-environment, a proof of concept experiment could entail intentional planting in 'light density' and 'heavy density' weed competition that are common to western Canadian farmers [ex. Canada thistle (*Cirsium arvense* L.), volunteer alfalfa (*Medicago sativa* L.), Wild mustard (*Sinapsis arvenis* L.), green foxtail (S*etaria viridis* L. (Beauv.), Wild oat (A*vena fatua* L.), and Redroot pigweed (*Amaranthus retroflexus* L.) (Van Acker et al., 2000)]. A smaller number of site-years may be required for identifying the impact weed genotypes may have on performance yields, Kissing Kucek et al., (2019) judged that eight site-years was the minimum number for organic wheat mega-environmental analysis, but was not testing for site discriminatory power. Therefore, five site-years of each weed treatment may be enough to test the present theory using 20 cultivars.

Figure 5-4. Heavy wild oat (*Avena fatua* L.) competition with spring wheat genotypes in Oxbow 2022 (left). Photo credit: Deb Tuchelt. Heavy alfalfa (*Medicago sativa* L.) pressure with spring wheat genotypes in Roblin 2021 (right). Photo credit: James Frey

Other selection and test environments could include ecological nurseries with varying levels of microbial biomass or varying levels of nitrogen and/or phosphorus supply to examine nutrient use efficiency, or factors that enhance uptake (eg. arbuscular mycorrhizal infection). Ecological nurseries and selection environment are explored in more detail in the future sections. Ecological nurseries could serve the greater agricultural community, as external inputs may become scarce and cost prohibitive for field crops.

5.1.3. Release, distribution, and adoption of new cultivars

Part of the success of a plant breeding program is the adoption and distribution of new cultivars created from that program. There are institutional challenges for the release and adoption of genotypes from organic plant breeding and PPB programs.

In 1923 the legislation, *Seeds Act* (then name *Seed Control* Act), required all new cultivars to be tested either at a research station or privately, and approved for registration by a Committee of Plant

Breeders of the Canadian Seed Grower's Association (Agriculture and Agri-Food Canada, 2013). Currently, the *Seeds Act* legislation is carried out by the Canadian Food Inspection Agency. The legislation's goal is to provide government oversight to ensure: i) health and safety requirements are met and, ii) variety information related to the variety identity is available (Agriculture and Agri-Food Canada, 2013).

The recommending committee that carries out the approval of new wheat cultivars in Western Canada is the Prairie Recommending Committee (Wheat, Rye, and Triticale) (PRCWRT) and recommends cultivars to CFIA for registration. A variety must be *distinguishable and have stable reproducibility* (Forhan, 2023). The PRCWRT consists of variety/trait developers (plant breeders and industry), producer representatives, and end-user representatives (PRCWRT, 2018). Data for variety registration requires multiple years of disease, agronomic, and baking quality testing. The cost to acquire the data needed to recommend a variety for registration may be prohibitive if the genotypes are not part of a well-funded breeding program.

If a variety is not registered, the sale of the seed is illegal, but the sale of the harvested grain is allowed. Despite this, many farmers are unable to market their harvested grain to the export market if it is not a registered variety. Grain marketers need quality data to properly market the grain and the buyer may blend the grain in accordance with their processing needs. Some PPB farmers have taken advantage of nearby local markets, creating a new, alternative grain marketing opportunity. Farmers have taken the initiative to start Grain Community Supported Agriculture initiatives (Grieshaber-Otto, 2020), others sell their grain to local bakeries in nearby urban centres (Moyles, 2018), and finally, some farms have vertically integrated and directly sell their flour products to the public and to bakeries (Dewavrin, 2023).

The following sections will outline the considerations plant breeders, farmer breeders, and organizations need to consider if they want to pursue variety registration within the Canadian registration system. While the Canadian registration system allows for heterogenous material, the common channels

in place are not designed to accommodate heterogenous material specifically bred for organic production systems. Chapter 2 demonstrated that current cultivars widely grown by organic farmers (eg. AAC Brandon) may be suitable for high yielding organic environments but are generally poor performers for many organic environments. Currently, only a handful of wheat cultivars are adopted on an annual basis, despite numerous new variety releases each year (Syme et al., 2023). PPB programs have been shown to increase variety adoption in many countries and increase breeding efficiency (Ceccarelli, 2015).

5.1.3.1. Registration considerations for organic wheat breeding programs

Given the economic importance of organic wheat to Canadian agriculture, it is disappointing that only one organically bred variety has ever been released, AAC Tradition (Canadian Food Inspection Agency, 2017). The major challenge for organic wheat breeding programs is a lack of plant breeders conducting early generation selection under organic management, and inappropriate testing sites for registration recommendation. The data generated for variety registration is still required under conventional management, and the recommending committee must approve a variety evaluation locations and management, which is then usually carried out by approved research stations. Given the established differences between organic and conventional production systems, cultivars that are well-adapted to organic production may not result in final registration. One way to overcome this challenge is by justifying to the recommending committee that organically bred cultivars will possess traits that are different than conventionally bred cultivars (eg. Plant Height) and may not perform optimally because the plant material was specifically bred for organic production but tested under conventional management (Kumar, 2023, pers. comm.). It would be up to the recommending committee to decide if the variety is worthy of registration, and for the breeder to convince seed companies that a robust organic market exists to grow and sell certified seed or employ a certified seed grower. Organic plant breeders are recommended to submit their genotypes to the General Purpose Class and Co-op trials unless quality traits fit extremely well within the CWRS class. In the future, a separate registration stream or class for

ecological production systems is recommended to be set in place, where testing sites reduce or eliminate external chemical intervention to accurately represent genotypes' performance.

5.1.3.2. Considerations for participatory plant breeding programs, heterogenous material

In recognition of the adaption value heterogenous genotypes can provide, the European Union (EU) has recently amended their legislation. The EU requires *distinctive, stable reproducibility and uniformity*. Canada's registration system does not require *uniformity.* Since January 2020, new seed regulations allow for organic seed genotypes to be sold through the EU, and has enabled the approval process to be faster and cheaper (European Commission, 2020).

Single head selection was not conducted on the participatory genotypes, and generally, the genotypes are visually more diverse than registered cultivars based on height, awn length and presence, and spike colour and shape. However, genetic diversity is important to create stability in stressful environments (van Frank et al., 2020). The population is meant to evolve as different abiotic and biotic stresses place natural selection pressure on the population. Taken together, the diversity of a PPB genotype may be its strength for organic farmers. A recent CFIA task force report indicated that the registration system as it stands could accommodate heterogenous material (Forhan, 2023). The Canadian registration legislation requires *distinguishable* and *stable reproducibility*, not *uniformity,* like the EU. This is good news for PPB programs, however, because the wheat registration system has exclusive experience with homogenous cultivars, submitting a heterogenous population for registration requires an extra step. The genotypes are specifically designed to evolve and change over time, therefore, do not possess stable reproducibility. During inspection within the variety registration system and later with the Canadian Seed Growers Association, the breeder would need to specify how much a population may change over time and explain the possible expressions that may change over time depending on a trait's penetrance (Kumar,

2023, per. comm.). Therefore, there is potential for PPB genotypes to enter the registration system if there is plant breeder motivation and financial support.

5.2. Soil phosphorus dynamics in organic production systems

5.2.1. Partnering genotypes with the environment

Production of organic anions and phosphatases by both plants and microorganisms directly and indirectly affect organic P pool as a nutrient source to plants (Hinsinger, 2001). Plants exudate 5-20% of photosynthetically fixed C from their roots as mucilage, and of those, organic anions/carboxylates directly influence organic P soil availability (Bolan, 1991; Oehl et al., 2001b). The ability to transform organic P into plant available P is dependant on the size of the microbial P pool and the time required for the nutrient pool to renew itself (Oberson et al., 2001). The form of P inputs (manure vs. fertilizer), input rates, and tillage regimes impact the organic P dynamics and availability. Microbial P dynamics in relation to crop type grown is poorly understood and understudied. Genotypic differences among multiple species have been shown to have differential root exudate characteristics therefore increase P uptake under low P conditions (Liu et al., 2004; Walters et al., 2018; Chang et al., 2019; Nguyen et al., 2019).

Furthermore, new P inputs that recycle P from urban settings back into agricultural systems are emerging. Products such as frass from black soldier fly (BSF; *Hermetia illucens*) larvae, anaerobically digested urban food or manure waste (digestate), and struvite ($NH_4MgPO_4 \cdot 6H_2O$) which is a mineral extracted from municipal wastewater streams or manure (Nicksy and Entz, 2021; Thiessen Martens et al., 2021). Little known about how these products cycle through the soil systems, how plant roots respond to these products, and if there is genotypic variation in response. For example, struvite is water-insoluble, and must come into contact with acid to solubilize (Degryse et al., 2017). Therefore, genotypes that exudate high amounts of organic acid and protons are hypothesized to be the best match for struvite use.

Future research aimed at investigating how breeding efforts can be targeted for efficient use of these new products is imperative for future productivity of all agricultural systems.

5.2.2. Domestication impacts on the root-soil interface under deficient available soil P

Domestication is the evolution of trait selection suited to human-centred environments rather than wild environments (Charmet, 2011). Domestication of crop species has been shown to influence the rhizosphere microbiome in barley (Bulgarelli et al., 2015), emmer wheat (*Triticum turgidum* L.) (Iannucci et al., 2017; Spor et al., 2020), and maize (Brisson et al., 2019). The organic P pool has the potential to contribute significantly to plant nutrition in cropping systems (Schneider et al., 2017). Phosphorus is often limited in natural ecosystems; therefore, plant roots and microorganisms have evolved to mineralize organic-P to soluble forms of P. Plant roots and microorganisms exudate carboxylates, phosphatases, and acidify the rhizosphere (Jakobsen et al., 2005; Richardson et al., 2011; Fraser et al., 2015; Campos et al., 2018). Additionally, plant roots can attract P-solubilizing microorganisms towards the rhizosphere via polysaccharide, protein, amino acids, sugars, phenolics, and organic acids (Chaparro et al., 2014). It has been hypothesized that selection under high fertility and intense artificial selection may come at the cost of rhizosphere-microbial interactions from wild relatives (Porter and Sachs, 2020). Work in corn and teosinte rhizosphere soil sampled from agricultural fields reported that modern corn inbreds and teosinte recruited different microbial communities (Brisson et al., 2019). The same lab tested teosinte and modern maize under autoclaved sand under greenhouse conditions, and found that the genotypes responded similarly to added mineral-P, and had similar microbial communities (Brisson et al., 2022). Taken together, domesticated, and wild-type genotypes may *recruit* different microbial communities rather than exudate different solubilizers. The interaction between the rhizosphere and microorganisms is complex; little is known about what influence domestication had on how plants engage with the rhizosphere community under constrained nutrient supply in combination with enhanced ecological practices (Isaac et al., 2021).

More specifically, how plants engage with the rhizosphere community in response to low phosphorus conditions in still unknown.

5.2.2.1. Future research proposal

Future research efforts could focus on testing different domestication series groups. For example, in wheat; (1) Ancestral: *Triticum turgidum* ssp. *Durum, Aegilops tauschii*, and *Triticum urartu;* (2) First domesticated: *Triticum monococcum,* (3) Pre-green revolution (landrace) cultivars (pre-1970s): Red Fife (1885), Ladoga (1888), Hard Red Calcutta (1890), Preston (1895), Marquis (1910), Ruby (1920), Thatcher (1935), Saunders (1947), Park (1963); (4) Post-Green Revolution (1980s-2023): Columbus (1980), Roblin (1986), Carberry (semi-dwarf) (2011), Lillian (bred using marker-assisted breeding, 2003), AAC Tradition (organically bred, 2015), AAC Brandon (semi-dwarf (2016), BL23-AS (bred using participatory breeding, 2017), Starbuck (2020), AAC Walker (bred using double haploid technology) (2023). The genotypes could be tested under conventional-low and high-P, organic-low and high-P soil environments. Rhizosphere soil can then be sampled for the purpose of enzyme activity, carboxylates, and hydrogen ions. Additionally, rhizosphere soil will be samples for amplicon generation and MiSeq sequencing of 16S rRNA using PCR to determine microbial (bacterial and fungi) (Spor et al., 2020).

5.2.3. Greater phosphorus cycling efficiency through breeding environments

Phosphorus exists in agroecosystems in multiple 'pools' that differ in their immediate availability to crops (Kovar and Claassen, 2005). Current conventional management strategies have been focussed on managing the inorganic P pool, to optimize fertilizer rates and managing the crop to be a strong sink (ie. very responsive), however, excess inorganic P can be lost quickly from the soil system via erosion (Drinkwater et al., 2017). The ability to tap into the Canadian prairie's microbial P pool's P supply potential for crop use is still not well understood. For example, working in Switzerland using radiotracer techniques, Bünemann et al. (2004) reported that P recovery in the microbial biomass P pools from legume residues was 15%, where as only about 5% of the P in mineral fertilizer was recovered in the microbial biomass.

This demonstrates that we may be able to operate at lower STP levels and source the majority the P for cash crops from biological fixation. Is it possible to maintain soil P levels at 5-10 ppm to reduce off-farm losses and the overuse of a non-renewable source? Research has already shown the potential for organic pool P to supply agricultural systems to a greater degree than previously thought (Oehl et al., 2002; Bünemann, 2015; Drinkwater et al., 2017), and specifically in Canada (Schneider et al., 2017). Using fumigation methodology to assess microbial P content and turnover, Oehl et al. (2001a) reported higher microbial biomass P pools and faster turnover rates in the organic treatments compared to conventional treatments in a long-term trial in Switzerland. The organic and conventional treatments did not differ in available P, the main difference was that the organic rotation received manure as a fertility source whereas the conventional received mineral fertilizer (Oehl et al., 2001a). Working in the same long-term trial, Oehl et al., (2004) confirmed these results with $^{33}PO_4$ isotopic dilution techniques. Therefore, the form of P inputs (residues vs. manure vs. fertilizer) and the rate of inputs impacts organic P dynamics and availability. When Bünemann (2015) reviewed organic P dynamic studies, it was reported that the relative contribution of biological and biochemical mineralization of organic P to plant available P ranged between 20 and 35% in arable soils, and 50 to 70% under permanent grassland. Only managing inorganic P pools leaves out the important role microbial communities play in mediating nutrient supply to plants (Richardson and Simpson, 2011; Simpson et al., 2011). Research is required for greater understanding of what ecological practices create efficient P nutrient cycling such that inorganic P is captured by either plants and/or microbes to enhance P uptake under low-P supply and reduce losses at higher inorganic STP.

5.2.3.1. Future research proposal

Future research could explore using different levels of microbially-sourced phosphorus and mineral fertilizer-based phosphorus at the early-generation selection stage for crops with different P uptake strategies. Corn is highly mycorrhizal (Deguchi et al., 2012), canola is non-mycorrhizal and shown

to exudate acid phosphatase (Hunter et al., 2014), and soybean roots fix their own nitrogen as well as exude acid phosphatases (Mo et al., 2022). Subsequently, the genotypes could then be evaluated under the different fertility of the sourced environments and the interactions between genetics and selection environment could be examined. An illustrative guide is shown in Figure 5-5.

Briefly, early generation selection experiments could take place in field trial experiments with different ratios of organic-P to mineral-P soils with STP-Olsen under 8ppm (Heard et al., 2015). Soil could be recruited from long-term research trials (Eg. Organic vs. Conventional Glenlea Long-term Trial). Selection between F_3-F_5 generations would occur by selecting 300 plants in each year, and in year F_5 the top yielding plants would be chosen for test environments. Resulting F_6 genotypes of corn, canola, and soybean can be tested under the same soils to detect interactions between the selection environment and the test environment. Rhizosphere soil and roots would be sampled at 3 sampling times in both experiments in each year: 4 weeks, 6 weeks, and 8 weeks after emergence. Rhizosphere soil would be used to analyze enzyme and carboxylase activity, and microbial community presence. Roots would be used for mycorrhizal infection using staining and microscopy. Currently, research strategies interested in achieving greater phosphorus use efficiency and uptake under low-P soils are primarily concerned with how to change the plant's physiology through molecular genetic techniques. The genotypes are usually tested under controlled environments using sterilized soil or hydroponic mediums, often sourcing genotypes that were not selected for the targeted environment (George et al., 2008; Rose et al., 2016; Wang et al., 2016a; Hari-Gowthem et al., 2019; Nguyen and Stangoulis, 2019; Wacker-Fester et al., 2019; El Mazlouzi et al., 2020b). The interplay between the native microorganisms and crop response is often downplayed, instead, this connection needs to be highlighted. Greenhouse and hydroponic experimental designs do not consider microbial community recruitment in the rhizosphere, and the ability for crops to adapt to a specific microbial community. Similar research could be carried out comparing nitrogen mineral fertilizer and legume-source nitrogen nutrition.

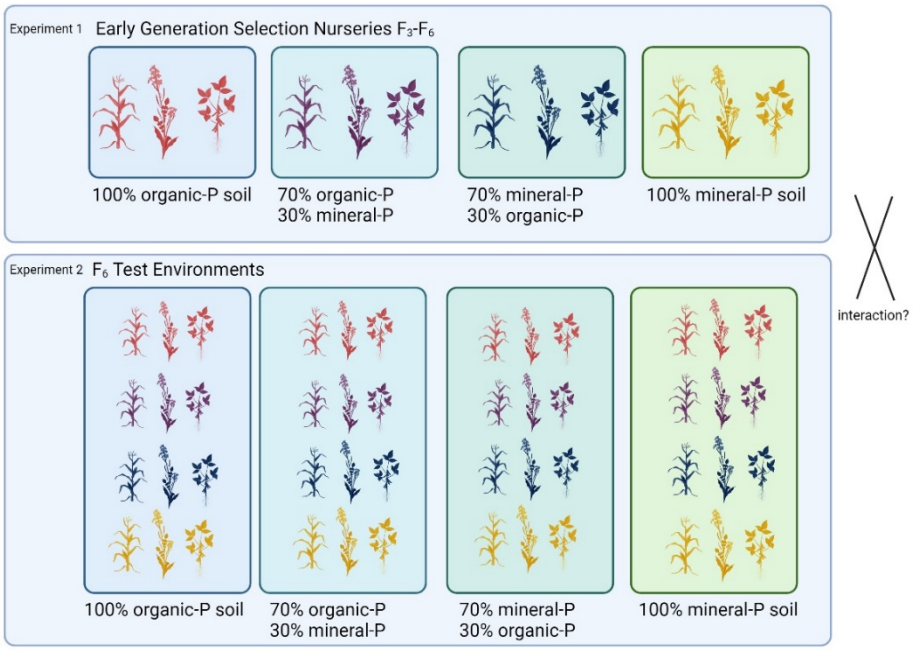


Figure 5-5. An illustrative guide for future research testing the impact of selection environment on phosphorus uptake strategies and performance within the target test environment under different ratios of organic and mineral-sourced soil phosphorus (P) levels. Experiment one involves the crops' selection process, samples would be taken to record genetic changes from the F_3-F_6 generations. The corresponding colour of the selection environment is shown in Experiment 2, where each genotype of each crop would be represented in each test environment to test for interactions. This image was created in BioRender.

5.3. Conclusions and contributions to knowledge

This thesis evaluated the performance of wheat genotypes that were distributed and selected by a diverse group of farmers from across Canada. More specifically, the goal of this research was to evaluate the performance of the genotypes under environmental conditions unique to organic farmers and identify what phenotypic traits are valuable to organic production systems and organic breeding programs.

Of specific concern is the continued unique challenge of low available soil P for stockless organic farms on the Canadian prairies. Phosphorus use efficiency and its associated traits have been explored around the world in weathered soils (Rose et al., 2013), however, Chapter 1 proposes a new ideotype for organic production systems that uses a phosphorus 'catch and release' mechanism which has never been explored in the literature.

The participatory breeding model used in Canada resulted in genotypes that performed distinctly from each other, and in many cases, demonstrated greater yield and yield stability than commercial cultivars widely grown by organic farmers on the Canadian prairies (Chapter 2). The phenotypic traits that were associated with yield were height, anthesis biomass, mature biomass, and kernel number per unit area. Yield performance and stability were closely associated with orange blossom wheat midge resistance, as the majority of the top performers with high stability possessed parental material with *Sm1* genes. Populations derived from a PPB model or similar models have been evaluated elsewhere around the world (Murphy et al., 2005; Ceccarelli and Grando, 2007; Dawson et al., 2008; Desclaux et al., 2012; Colley et al., 2021; Sandro et al., 2022), and in Canada (Entz et al., 2018), however, no other work has evaluated the scale of geographic diversity of participating farmers, in combination with a wide geographic range of organic test environments in Canada. Additionally, it is recognized that the environment contributes to the variance in organic trial yield data deriving from more stressful conditions, which would suggest that organic breeding programs need more testing environments than conventional breeding programs (Bocci et al., 2020; Kissing Kucek et al., 2021a). However, this research argues that it may not be the amount of testing environments that needs to be considered in organic breeding programs, but the characteristics of the test environment to enhance the discriminatory qualities of the environment, as well as be representativeness of the target environment. To the author's knowledge, this concept has not been proposed before in the literature for organic breeding programs. Overall, this research contributes to a greater understanding of the impact of early generation farmer selection in PPB programs. Seasonal extremes and stressful growing conditions are expected to increase with the effects of climate change, and PPB programs may be part of the solution to create resilient food production systems.

Chapter 3 outlines agronomic differences between parents with different breeding histories and how farmer selection from different geographic regions in Canada shaped the genotypes. Despite

different breeding histories, the modern and landrace cultivars did not yield differently from each other under organic management. However, the modern cultivar, 5602HR did have significantly greater protein than the landrace, Red Fife. Farmer genotypes did not yield differently from either parent, nonetheless, both genotypes had greater protein than Red Fife. Both farmer genotypes were taller than 5602HR, and earlier maturing than Red Fife. Farm2 and Farm1 had similar height to Red Fife, yet Farm2 had better lodging resistance than both Red Fife and Farm1 under amended P conditions. This difference could be due to different wind speeds in the selection environments. Overall, we learned that breeding progress has been substantially slower under organic production systems, and farmer selection can have a positive result combining valuable traits from parental material from different breeding histories. Evaluation of a second PPB family (AAC Scotia x Norwell, explored in Appendix B) with different yielding parents demonstrated that while some farmers selected genotypes that were in between both parents, one farmer selected genotype had high yield and high protein (Table B-3). A future opportunity would be to test the same treatments under the selection environment the genotypes were organically grown to evaluate local adaptation of genotypes.

Farm2 and Red Fife exhibited greater P yield efficiency than 5602HR and Farm1. Phosphorus use and uptake efficiency differed among parents and farmer selections depending on the environmental conditions. 5602HR and the farmer genotypes were more responsive to amended P conditions when considering P accumulation and uptake efficiency than Red Fife. Under high precipitation conditions in 2022, Farm1 demonstrated 128% P uptake efficiency, greater than all other genotypes tested. With respect to the ideotype proposed in Chapter 1, greater P uptake efficiency was achieved by one farmer genotype, and yield efficiency was achieved by another farmer genotype. One of the most important traits to the P ideotype is low phosphorus harvest index (PHI). Lower PHI was not achieved with the PPB model in this study. Belowground P uptake dynamics such as phosphatase exudation and arbuscular mycorrhizal infection did not differ among treatments. Testing for other belowground dynamics such as organic acid

exudation and direct acidification of the rhizosphere would be valuable. This research contributes to greater knowledge of selection environment on traits that are not directly observable, such as P uptake efficiency and P yield efficiency. The next logical step would be to test the genotypes in their specific selection environment for local adaptation, and in addition, to test a farmer genotype that was specifically selected under deficient P conditions.

Embracing diversity within breeding programs and breeding specifically for production systems that emphasizes ecological management of nutrients, diseases, and weeds, whether organic or conventional, is an essential feature of future resilient food production systems. This research demonstrates the participatory plant breeding model with the direct selection in the target environment can produce high quality, high yielding, stable genotypes under organic production.

Table B-3. A comparison table of study objectives between two spring wheat 'PPB Families': Family 1 and Family 2. Family 1 parental cross was between a modern (5602HR) and a landrace (Red Fife) cultivars. Family 2 parental cross was between two modern cultivars (AAC Scotia and Norwell). The experiment was conducted under organic conditions in Libau, Manitoba in 2020, 2021, and 2022 under limited soil test phosphorus (3ppm); and amended soil with composted manure at 25 kg P ha^{-1}

Objective	Family 1: Red Fife x 5602HR	Family 2: AAC Scotia x Norwell
Suitability of combining wheat genotypes as parental material for an organic participatory plant breeding program by comparing the performance under organic conditions.	Red Fife was taller, greater lodging potential, larger kernel mass, and lower protein than 5602HR. Parental cultivars did not differ in yield.	AAC Scotia was taller, had greater lodging potential, higher yield, larger kernel mass, and lower protein than Norwell.
How farmer genotypes differed in their performance from their parents under a range of organic growing conditions.	Farmer genotypes did not differ from parents in biomass accumulation, and yield. Farmer genotypes resembled Red Fife in height and kernel mass. Farmer genotypes had similar grain protein and lodging resistance to 5602HR.	Farmer genotypes did not differ from the parents in biomass accumulation. FarmC was more similar to AAC Scotia in height, lodging severity, kernel mass, and grain protein. Farmer genotypes FarmA and FarmB were taller than Norwell, but shorter than AAC Scotia. FarmA and FarmB yields were lower than AAC Scotia and similar to Norwell. Farmer genotypes FarmA and FarmB fell between AAC Scotia and Norwell in height and protein.
Evaluate the impact geographically divergent farmers and their respective environments had on full sibling derived genotypes	Farmer genotypes did not differ in biomass accumulation, height, yield, and kernel mass. Farm2 had greater lodging resistance and lower protein levels than Farm1 under high fertility and high precipitation conditions.	Farmer genotypes did not differ in biomass accumulation and yield. FarmC was taller, had greater lodging severity, and lower grain protein than FarmA and FarmB. Under high fertility, high precipitation conditions, FarmC had significantly greater kernel mass than farmer genotypes FarmA and FarmB.

RESPONSES TO THE EXTERNAL EXAMINER

The external examiner raised some important considerations for the thesis, and I would like to take this opportunity to address the most pertinent subjects.

The reviewer made note of the small plot size used for the experiments which may have led to the experimental units to be influenced by edge effects. I recognize this is a challenge in our experimental design. To combat edge effects, rows of fall rye were seeded between each plot, and an extra wheat plot was seeded at the end of each block. This was not mentioned in the initial draft of the thesis and has now been rectified. The reason for our small plot size was because of a combination of reasons. In the first year of all experiments, we were limited by the amount of seed stock available. Plot sizes in Chapter 3 and 4 experimental plots were doubled with two rows of fall rye in between each plot in years 2021 and 2022. Chapter 2's small plot size beyond the first year of the experiment increased in length by 1m but did not change substantially. To reduce mixing between genotypes during harvest, physically separating the plots by four rows of fall rye was essential. In our experience, when lodging occurs it is very difficult to separate the plots from each other to avoid mixing if the plots are too close to each other. Additionally, given the number of treatments and replicates, larger plots would have doubled the size of the experiment which would have introduce another level of variability due to spatial heterogeneity. The harvested area of Oxbow 2021 and 2022 was small because the plots were geographically far away from the University (approximately 5 hours drive). Therefore, the plots were hand-harvested, transported back to the university and threshed with a stationary combine. We harvested the maximum amount that could be safely transported over a long distance.

We used composted manure as a treatment to replenish the available phosphorus for crop growth. We recognize that adding composted manure, as opposed to a singular source of phosphorus fertilizer, also introduced nitrogen to the system. We decided to use compost manure to capture

phosphorus mineralization from the composted manure. To ensure the crop response to composted manure was due to phosphorus addition, we conducted the experiment on land with excess nitrogen status and extremely low-test phosphorus. When soil tests were taken at anthesis in 2020 and 2021 (very dry years), we observed an increase in Olsen-P from 3ppm to 8 and 9 ppm, respectively, indicating that phosphorus mineralization occurred. In 2022, we observed a change from spring sampling to anthesis sampling from 3 to 4ppm. Presumably because the crop had taken up the mineralized phosphorus.

The external examiner was interested in participatory evolutionary breeding practices. Evolutionary breeding occurs through mass selection continuously harvested in the same environment for approximately 10 years. The principal difference between the Canadian PPB program and evolutionary breeding is that farmers made intentional selections from the populations each year for three years instead of mass selection over 10 years. I would argue that the Canadian program made excellent genetic progress adapted to organic conditions on the Canadian prairies and relatively short amount of time. Additionally, we did not plant the populations on the farms in which the populations were was selected on because the goal of this thesis was not to observe local adaptation to the selection environment, but broad adaption to organic production systems. In the general discussion, I proposed future research questions that attempts to more closely connect the selection environment to the target environment, in the PPB program but also more broadly in other crops.

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APPENDIX A. CHAPTER 2.

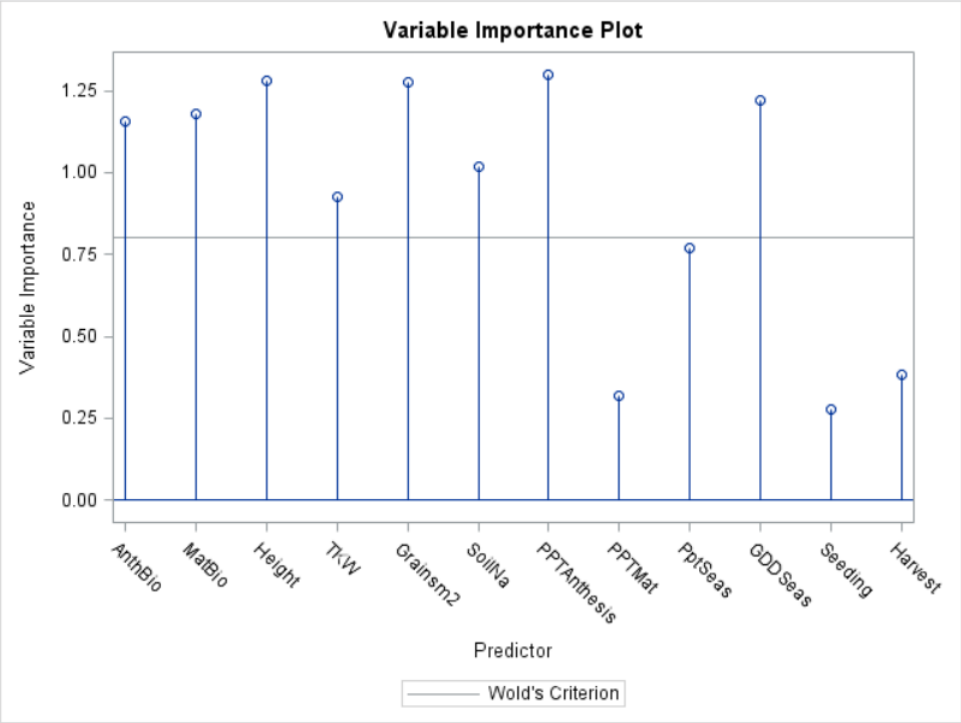


Figure A-1. The partial least squares regression variable importance in the projection plot for final grain yield among 25 spring wheat farmer genotypes and 6 spring wheat check cultivars seven organic environments (Carman (2020, 2021, 2022), Libau (2020, 2021, 2022), and Oxbow (2021)). The black line denotes the variable importance value of 0.8. Any parameter >0.8 is considered a significant contributor to the model. AnthBio, biomass accumulation at anthesis stage; MatBio, biomass accumulation; Height, plant height; TKW, thousand kernel weight; grainsm2, kernels per unit area; SoilNa, soil test sodium; PPTAnthesis, precipitation between stem elongation and anthesis growth stages; PPTMat, precipitation between anthesis and maturity, PptSeas, precipitation from seeding to harvest; GDDSeas, growing degree days from seeding to harvest; Seeding, seeding date; Harvest, harvest date.

APPENDIX B. CHAPTER 3.

B1. Introduction of a Second PPB Family

A second attempt to test the proof of concept was explored with another PPB family, AAC Scotia and Norwell. Both parents are modern cultivars with different yield performance and grain protein levels under organic conditions (Table B-1). Both parental cultivars were bred for eastern Canadian (Ontario, Quebec, and the Maritimes provinces) growing conditions. AAC Scotia was registered 2018 (Canadian Food Inspection Agency, 2018), and Norwell was registered in 2009 (Canadian Food Inspection Agency, 2009). Crossing method, distribution history, and selection procedure followed the same methodology as Red Fife and 5602HR cross. The cross was distributed and selected by three organic farmers; FarmA (located in Les Cedres, Quebec), FarmB (located in Freetown, Prince Edward Island), and FarmC (Saint-Gérard-Majella, Quebec). The parental cultivars and farmers selected genotypes were evaluated in the same factorial randomized complete block design as the Red Fife and 5602HR family in Libau, Manitoba in 2020, 2021, and 2022.

B1.2. Results

Results of biomass accumulation, plant height, lodging severity, yield, and protein will be based on a combined analysis of years and manure treatments, since there was no genotype x manure, genotype x year, or genotype x manure x year. There was a significant genotype x year interaction for kernel mass (Figure B-1).

B1.2.1. Evaluation of Parental Differences

AAC Scotia and Norwell performed similarly in early, anthesis, and mature growth stages of biomass accumulation across all years and fertility treatments (Table B-1). AAC Scotia was significantly taller than Norwell by 15cm (Table B-1). When conditions were favorable for lodging potential in 2022, AAC Scotia had significantly greater lodging risk than Norwell under both fertility treatments (Table B-1).

Yield performance significantly differed among parental cultivars; AAC Scotia had significantly greater yield than Norwell by 492 kg ha^{-1} (*P>F* 0.006) when averaged across all years and manure treatments. AAC Scotia had greater kernel mass than Norwell by 2 g $1000seeds^{-1}$. Norwell had significantly greater grain protein than AAC Scotia by 2% (*P>F* <.0001) (Table B-1). Therefore, AAC Scotia taller, greater lodging potential, higher yielding, but lower protein than Norwell under a range of organic conditions.

B1.2.2 Farmer Selected Population Comparison to Parents

There were no genotypic differences between the farmer genotypes and the parents in early, anthesis, and mature biomass accumulation (Table B-1). FarmA and FarmB were taller than Norwell by 9cm and shorter than AAC Scotia by 8cm. FarmA and FarmB had lower lodging potential than AAC Scotia, and more similar to Norwell (Table B-2). FarmC was similar height and lodging potential to AAC Scotia. FarmC was taller than Norwell by 18cm and greater lodging potential. FarmA and FarmB did not yield differently from Norwell and had significantly lower yield than AAC Scotia by 310 and 424 kg ha^{-1}, respectively. FarmC yield was not significantly different from both AAC Scotia and Norwell (2481 kg ha^{-1}). Farmers genotypes' kernel masses were not different from AAC Scotia and significantly higher than Norwell by 3 (FarmA and FarmB) and 4 (FarmC) g $1000seeds^{-1}$. FarmA and FarmB's grain protein were lower than Norwell by 1 and 0.7%, respectively, and higher than AAC Scotia by 1 and 1.3%, respectively. FarmC had similar grain protein levels to AAC Scotia, and significantly lower protein levels than Norwell by 0.8%.

B1.2.3. Farmer Selection Populations Analyzed as a Group Compared to Parents

When farmer genotypes were analyzed as a group, farmer genotypes were 13 cm taller (*P>F* <.0001) than Norwell and 5 cm shorter (*P>F* 0.0127) than AAC Scotia (Table B-1). As a group, farmer genotypes were not different from AAC Scotia and Norwell in biomass accumulation and yield (Table B-1). As a group, farmer genotypes had similar height, lodging severity, and kernel mass to AAC Scotia, but

had higher grain protein by 0.81% (*P>F* 0.0254) (Table B-1). Farmer genotypes were taller than Norwell by 13 cm (Table B-1), had greater lodging potential (Table B-2), larger kernel mass by 5 g 1000seeds^{-1}, and significantly lower grain protein by 1.2% (*P>F* 0.0014).

Taken together, the farmer selected genotype FarmA, reflected AAC Scotia moreso than Norwell in height, lodging potential, yield, kernel mass, and protein. Farmer genotypes FarmA and FarmB were more similar to Norwell than AAC Scotia in lodging potential and yield. Farmers FarmA and FarmB fell in between AAC Scotia and Norwell in height, kernel mass, and protein.

B1.2.4. Performance between farmer genotypes

There were no differences between farmer genotypes in early, anthesis, and mature biomass accumulation, yield, and kernel mass (Table B-1). FarmA and FarmB did not differ from each other for plant height and were 6 cm shorter than farmer selection FarmC. FarmC had greater lodging potential than both FarmA and FamrB (Table B-2). FarmA and FarmB had greater grain protein levels than FarmC by 0.8 and 1.1%, respectively. There was a genotype x year interaction, deriving from farmer genotypes FarmA and FarmB's kernel mass in 2022 (Figure B-1). In 2020 and 2021, FarmA and FarmB had similar kernel mass to FarmC. In 2022, however, FarmA and FarmB kernel masses were significantly lower than FarmC by 2.2 and 2.5 g 1000seeds^{-1}, respectively.

B1.3. Conclusions Integrating Family 1 and Family 2

A table comparing the objective outcomes of Family 1 and Family 2 is shown in Figure B-3. Family 1 (Red Fife and 5602HR) involved crossing a modern and a landrace genotype that have different breeding histories and agronomic traits but did not yield differently from each other. Family 2 parents (AAC Scotia and Norwell) were both modern genotypes that had different agronomic traits, and more specifically, yielded differently from each other under organic conditions. The purpose of testing a second family was to observe the impact of different yielding parents on farmer genotypes. Farmer genotypes from the

Family 1 cross were more similar to the landrace genotypes in many agronomic traits, however, had higher protein. In the case of the Family 2 cross, one farmer genotype (FarmC) resembled the higher-yielding parent AAC Scotia, and two farmer genotypes (FarmA and FarmB) resembled the lower-yielding parent Norwell. Farmer genotypes from both families (Family 1 and Family 2) produced genotypes that incorporated important traits from parental cultivars for modern organic grain production (tall, maintained yield, greater kernel mass, and maintained or increased protein). Taken together, the correct selection of divergent parental cultivars for use in PPB programs is essential. Finally, lessons learned from the Family 2 demonstrated that farmers created genotypes that performed in a different way, using two modern, differently yielding parents.

Table B-1. Lsmeans and combined analysis of variance comparing agronomic parameters from three years of data (2020, 2021, 2022) collected under organic conditions in Libau, Manitoba among two spring wheat cultivars (AAC Scotia and Norwell) and three spring wheat farmer genotypes (FarmA, FarmB, and FarmC) under limited soil test phosphorus (3ppm) and amended soil with composted manure at 25 kg P ha^{-1}

	Early Biomass	Anthesis Biomass	Maturity Biomass	Plant Height	Yield	Seed Mass	Protein
Year (Y)	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	cm	kg ha^{-1}	g $1000seeds^{-1}$	%
2020	737a	3538	6498b	77b	2480b	35a	12.2c
2021	426b	2935	4804c	74b	1848c	29c	12.8b
2022	810a	3649	9632a	98a	2941a	33b	14.9a
Year *P>F**	0.0059	0.0763	<.0001	<.0001	0.0001	<.0001	<.0001
Genotype (-P,+P)[Ŧ] (G)							
FarmA	651	3388	7169	82b	2405b	33a	13.4b
FarmB	662	3143	6820	82b	2291b	33a	13.7b
FarmC	706	3482	7235	88a	2481ab	34a	12.6c
AAC Scotia	633	3400	7094	89a	2715a	32a	12.4c
Norwell	637	3457	6572	74c	2223b	30b	14.4a
Genotype *P>F*	0.7261	0.9109	0.8334	<.0001	0.0060	<.0001	<.0001
Manure (M)							
(+)P	763a	4026a	7539a	85a	2863a	34a	12.9b
(-)P	553b	2722b	6417b	81b	1983b	32b	13.6a
Manure *P>F*	<.00001	<.0001	0.0078	<.0001	<.0001	<.0001	<.0001
Interactions *P>F*							
G x M	0.1902	0.9333	0.9155	0.6752	0.2405	0.8248	0.0672
G x Y	0.4839	0.8604	0.8847	0.6579	0.9877	<.0001	0.1004
M x Y	0.0007	0.0105	0.5065	0.1398	0.0002	<.0001	0.056
G x M x Y	0.068	0.9981	0.3988	0.6437	0.5772	0.2054	0.1065
Coeff. Of Variation (%)	34	37	30	7	19	7	5.4
Farmer Genotypes Lsmeans	665	3321	7107	84	2399	33a	13.3
Parental Cultivars Lsmeans	628	3416	6863	82	2465	31b	13.5
Contrasts							
Farmer Genotypes vs. Parents *P>F*	0.5355	0.7287	0.677	0.3243	0.6746	0.0336	0.5224
Estimate	37	-95	243	2	-66	2	-0.18
Farmer Genotypes vs. AAC Scotia *P>F*	0.6606	0.8316	0.9688	0.1255	0.1259	0.9425	0.0254
Estimate	33	-73	28	-5	-307	0.02	0.81
Farmer Genotypes vs. Norwell *P>F*	0.5885	0.7369	0.5352	0.0024	0.3829	0.0011	0.0014
Estimate	41	-116	458	10	174	2.8	-1.2

*Lsmeans within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test (P≥ 0.05);
[Ŧ]Limited P treatment, (-)P; P-Amended treatment, (+)P

Table B-2. Lsmeans and analysis of variance comparing lodging severity from 2022 under organic conditions in Libau, Manitoba among two spring wheat cultivars (AAC Scotia and Norwell) and three spring wheat farmer genotypes (FarmA, FarmB, FarmC) under limited soil test phosphorus (3ppm) and amended soil with composted manure at 25 kg ha^{-1}

	Lodging Severity
Genotype (-P,+P)[Ŧ] (G)	1-9
FarmA	0.6bc
FarmB	0.4c
FarmC	1.3a
AAC Scotia	1.1ab
Norwell	0c
Genotype *P>F**	0.0038
Manure (M)	
(+)P	0.9
(-)P	0.6
Manure *P>F*	0.1893
Interaction *P>F*	0.5322
Coeff. Variation (%)	124
Farmer Genotypes Lsmeans	0.8
Parental Cultivars Lsmeans	0.6
Contrasts	
Farmer Genotypes vs. Parents *P>F*	0.42
Estimate	0.22
Farmer Genotypes vs. AAC Scotia *P>F*	0.35
Estimate	-0.33
Farmer Genotypes vs. Norwell *P>F*	0.0324
Estimate	0.79

*Lsmeans within the same column followed by the same letter are not significantly different with groups of treatments by an analysis of variance test (P ≥ 0.05);
[Ŧ]Limited P treatment, (-)P; P-Amended treatment, (+)P

0

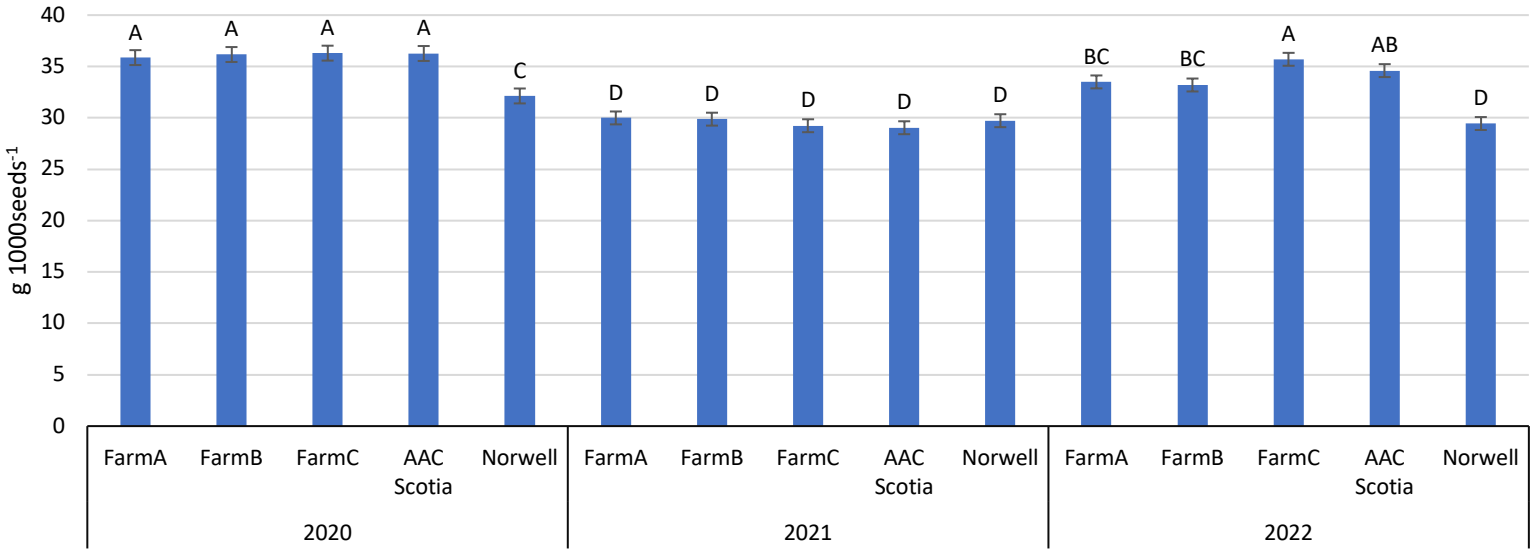


Figure B-1. Kernel mass genotype x year interaction effects collected under organic conditions in 2020, 2021, and 2022 in Libau, Manitoba among two spring wheat cultivars (AAC Scotia and Norwell) and three spring wheat farmer genotypes (FarmA, FarmB, and FarmC) under limited soil test phosphorus (3ppm), (-)P; and amended soil with composted manure at 25 kg P ha^{-1}, (+)P.
Treatments with the same letter are not significantly different by an analysis of variance test ($P \leq 0.05$).

Table B-3. A comparison table of study objectives between two spring wheat 'PPB Families': Family 1 and Family 2. Family 1 parental cross was between a modern (5602HR) and a landrace (Red Fife) cultivars. Family 2 parental cross was between two modern cultivars (AAC Scotia and Norwell). The experiment was conducted under organic conditions in Libau, Manitoba in 2020, 2021, and 2022 under limited soil test phosphorus (3ppm); and amended soil with composted manure at 25 kg P ha^{-1}

Objective	Family 1: Red Fife x 5602HR	Family 2: AAC Scotia x Norwell
Suitability of combining wheat genotypes as parental material for an organic participatory plant breeding program by comparing the performance under organic conditions.	Red Fife was taller, greater lodging potential, larger kernel mass, and lower protein than 5602HR. Parental cultivars did not differ in yield.	AAC Scotia was taller, had greater lodging potential, higher yield, larger kernel mass, and lower protein than Norwell.
How farmer genotypes differed in their performance from their parents under a range of organic growing conditions.	Farmer genotypes did not differ from parents in biomass accumulation, and yield. Farmer genotypes resembled Red Fife in height and kernel mass had similar grain protein and lodging resistance to 5602HR.	Farmer genotypes did not differ from the parents in biomass accumulation. FarmC was more similar to AAC Scotia in height, lodging severity, kernel mass, and grain protein. Farmer genotypes FarmA and FarmB were taller than Norwell, but shorter than AAC Scotia. FarmA and FarmB yields were lower than AAC Scotia and similar to Norwell. Farmer genotypes FarmA and FarmB fell between AAC Scotia and Norwell in height and protein.
Evaluate the impact geographically divergent farmers and their respective environments had on full sibling derived genotypes	Farmer genotypes did not differ in biomass accumulation, height, yield, and kernel mass. Farm2 had greater lodging resistance and lower protein levels than Farm1 under high fertility and high precipitation conditions.	Farmer genotypes did not differ in biomass accumulation and yield. FarmC was taller, had greater lodging severity, and lower grain protein than FarmA and FarmB. Under high fertility, high precipitation conditions, FarmC had significantly greater kernel mass than farmer genotypes FarmA and FarmB.

Other Tables

Table B-4. Lsmeans and combined analysis of variance comparing agronomic parameters in 2020 collected under organic conditions in Libau, Manitoba among two spring wheat cultivars (AAC Scotia and Norwell) and three spring wheat farmer genotypes (FarmA, FarmB, and FarmC) under limited soil test phosphorus (3ppm) and amended soil with composted manure at 25 kg P ha^{-1}

	Early Biomass	Anthesis Biomass	Maturity Biomass	Plant Height	Yield	Seed Mass	Protein
Genotype (-P,+P)[Ŧ] (G)	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	cm	kg ha^{-1}	g $1000seeds^{-1}$	%
FarmA	758	3200	6886	78.2ab	2525b	36a	11.8ab
FarmB	668	3394	5863	75.3b	2327bc	36a	12.7a
FarmC	777	3962	6733	81.3a	2526b	36a	11.3bc
AAC Scotia	626	3462	7275	82.8a	2806a	36a	10.7c
Norwell	758	3672	5732	69.7c	2215c	32b	12.4a
Genotype *P>F*	0.2264	0.9567	0.5304	<.0001	0.0101	<.0001	0.0069
Manure (M)							
(+)P	804.3	4321a	7581a	79.4a	2913a	35a	12.1a
(-)P	575.1	2619b	5488b	74.5b	1965b	34b	11.4b
Manure *P>F*	0.0005	0.0003	0.0001	0.0002	<.0001	<.0001	0.0307
G x M *P>F*	0.2114	0.7493	0.5457	0.7242	0.5006	0.9735	0.8759
Coeff. Of Variation (%)	32	45	27	6	24	2	9
Farmer Genotypes Lsmeans	754	3519	6494	78	2459	36a	12.3
Parental Cultivars Lsmeans	712	3567	6504	76	2510	34b	11.9
Contrasts							
Farmer Genotypes vs. Parents *P>F*	0.6241	0.9343	0.9882	0.2968	0.8147	<.0001	0.3654
Estimate	42	-48	-9	2	-51	1.9	0.39
Farmer Genotypes vs. AAC Scotia *P>F*	0.3265	0.9384	0.3476	0.0752	0.2176	0.7322	0.0293
Estimate	107	56	-780	-5	-346	-0.15	1.26
Farmer Genotypes vs. Norwell *P>F*	0.8304	0.8355	0.3594	0.0016	0.3818	<.0001	0.4012
Estimate	-23	-153	761	9	244	3.9	-0.466

*Lsmeans within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test (P≥ 0.05);
[Ŧ]Limited P treatment, (-)P; P-amended treatment, (+)P

Table B-5. Lsmeans and combined analysis of variance comparing agronomic parameters in 2021 collected under organic conditions in Libau, Manitoba among two spring wheat cultivars (AAC Scotia and Norwell) and three spring wheat farmer genotypes (FarmA, FarmB, and FarmC) under limited soil test phosphorus (3ppm) and amended soil with composted manure at 25 kg P ha^{-1}

	Early Biomass	Anthesis Biomass	Maturity Biomass	Plant Height	Yield	Seed Mass	Protein
Genotype (-P,+P)[Ŧ] (G)	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	cm	kg ha^{-1}	g $1000seeds^{-1}$	%
FarmA	369	2967	4435	71bc	1804	30	12.8b
FarmB	383	2607	4614	73abc	1722	30	13.3b
FarmC	517	3300	5423	79ab	1839	29	11.9c
AAC Scotia	435	3003	4650	79a	2083	29	11.9c
Norwell	415	2798	4896	67c	1782	30	14.2a
Genotype *P>F*	0.4615	0.7686	0.7157	0.0108	0.6513	0.8307	<.0001
Manure (M)							
(+)P	436	3071	5281	75	2041a	30	12.7
(-)P	416	2799	4327	73	1651b	29	13
Manure *P>F*	0.7023	0.4342	0.0532	0.3487	0.0227	0.3158	0.2417
G x M *P>F*	0.2114	0.7493	0.5457	0.7242	0.5006	0.9735	0.8759
Coeff. Of Variation (%)	41	37	32	10	29	6.5	6
Farmer Genotypes Lsmeans	427	2958	4824	74	1801	29	12.7
Parental Cultivars Lsmeans	425	2900	4773	73	1932	29	13.1
Contrasts							
Farmer Genotypes vs. Parents *P>F*	0.9793	0.8653	0.9209	0.5455	0.4787	0.5952	0.1019
Estimate	1.4	57	51	1.4	-131	0.3	-0.4
Farmer Genotypes vs. AAC Scotia *P>F*	0.9048	0.9178	0.7905	0.1315	0.2313	0.3855	0.0213
Estimate	-8.6	-44	174	-5	-282	0.6	0.72
Farmer Genotypes vs. Norwell *P>F*	0.8723	0.7105	0.9134	0.0169	0.9349	0.9755	<.0001
Estimate	11.6	160	-71	8	19	-0.02	-1.5

*Lsmeans within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test (P≥ 0.05);

[Ŧ]Limited P treatment, (-)P; P-amended treatment, (+)P

Table B-6. Lsmeans and combined analysis of variance comparing agronomic parameters in 2021 collected under organic conditions in Libau, Manitoba among two spring wheat cultivars (AAC Scotia and Norwell) and three spring wheat farmer genotypes (FarmA, FarmB, and FarmC) under limited soil test phosphorus (3ppm) and amended soil with composted manure at 25 kg P ha^{-1}

	Early Biomass	Anthesis Biomass	Maturity Biomass	Plant Height	Yield	Seed Mass	Protein
Genotype (-P,+P)[Ŧ] (G)	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	cm	kg ha^{-1}	g 1000$seeds^{-1}$	%
FarmA	805	3997	10186	98b	2887	34b	15.1b
FarmB	905	3428	9982	98b	2824	33b	14.9b
FarmC	803	3182	9547	105a	3065	36a	14.3c
AAC Scotia	819	3737	9357	106a	3256	35ab	14.2c
Norwell	719	3902	9088	87c	2673	29c	16.2a
Genotype *P>F*	0.5691	0.7415	0.293	<.0001	0.1973	<.0001	<.0001
Manure (M)							
(+)P	984a	4587a	9938	102a	3583a	35a	14.2b
(-)P	636b	2711b	9328	96b	2300b	31b	15.5a
Manure *P>F*	<.0001	0.0002	0.2916	<.0001	<.0001	<.0001	<.0001
G x M *P>F*	0.1201	0.9971	0.4671	0.2408	0.3964	0.0746	0.2439
Coeff. Of Variation (%)	34	37	32	3.5	16	6	2.6
Farmer Genotypes Lsmeans	838	3535	9951	100a	2925	34a	15
Parental Cultivars Lsmeans	769	3819	9223	96b	2965	32b	15
Contrasts							
Farmer Genotypes vs. Parents *P>F*	0.5289	0.5901	0.4335	0.0243	0.8862	0.034	0.0729
Estimate	68	-283	728	4	-39	2	-0.4
Farmer Genotypes vs. AAC Scotia *P>F*	0.8899	0.7625	0.6117	0.0127	0.3445	0.7017	0.0446
Estimate	19	-201	593	-5	-330	-0.5	0.5
Farmer Genotypes vs. Norwell *P>F*	0.3923	0.5823	0.4612	<.0001	0.4701	0.004	<.0001
Estimate	118	-366	863	13	252	4.6	-1.3

*Lsmeans within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test (P≥ 0.05);

[Ŧ]Limited P treatment, (-)P; P-amended treatment, (+)P

APPENDIX C. CHAPTER 4.

C1. Proof of concept exploration with Family 2

Family 2 consists of two differently yielding modern parents, AAC Scotia and Norwell. AAC Scotia and Norwell were selected and bred for eastern Canadian conditions (Ontario, Quebec, and the Maritime provinces). Three farmer genotypes were generated from the parental cross. An overview of the breeding history of the parents, and farmer selection locations are outlined in Appendix B, Chapter 3. The parental cultivars and farmer genotypes were evaluated in the same factorial randomized complete block design as the Red Fife and 5602HR family in Libau, Manitoba in 2020, 2021, and 2022.

C1.2. Results

There were no genotype x manure, genotype x year, or genotype x manure x year interactions detected for straw P concentration and accumulation, grain P accumulation, total plant P accumulation, PUptE, PHI and PRE (Table C-1). There was a significant genotype x year interaction for grain P concentration (Figure C-1) and GrainN:P ratio (Figure C-2). Additionally, there was a genotype x manure x year interaction for hyphae (Figure C-3) and total colonization (Figure C-4), but no other interactions were shown for belowground biological parameters (Table C-2). Therefore, results will be discussed as a combined analysis of manure treatments and years except where an interaction exists.

C1.2.1. Evaluations of parental differences

AAC Scotia and Norwell were similar to each other for straw P concentration and accumulation, grain P accumulation, total P accumulation, PUptE, PHI, PRE, and GrainN:P ratio (Table C-1). Norwell had significantly greater grain P concentration than AAC Scotia. There was a significant genotype x year interaction for grain P concentration (Figure C-1) and grain N:P ratio (Figure C-2). However, the interactions derived from farmer selection differences in relation to Norwell. AAC Scotia had significantly greater PYE than Norwell by 71 kg ha^{-1} (Table C-1).

Parental cultivars did not significantly differ from each other for Ptase, RhWEP, arbuscule colonization, hyphae colonization, or total colonization. There was a significant genotype x manure x year interaction for hyphae (Figure C-3) and total colonization (Figure C-4). The interaction sources were derived from farmer selection differences. Therefore, AAC Scotia had lower grain P concentration and greater PYE than Norwell under a range of organic conditions. Parental cultivars did not differ in belowground parameters measured.

Table C-1. Lsmeans and combined analysis of variance comparing phosphorus parameters from three years of data (2020, 2021, 2022) collected under organic conditions in Libau, Manitoba among two spring wheat cultivars (AAC Scotia and Norwell) and three spring wheat farmer genotypes (FarmA, FarmB, and FarmC) under limited soil test Olsen-phosphorus (3ppm) and amended with composted manure at 25 kg P ha^{-1}

	Straw P[a] Conc.[₮]	Straw P Acc.[¥Δ]	Grain P Conc.	Grain P Acc.	Total P Acc.	PUptE[b]	PHI[c]	PYE[d]	PRE[eΔ]	GrainN:P Ratio[f]
Year (Y)	mg g^{-1}	kg ha^{-1}	mg g^{-1}	kg ha^{-1}	kg ha^{-1}	%	%	kg ha^{-1}	kg ha^{-1}	
2020	2.4b	0.98b	2.3b	5.3b	6.2b	46b	84b	400b	0.041b	8.5b
2021	1.2c	0.35c	1.6c	2.7c	3.1c	31c	88a	604a	0.020c	12.8a
2022	4.5a	2.7a	3.1a	8.4a	11.2a	77a	75c	247c	0.10a	7.8b
Year *P>F**	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Genotype (G) (-P,+P)[β]										
FarmA	2.5	1.3	2.3b	5.4	6.7	52	84	417b	0.051	9.7
FarmB	2.8	1.5	2.5a	5.6	7.1	53	81	386c	0.06	9.6
FarmC	2.7	1.4	2.2b	5.4	6.9	51	82	430b	0.053	9.6
AAC Scotia	2.5	1.4	2.1c	5.4	6.7	50	82	462a	0.056	10.2
Norwell	2.9	1.2	2.6a	5.5	6.7	51	84	391c	0.052	9.8
Genotype *P>F*	0.242	0.6134	<.0001	0.9878	0.8818	0.8801	0.3520	<.0001	0.7113	0.3225
Manure (M)										
(+)P	2.8	1.7a	2.5a	6.8a	8.5a	26b	83	403b	0.058	9.1b
(-)P	2.5	1b	2.2b	4.1b	5.2b	75a	82	432a	0.051	10.4a
Manure *P>F*	0.0528	<.0001	<.0001	<.0001	<.0001	<.0001	0.4403	<.0001	0.1567	<.0001
Interactions *P>F*										
G x M	0.31	0.3298	0.0952	0.7367	0.5762	0.6299	0.2177	0.4248	0.4356	0.0891
G x Y	0.351	0.9185	0.0226	0.9631	0.7952	0.5538	0.9381	0.0608	0.9597	0.0437
M x Y	0.0243	<.0001	0.0002	<.0001	<.0001	<.0001	0.1351	0.0556	0.0079	0.01
G x M x Y	0.8912	0.4764	0.3012	0.7467	0.4788	0.2962	0.8175	0.3896	0.8016	0.0559
Coeff. Variation (%)	34	47	10	24	21	20	7	10	50	12
Farmer Genotypes Lsmeans	2.6	1.5	2.4	5.5	7	51	82	413	0.056	9.7
Parental Cultivars Lsmeans	2.6	1.3	2.3	5.5	6.6	50	83	435	0.053	10.1
Contrasts										
Farm Genotypes vs. Parents *P>F*	1	0.5356	0.7649	0.9471	0.6470	0.8904	0.5957	0.4915	0.7142	0.4345
Estimate	0	0.16	0.4	0.04	0.4	0.92	-0.8	-22	0.003	-0.4
Farm Genotypes vs. AAC Scotia *P>F*	0.4725	0.7686	0.1203	0.9291	0.6305	0.8760	0.9677	0.1261	0.7802	0.4015
Estimate	0.03	0.101	2.7	0.07	0.5	1.3	0.08	-62	0.003	-0.5
Farm Genotypes vs. Norwell *P>F*	0.4648	0.4906	0.2775	0.9871	0.8084	0.9510	0.3746	0.6422	0.7636	0.6903
Estimate	-0.03	0.23	-1.9	0.013	0.3	0.51	-1.7	18	0.003	-0.27

*Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.05$); [Δ] Data transformed natural log for normality; [β](-)P, limited P treatment; (+)P, P-amended treatment; [a]P, phosphorus; [b]PUptE, Phosphorus Uptake Efficiency; [c]PHI, Phosphorus Harvest Index; [d]PUE, Phosphorus Utilization Efficiency; [e]PRE, phosphorus return efficiency; [f]GrainN:P Ratio, Ratio of Grain N Concentration to Grain P Concentration; [₮]Conc., concentration; [¥]Acc. accumulation

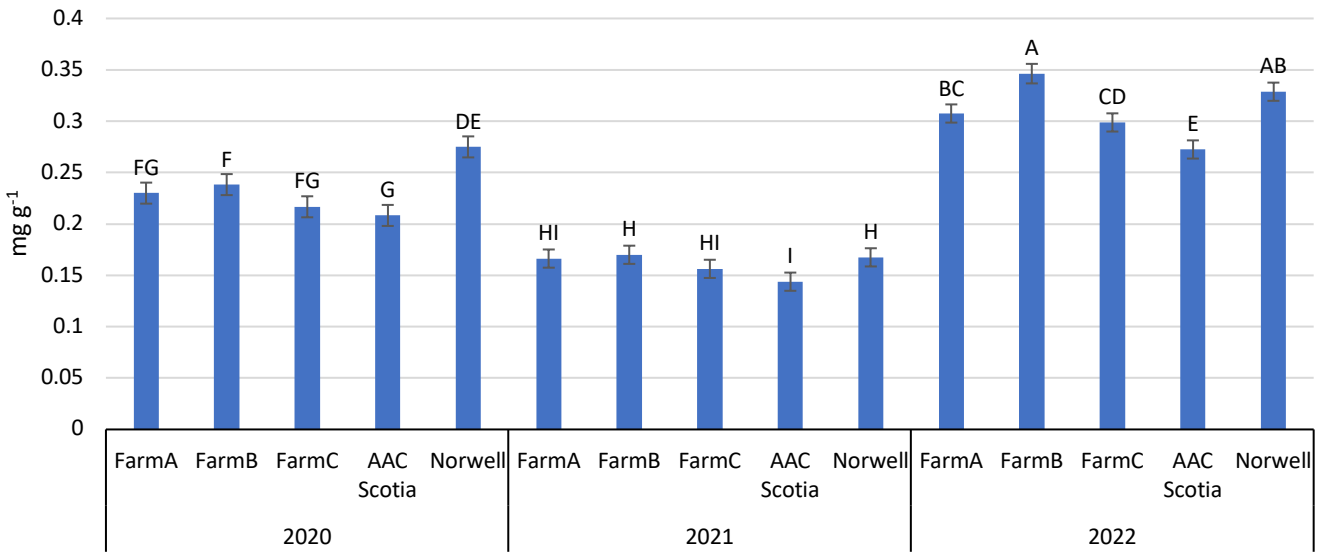


Figure C-1. Grain P concentration genotype x year interaction under organic conditions in Libau, Manitoba in 2020, 2021, and 2022 among two spring wheat cultivars and three spring wheat farmer genotypes under limited soil test phosphorus (3ppm), (-)P; and amended soil with composted manure at 25 kg ha^{-1}, (+)P.
Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.05$)

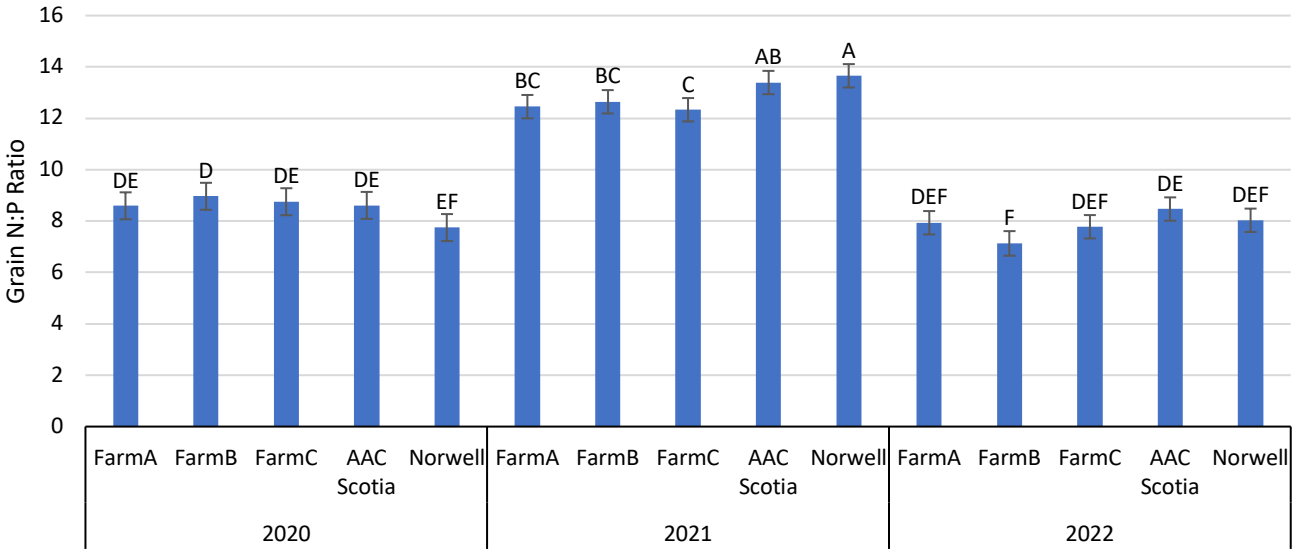


Figure C-2. Grain N:P Ratio genotype x year interaction under organic conditions in Libau, Manitoba in 2020, 2021, and 2022 among two spring wheat cultivars and three spring wheat farmer genotypes under limited soil test phosphorus (3ppm), (-)P; and amended soil with composted manure at 25 kg ha^{-1}, (+)P.
Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.05$)

Table C-2. Lsmeans and combined analysis of variance comparing belowground microbial activity from three years (2020, 2021, 2022) collected under organic conditions in Libau, Manitoba among two spring wheat cultivars (AAC Scotia and Norwell) and three spring wheat farmer selected gentypes (FarmA, FarmB, and FarmC) under limited soil test Olsen-phosphorus (3ppm) and amended with composted manure at 25 kg P ha^{-1}

	Rhizosphere Acid Phosphatase$^{\alpha}$	Rhizosphere WEP$^{\text{Ŧ}\alpha}$	Arbuscule Infection	Hyphae	Arbuscules + Hyphae
Year (Y)	pmol MUF g^{-1} soil h^{-1}	mg P kg^{-1} soil	%	%	%
2020	930	9.8a	8b	5.8b	13.5b
2021	719	2.7b	7b	12.9a	21a
2022	367	9.9a	15a	6.9b	22a
Year *P>F**	0.1429	0.0216	<.0001	0.0002	0.0108
Genotype (-P,+P)$^{\beta}$(G)					
FarmA	516	5.3	11	10.8a	22a
FarmB	880	7.2	8.9	7.1b	16b
FarmC	454	7.7	7.8	8.1b	16b
AAC Scotia	792	7.5	9.7	8.4b	18ab
Norwell	718	9.9	12.2	8.3b	20ab
Genotype *P>F**	0.9220	0.5830	0.2178	0.0440	0.0899
Manure (M)					
(+)P	576	8.5	9.9	7.7b	17
(-)P	768	6.5	10.8	9.4a	20
Manure *P>F*	0.8295	0.1558	0.5379	0.0415	0.1127
Interactions *P>F*					
G x M	0.5912	0.3729	0.698	0.5403	0.6161
G x Y	0.3377	0.8911	0.8309	0.1552	0.5246
M x Y	0.6878	0.9007	0.2059	0.3993	0.3257
G x M x Y	0.8853	0.5249	0.9498	0.0079	0.0591
Coeff. of Variation (%)	14	81	65		
Farmer Genotypes Lsmeans	583	6.4	9.7	8.9	19
Parental cultivars Lsmeans	779	8.2	10.9	8.5	20
Contrasts					
Farmer Genotypes vs. Parents *P>F*	0.7061	0.3685	0.3526	0.6919	0.6688
Estimate	-195	-1.8	-1.2	0.43	-0.8
Farmer Genotypes vs. AAC Scotia *P>F*	0.9593	0.5818	0.8789	0.6073	0.8503
Estimate	-240	-1.5	-0.2	0.73	0.4
Farmer Genotypes vs. Norwell *P>F*	0.5946	0.3847	0.1885	0.9174	0.3849
Estimate	-149	-2.2	-2.2	0.14	-2.1

*Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.05$)

$^{\text{Ŧ}}$WEP, water extractable phosphate; $^{\alpha}$data natural log-transformed; $^{\beta}$(-)P, Limited P treatment; (+)P, P-amended treatment

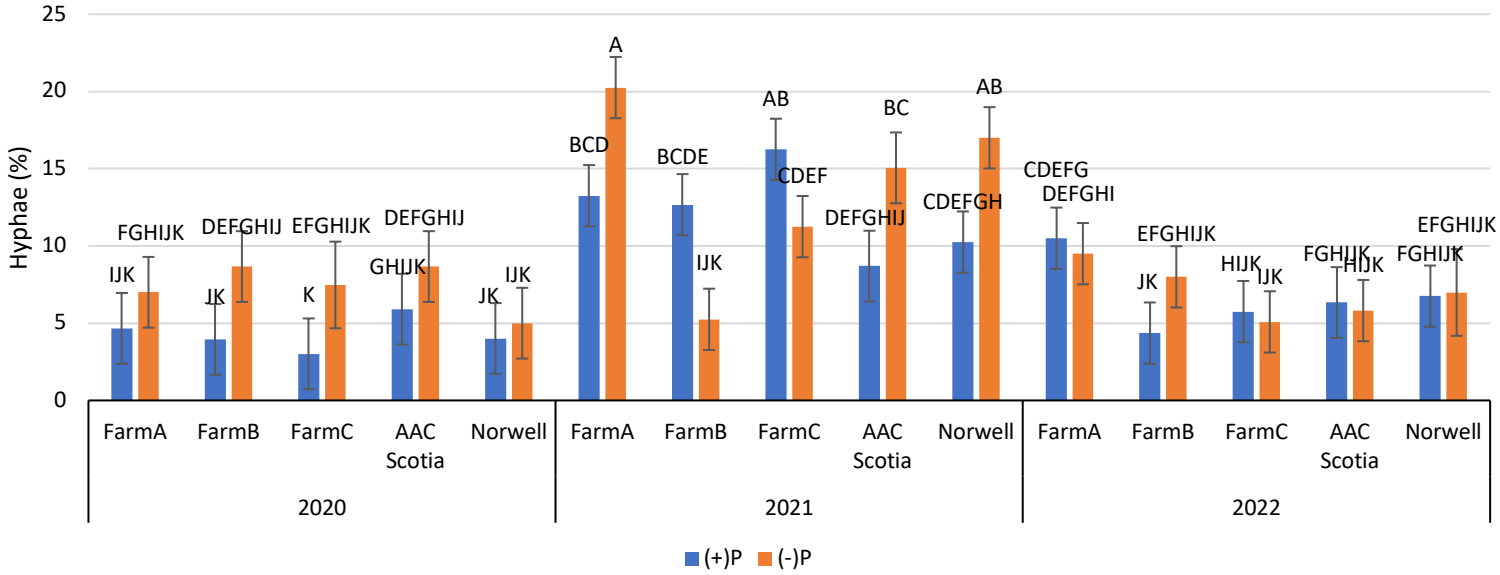


Figure C-3. Hyphal percent colonization genotype x manure x year interaction under organic conditions in Libau, Manitoba in 2020, 2021, and 2022 among two spring wheat cultivars and three spring wheat farmer genotypes under limited soil test phosphorus (3ppm), (-)P; and amended soil with composted manure at 25 kg ha^{-1}, (+)P.
Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test (P ≤ 0.10)

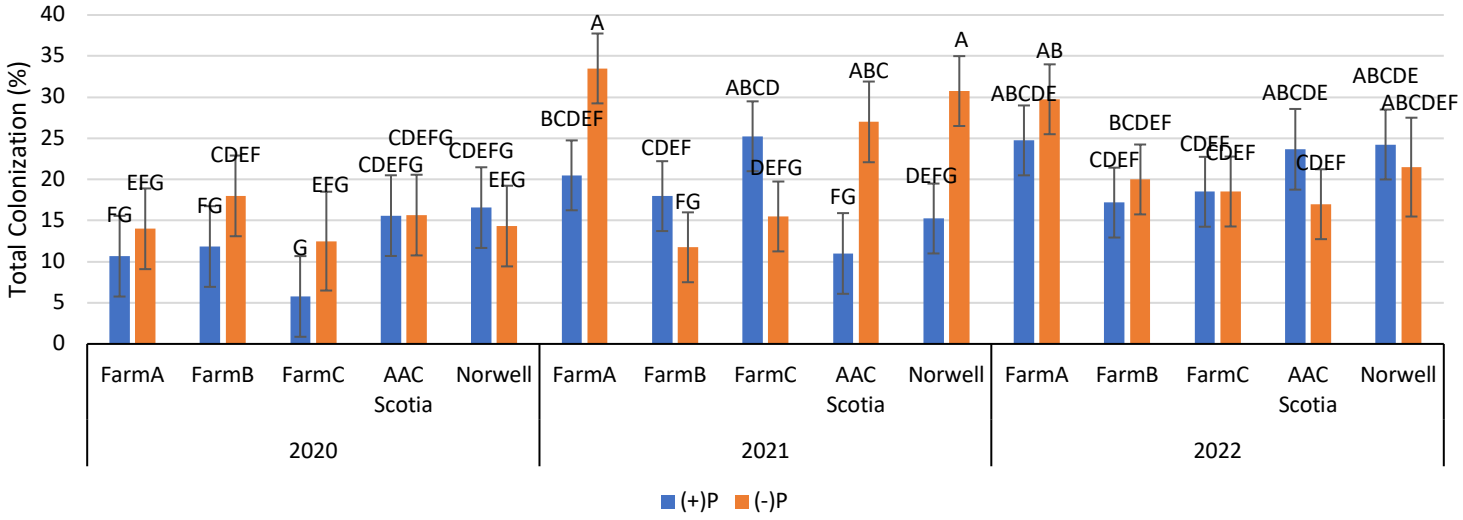


Figure C-4. Total (arbuscule and hyphae) percent colonization genotype x manure x year interaction under organic conditions in Libau, Manitoba in 2020, 2021, and 2022 among two spring wheat cultivars and three spring wheat farmer genotypes under limited soil test phosphorus (3ppm), (-)P; and amended soil with composted manure at 25 kg ha^{-1}, (+)P.
Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test (P ≤ 0.10)

C1.2.2. Farmer genotypes compared to the parental cultivars

There were no genotypic differences between farmer genotypes and parental cultivars in straw P concentration and accumulation, grain P accumulation, total P accumulation, PUptE, PHI, PRE, and grain N:P ratio (Table C-1). FarmB had similar grain P concentration to Norwell, and greater concentration than AAC Scotia. FarmA and FarmC had lower grain P concentration than Norwell, but greater than AAC Scotia. A significant genotype x year interaction revealed that in Norwell had significantly greater grain P concentration than all farmer genotypes in 2020, but similar concentrations in 2021 to farmer genotypes. In 2022, under favourable growing conditions, FarmA and FarmB had similar grain P concentration to Norwell (Figure C-1). AAC Scotia had greater PYE than all farmer genotypes. FarmA and FarmC had similar PYE to Norwell, and FarmB had lower PYE than both AAC Scotia and Norwell. A slightly significant (*P>F* 0.0437) genotype x year interaction was detected (Figure C-2). Farmer genotypes had similar ratios to both parents in 2020 and 2022. However, under drought conditions in 2021, farmer selections had lower ratios than Norwell, but remained similar to AAC Scotia.

No genotypic differences between the farmer genotypes and parental cultivars were detected for Ptase, RhWEP, and arbuscular colonization. However, FarmA had greater hyphae percent colonization than both parents, and similar total colonization to both parents (Table C-2). The other farmer genotypes (FarmB and FarmC) were similar to both parents for both parameters. There was a significant genotype x manure x year interaction for hyphae percent colonization (Figure C-3). Norwell's hyphae colonization was significantly greater under limited P treatments, and the opposite was shown for farmer genotypes FarmB and FarmC. Taken together, farmer genotypes had greater grain P concentration than AAC Scotia, but some farmer genotypes were similar to Norwell. Farmer genotypes had lower PYE than AAC Scotia and were similar to Norwell. FarmA had greater hyphae percent colonization than both parents, and Norwell differed from FarmB and FarmC depending on the year and the fertility treatments observed.

C1.2.3. Performance between farmer genotypes

Farmer genotypes were not different from each other for straw P concentration and accumulation, grain P accumulation, total P accumulation, PUptE, PHI, PRE, and grain N:P ratios (Table C-1). FarmB had significantly greater grain P concentration than farmer selections FarmA and FarmC. Conversely, FarmA and FarmC had significantly greater PYE than farmer FarmB. The grain P concentration genotype x year interaction indicated that FarmB had greater grain P concentration than the other two farmer genotypes in 2022, only (Figure C-1). The significant genotype x year interaction for grain N:P ratio did not reveal interactions between farmer genotypes (Figure C-2).

Farmer genotypes did not differ in Ptase, RhWEP, and arbuscule colonization (Table C-2). FarmA had significantly greater hyphae percent colonization than both FarmB and FarmC by 6%. There was a significant genotype x manure x year interaction, in 2020 and 2022, hyphae percent colonization did not differ for any farmer genotype between manure treatments (Figure C-3), however, FarmA had significantly greater hyphae colonization under limited P treatments than in 2021. In addition, the opposite result was observed for FarmB and FarmC in 2021, hyphae colonization was significantly greater under P-amended conditions. Total colonization genotype x manure x year interaction demonstrated similar dynamics as hyphae percent colonization, indicated that it was mainly hyphae activity that was driving the total colonization genotype x manure x year interaction.

Generally, FarmA and FarmC had greater PYE and lower grain P concentration than FarmB. FarmA had demonstrated greater hyphae colonization than both FarmB and FarmC in 2021. Additionally, FarmB and FarmC had greater hyphae colonization under P-amended treatments, opposite of FarmA.

C1.3. Conclusions integrating Family 1 and Family 2 together

Family 1 (Red Fife x 5602HR) was derived from a modern and a landrace cultivar cross and Family 2 (AAC Scotia x Norwell) was a cross between two modern cultivars. Although the Family 1 had parents

with very different breeding histories, the cultivars did not yield differently in a previous experiment (Chapter 3, Table 3-4). Parental cultivars from Family 2 did yield significantly different from each other (Appendix B, Chapter 3). The goal of incorporating a second family was to observe how two different yielding parents impacted farmer genotypes, and how three farmer genotypes differed from each other. A table comparing the objective outcomes of the Family 1 and Family 2 is shown in Table C-3.

Table C-3. A comparison table of study objectives between two spring wheat 'PPB Families': Family 1 and Family 2. Family 1 parental cross was between a modern (5602HR) and a landrace (Red Fife) cultivars. Family 2 parental cross was between two modern cultivars (AAC Scotia and Norwell). The experiment was conducted under organic conditions in Libau, Manitoba in 2020, 2021, and 2022 under limited soil test phosphorus (3ppm); and amended soil with composted manure at 25 kg P ha^{-1}

Objective	Family 1: Red Fife x 5602HR	Family 2: AAC Scotia x Norwell
Evaluate phosphorus uptake, yield efficiency and belowground traits that facilitate P uptake of two parental cultivars used to generate genotypes for the PPB program.	5602HR was more responsive to manure than Red Fife for total P accumulation, and had greater grain N:P ratios, especially under drought conditions. Red Fife demonstrated greater PYE than 5602HR. Red Fife had greater Ptase activity than 5602HR under limited P conditions.	Norwell had significantly greater grain P concentration than AAC Scotia. AAC Scotia demonstrated greater overall PYE than Norwell.
How farmer genotypes differed in their phosphorus dynamics from their parents under a range of organic growing conditions.	Farm1 was similar to 5602HR's responsiveness to manure for PUptE, and total P accumulation. Farm2 was similar to Red Fife for PUptE, total P accumulation, and PYE. Farm1 demonstrated greater PUptE and total P accumulation in 2022. Farmer genotypes had greater APase activity than 5602HR, and similar values to Red Fife.	Farmer genotypes had greater grain P concentration than AAC Scotia, and FarmB had similar grain P concentration to Norwell. Farmer genotypes had lower PYE than AAC Scotia, reflecting Norwell. FarmB had lower PYE than both parents. FarmA had greater hyphae colonization than both parents, especially under drought conditions.
Evaluate the impact geographically divergent farmers and their respective environments had on the phosphorus dynamics from full sibling derived genotypes	Majority of differences between farmer genotypes were apparent in 2022. Farm1 had greater grain P concentration, total P accumulation, and PUptE than Farm2 in 2022.	Farmer genotypes FarmA and FarmC had greater PYE than FarmB but lower grain P concentration. FarmA had greater hyphae colonization than FarmC and FarmB under drought conditions. Farmer genotypes FarmC and FarmB greater hyphae colonization under amended P treatments in drought, the opposite of FarmA.

C2. Root Greenhouse Trial

A separate greenhouse trial was conducted to evaluate genotypic root differences within Family 1 (Red Fife x 5602HR cross). Greater P uptake has been associated with wide root angle in the top 15cm, wider root system width, greater root biomass, and larger root-to-shoot ratios (Lynch and Brown, 2001; Lynch, 2011).

C2.1. Materials and Methods

C2.1.1. Experimental design and treatments

The pot study was carried out in greenhouse settings with ventilation on limited P soil sampled from the Libau 2022 field trial, all soil characteristics and nutrient sources are described in Chapter 3, Table 3-2. The soil was collected from 0-15 cm layer, dried at room temperature, and sieved to 5mm. The soil was homogenized and added to 22-cm diameter pots at a rate of 4300 g dry soil per pot. A metal pie plate was set at the bottom of the pot to prevent water draining.

The experiment was a randomized complete block design with four blocks as replicates. The treatments were Farm1, Farm2, Red Fife, and 5602HR. Seeds were sieved to 5-6 64^{th} x ¾ to ensure uniform seed mass. Twenty seeds of each treatment were rinsed with 1% bleach solution then reverse osmosis water. Seeds were placed on moist filter paper in petri dishes. Radicle was showing two days later, and ten seeds were placed 2-cm below the surface. After eight days, Seedlings were thinned to five plants per pot when pots were planned to be sampled at stem elongation, and three plants per pot a when pots were planned to be sampled at anthesis. Pots were watered by weight up to a target of 80% free-drained container water capacity using reverse osmosis water. Pots were watered every 1-3 days as needed. Every week pots were watered to their target weight and re-randomized within their blocks. Blocks were also randomized to avoid uneven soil drying and edge effects.

C2.1.2. Sampling and imaging protocol

When plants were at the stem elongation development and anthesis stages, they were measured for height by measuring the distance between the base of the soil to the top of the plant. The pots were then rinsed very carefully under water to remove the soil from the root system. Pots were soaked in water prior to rinsing when plants were at the anthesis stage. Plants were cut at the crown of the plant for drying and weighing. Roots and shoots were dried at 65°C for 72hrs and then weighed. Images were taken against a dark background using a Canon EOS 1000 digital camera with F-stop set to f/25, exposure time at 1/4s and ISO at 200. Digital images were then used to visually score root traits using ImageJ image analysis software available at (https://imagej.net/ij/index.html) (Schneider et al., 2012). The root architecture traits scored were root angle, system width, and crown width (York et al., 2018; Fradgley et al., 2020). Root traits are detailed further in Table C-4 and an illustrative guide is

shown in Figure C-5.

Table C-4. Description of wheat root traits scored in greenhouse study (York et al., 2018; Fradgley et al., 2020)

Trait	Description
Root Angle	The angle between two lines originating at the base of the plant at ground level which fits the angle of the outer most crown roots in a 2D image of the root system using the angle tool function within Image J analysis software
System Width	Width of the root system at the widest point with roots present at both sides
Crown width	Width of the plant where the stem and the root system connect

Figure C-5. Example images of wheat root sample at stem elongation, A; and anthesis, B; used in ImageJ to score root architecture traits in ImageJ. Measures include crown width (green line), system width (yellow line), and root angle (red arc).

C2.1.3. Statistical analysis

Data was analysed using PROC Mixed procedure with SAS program 9.4 (SAS, 2013a). Tests for normal distribution of data were carried out using PROC Univariate with Shapiro-Wilks values. Values greater than 0.9 were assumed to be normally distributed. Differences among genotypes were tested using the Least Significant Difference (LSD) test and considered significant at $p < 0.05$. Data shown in tables represents the Least Squares Means (lsmeans). To compare the farmer genotypes with the parents, treatments were combined and analyzed into three groups: Farmer genotypes contrasted with both parents, farmer genotypes contrasted with Red Fife, and farmer genotypes contrasted with 5602HR. Contrasts were carried out using PROC GLM procedure in SAS 9.4 (SAS, 2013a).

C2.3. Results and Discussion

No genotypic main effects were detected for shoot height, root and shoot biomass, root:shoot ratio, root angle, system and crown width at the stem elongation and anthesis stage (Tables C-5 and C-6). At anthesis, 5602HR, Farm1 and Farm2 were significantly taller than Red Fife. Other work has demonstrated genotypic differences in root architecture among wheat genotypes, specifically between landrace and modern genotypes (Fradgley et al., 2020; Boudiar et al., 2021). However, it is interesting that the root angle was greater at the stem elongation stage versus the at the anthesis sage. One reason we may not have observed differences may be due to drought stress. Despite daily waterings, the plants were under heat stress due to high temperatures in the greenhouse over the summer. This may have explained smaller root angles among the anthesis stage plants (Alahmad et al., 2019). Future research should be conducted either in a greenhouse with a cooling wall, or in a growth chamber where temperature and daylight can be regulated.

C2.4. Conclusions

The study's objective was to explore root biomass and architecture differences among genotypes that may facilitate greater P uptake efficiency and accumulation under low available P soil. The measurements taken to examine root architecture was not connected to greater P accumulation. Future research should examine more root architecture measurements such as seminal root number, nodal root number, root length, and root hairs (York et al., 2013; Wang et al., 2016b; Fradgley et al., 2020).

Table C-5. Lsmeans analysis of variance comparing shoot and root weight, and root architecture parameters at stem elongation development stage collected under greenhouse conditions using organic managed soils from Libau, Manitoba among two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) under limited soil test Olsen-phosphorus (3ppm)

	Shoot Height	Root Biomass	Shoot Biomass	Root:Shoot Ratio	Root Angle	System Width	Crown Width
Genotype	cm	g	g		degrees	cm	cm
Farm1	36	0.145	0.297	0.47	68	4.4	0.54
Farm2	38	0.153	0.312	0.49	71	3.9	0.54
Red Fife	34	0.129	0.258	0.52	77	4.6	0.47
5602HR	38	0.159	0.314	0.51	71	4.4	0.53
Genotype *P>F**	0.1218	0.7104	0.6882	0.8903	0.6357	0.1822	0.7356
Coeff. of Variation (%)	8	25	25	18	31	18	17
Farmer Genotypes Lsmeans	37	0.14	0.30	0.48	69	4.2	0.54
Parental Cultivars Lsmeans	36	0.14	0.28	0.52	74	4.5	0.5
Contrasts							
Farmer Genotypes vs. Parents *P>F*	0.6692	0.7930	0.6342	0.5047	0.6906	0.5521	0.4135
Estimate	0.61	0.005	0.02	-0.03	-4.8	-0.2	0.03
Farmer Genotypes vs. Red Fife *P>F*	0.1765	0.3923	0.3348	0.5036	0.5850	0.5564	0.2809
Estimate	2.5	0.02	0.05	-0.04	-8.6	-0.3	0.07
Farmer Genotypes vs. 5602HR *P>F*	0.4847	0.6613	0.8391	0.6723	0.9434	0.7172	0.8520
Estimate	-1.2	-0.01	-0.009	-0.02	-1	-0.18	0.01

*Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.05$)

Table C-6. Lsmeans analysis of variance comparing shoot and root weight, and root architecture parameters at the anthesis development stage collected under greenhouse conditions using organic managed soils from Libau, Manitoba among two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) under limited soil test Olsen-phosphorus (3ppm)

	Shoot Height	Root Biomass	Shoot Biomass	Root:Shoot Ratio	Root Angle	System Width	Crown Width
Genotype	cm	g	g		degrees	cm	cm
Farm1	57a	0.36	1.32	0.28	40	5.8	0.87
Farm2	58ab	0.30	1.24	0.28	33	4.6	0.83
Red Fife	53b	0.34	1.5	0.34	36	5.7	0.89
5602HR	61a	0.42	1.8	0.23	38	6.2	0.78
Genotype *P>F**	0.0333	0.2708	0.636	0.704	0.6523	0.0948	0.6884
Coeff. of Variation (%)	6	35	28	44	97	22	
							17
Farmer Genotypes Lsmeans	58	0.33	1.3	0.29	36	5.2	0.85
Parental Cultivars Lsmeans	57	0.38	1.7	0.23	37	5.9	0.84
Contrasts							
Farmer Genotypes vs. Parents *P>F*	0.4308	0.4557	0.0965	0.3667	0.9345	0.2507	0.809
Estimate	1.4	-0.04	-0.37	0.05	-0.3	-0.77	0.01
Farmer Genotypes vs. Red Fife *P>F*	0.0334	0.9069	0.4285	0.4328	0.8494	0.5012	0.6297
Estimate	5	-0.009	-0.2	0.05	0.9	-0.54	-0.04
Farmer Genotypes vs. 5602HR *P>F*	0.3054	0.2788	0.0550	0.4533	0.7463	0.2256	0.4584
Estimate	-2.2	-0.08	-0.53	0.06	-1.5	-1	0.06

*Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.05$)

C3. Seed Phosphorus Greenhouse Trial

A wheat ideotype for low available soil P has been proposed that includes limiting P translocation from the biomass into the grain to reduce off-farm P export (Carkner et al., 2023). However, plants rely on seed P reserves for nutrition at germination, therefore lower seed P reserves have the potential to reduce the germination speed and early crop vigour (Grant et al., 2001). Early season crop vigour is an important trait related to crop competitive ability (Mason and Spaner, 2006). Organic farmers rely on early season vigour to gain a competitive advantage over weeds. Early season vigour is of specific importance to Canadian organic prairie farmers because organic production systems heavily rely on biological activity for P supply through mineralization, which is slow in cold soils (Schneider et al., 2017). However, research in rice has demonstrated that seeds low in P reserves access external P as early as 3 days post germination (Julia et al., 2018) To test the impact low seed phosphorus as seed stock may have on early vigour and germination, a separate growth room trial was conducted.

C3.1. Materials and Methods

C3.1.1. Experimental treatments

The genotypes used were Farm1, Farm2, Red Fife, and 5602HR. Seed stock was taken from limited P and amended-P treatment seed stocks from the Libau 2020 field trial and assessed for seed phosphorus concentration analyzed by Agvise Laboratories in North Dakota, USA, using inductively couple plasma-optical emission spectroscopy (PerkinElmer) following digestion with HNO_3 and H_2O_2 at 150°C following the procedure by Havlin and Soltanpour (1980). Seeds within the amended P treatment ranged between 2.6-3.8 mg g^{-1}, and seed within the limited P treatment ranged between 2-2.2 mg g^{-1}. Seeds were sieved to 5-6 64^{th} x ¾ to ensure uniform seed mass. Seeds were sterilized with 1% bleach solution then reverse osmosis water.

C3.1.2. Germination Study

To test for germination impacts, 200 seeds were chosen randomly and separated into four 50 seed replicates. Each seed was equally spaced on one paper towel, folded to cover both sides of the seeds, wetted down with reverse osmosis water, and placed inside a plastic bag. The bags were stored in an opaque cover container in a dark refrigerator that was maintained at 4°C. Seeds were checked on every day at the same time of day for germination activity. Each block was taken out of the fridge to account for any temperature change when the seeds were removed for activity. Germination was considered to commence when the radicle was 2-cm long. Germination was represented as a percentage of germinated seeds.

C3.1.3. Early vigour Study

The early vigour study was carried out in a growth chamber with daytime temperature and humidity at 18°C, 60% humidity and night-time temperatures 15°C at 55% humidity. The study was a factorial complete block design with two seed P levels, four genotypes, and two samplings resulting in 64 pots. The pot study was carried out using P limited soil sampled from the Libau 2022 field trial, all soil characteristics and nutrient sources are described in Chapter 3, Table 3-2. The soil was collected from 0-15 cm layer, dried at room temperature, and sieved to 5mm. The soil was homogenized and added to 15-cm diameter pots at a rate of 740 g dry soil per pot. A plastic tray with no holes was set at the bottom of the six pots to prevent water draining.

Ten seeds of each treatment were rinsed with 1% bleach solution then reverse osmosis water. Seeds were placed on moist filter paper in petri dishes. Radicle was showing two days later, and ten seeds placed 2-cm below the surface. Seedlings were thinned to three plants per pot. Pots were watered by weight up to a target of 80% free-drained container water capacity using reverse osmosis water. Pots

were watered every 1-3 days as needed. Every week pots were watered to their target weight and re-randomized within their blocks. Blocks were also randomized to avoid uneven soil drying and edge effects.

C3.1.4. Sampling Protocol

Plants were sampled at five days after germination (DAG) and twelve days after germination. Plants were measured for height by measuring the distance between the base of the soil to the top of the plant. The pots were then rinsed very carefully under water to remove the soil from the root system. Pots were soaked in water prior to rinsing when plants were at the anthesis stage. To measure root length, images were taken against a dark background using a Canon EOS 1000 digital camera with F-stop set to f/25, exposure time at 1/4s and ISO at 200. Digital images were then used to measure the root length of the longest root using ImageJ image analysis software available at (https://imagej.net/ij/index.html) (Schneider et al., 2012). Root lengths are an average of three plants in each replicate. An example of the digital images created is shown in Figure C-6. Plants were cut at the crown of the plant for drying and weighing. Root and shoots were dried at 65°C for 72 hours and then weighed. Plants were cut at the crown of the plant for drying and weighing. Root and shoots were dried at 65°C for 72 hours and then weighed.

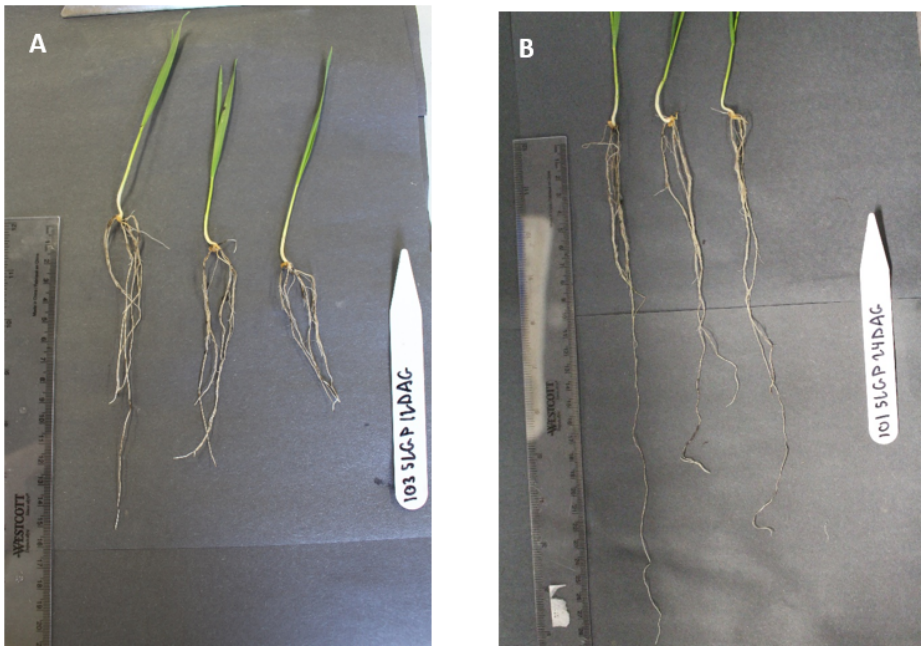


Figure C-6. Example images of wheat root sample at 6 days after germination, A; and 12 days after germination, B; used in ImageJ to measure root length.

C3.1.5. Statistical Analyses

Data was analysed using PROC Mixed procedure with SAS program 9.4 (SAS, 2013a). Tests for normal distribution of data were carried out using PROC Univariate with Shapiro-Wilks values. Values greater than 0.9 were assumed to be normally distributed. Differences among genotypes and seed P levels were tested using the Least Significant Difference (LSD) test and considered significant at $p < 0.05$. Data shown in tables represents the Least Squares Means (lsmeans). To compare the farmer genotypes with the parents, treatments were combined and analyzed into three groups: Farmer genotypes contrasted with both parents, farmer genotypes contrasted with Red Fife, and farmer genotypes contrasted with 5602HR. Contrasts were carried out using PROC GLM procedure in SAS 9.4 (SAS, 2013a).

C3. 2. Results and Discussion

C3.2.1. Germination Study

Germination commenced on day 3, and genotypes reached maximum germination at day seven. There were genotypic differences were detected for Days 4 to 7 (Table C-7) and a genotype x seed P level interaction was observed for Day 4, 5, and 6 (Figure C-7).

Farm1 and Red Fife demonstrated significantly faster germination under high grain P treatment, and Farm2 and 5602HR had faster germination under the low grain P treatment at 4 days after initiation (Figure C-7A). By day 5 after initiation, the majority of Farm1 had germinated, with no difference between seed P levels (Figure C-7B). Other genotypes at day 5 after initiation followed the same pattern as day 4 after initiation. By day 6 after initiation, the seed P levels were not different among any genotype except Red Fife (Figure C-7C). Red Fife's per cent germination with low seed P significantly lower than with high grain P. At day 7 after initiation, all genotypes had reached maximum germination. All genotypes were similarly germinated by day 7, except Farm2, which only reach 79% germination (Table C-7). No interaction was detected by day 7, indicating that under cold conditions by day 7 after initiation, the seed P level did not impact final germination levels.

Farmer genotypes germinated faster than the parental cultivars by 12% 4 days after initiation (Table C-7). This was mainly driven by Farm1. The only other difference between farmer genotypes and parental cultivars was 7 days after initiation, when 5602HR's per cent germination was greater than the farmer selections by almost 6%, however, this was mainly derived from Farm2's poor germination rate.

It is interesting that overall, Farm1 had the fastest germination than all other genotypes, farmers were not selecting for fast emergence, farmers were not rogueing slow emerging seedlings during the selection process. It is not clear why other treatments emerged faster when seed P was low, this may be because the range of seed P chosen for the experiment wasn't different enough to detect consistent

effects, or the seed P levels do not impact germination speed in a significant way. The next steps of this research would be to evaluate seeds at lower seed P levels that we could not obtain. For example, Yugandhar et al. (2022) consider low seed P to be below 1.2 mg g^{-1} using mutants in rice.

Table C-7. Lsmeans and combined analysis of variance comparing percent germination at 4°C among two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) from two different seed P levels.

	3 DAI[α]	4 DAI	5 DAI	6 DAI	7 DAI
Genotype (G)	%	%	%	%	%
Farm1	1.25	48a	83a	89a	91a
Farm2	0	14b	54c	75b	79b
Red Fife	0.42	21b	62bc	82ab	88a
5602HR	0	17b	70b	87a	91a
Genotype *P > F*	0.0536	<.0001	0.0002	0.0066	0.0002
Grain P Level (P)[β]					
(+)P	0.62	26	66	85	88
(-)P	0.21	24	69	82	87
Manure *P > F*	0.2342	0.4797	0.3266	0.2573	0.4625
G x P *P>F*	0.6863	<.0001	<.0001	0.0357	0.5711
Coefficient of Variation (%)	8	56	27	11	6
Farmer Genotypes Lsmeans	0.62	31a	69	82	85b
Parental Cultivars Lsmeans	0.2	19b	66	84	89a
Contrasts					
Farmer Genotypes vs. Parents *P>F*	0.2688	0.0193	0.7067	0.4927	0.0269
Estimate	0.416	12	2.5	-2.2	-4.2
Farmer Genotypes vs. 5602HR *P>F*	0.1780	0.0252	0.8778	0.2660	0.0127
Estimate	0.62	14	-1.2	-4.5	-5.8
Farmer Genotypes vs. Red Fife *P>F*	0.6487	0.1022	0.4442	1	0.2592
Estimate	0.2	10	6.2	0	-2.5

*Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test (P ≤ 0.05);
[α]DAG, Days after initiation; [β]LGP, low grain-P; HGP, high grain-P

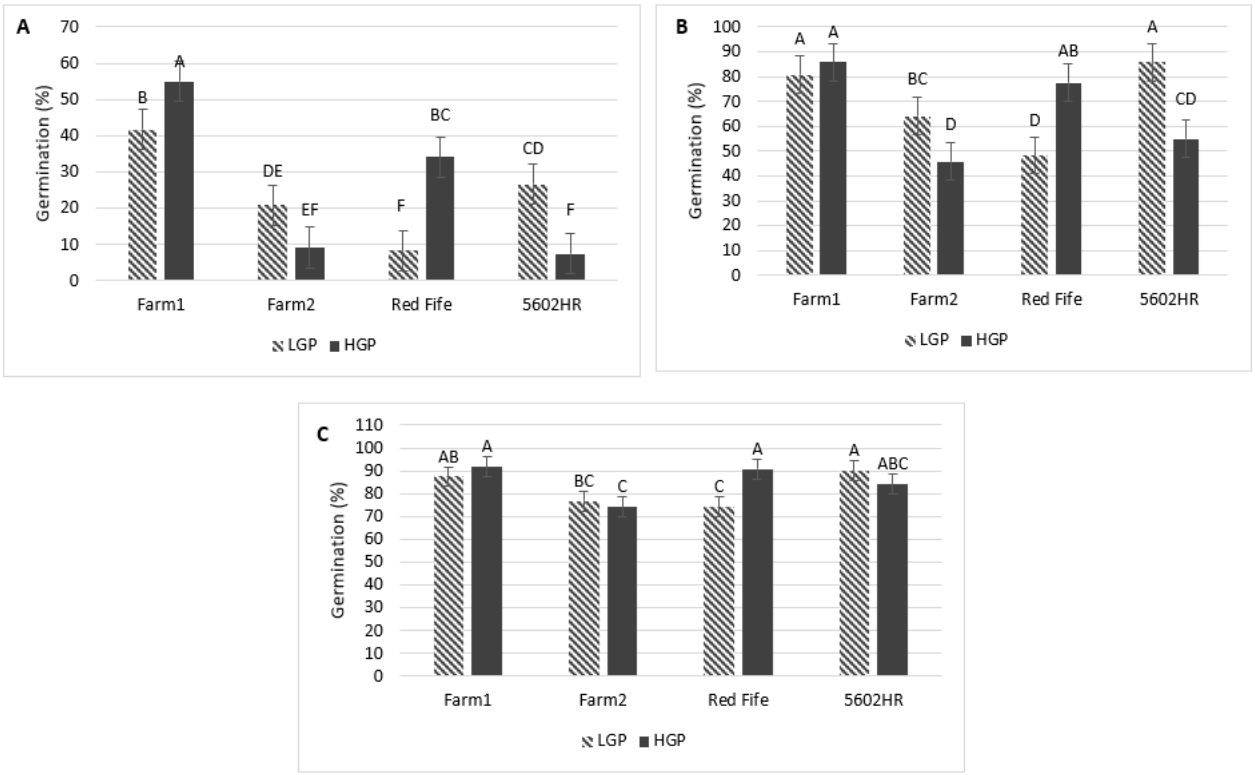


Figure C-7. Genotype x seed P level interaction for per cent germination at 4 days (A); 5 days (B); and 6 days (C) after initiation of the experiment. LGP, low grain-P; HGP, high grain-P.
Means with the same letter are not significantly different within groups of treatments by an analysis of variance test (P≤0.05)

C3.2.2. Early Vigour Study

No genotypic or manure main effects were detected for root and shoot biomass, shoot height, root:shoot ratio, or root length at 5 days after germination (Table C-8). A significant genotype x grain P level was detected for shoot height (Figure 3-8).

No differences between grain P levels were shown for Farm2 and Red Fife, however, Farm1 and 5602HR's shoot heights were significantly greater when grain P level was high (Figure C-8). The results indicate that at 5 days post germination, genotypes responded differently to grain P levels.

When treatments were sampled at 12 days after emergence, there were no significant differences among genotypes for root and shoot biomass, root:shoot ratios, shoot heights, and root lengths (Table C-9). There were manure main effects for shoot biomass and shoot height. Higher grain P levels resulted in shoots that were 5.2 mg greater than low grain P treatments, and 3.1 cm taller than low grain P treatments. This result implies that it may be advantageous for the genotypes to have high grain P for shoot height and biomass at as early as 12 days after germination.

Seed-placed banded phosphorus fertilizer application is a common practice in conventional agriculture, because the benefits of early season supplemental phosphorus nutrition is well known (Grant et al., 2001). However, others have noted that seed mass had a greater impact on early vigour than phosphorus concentration (Derrick and Ryan, 1998). We did not observed differences between grain P levels in all measurements, indicating that some early season vigour traits may be less sensitive to low grain P reserves as others.

C4. Conclusions and Future Directions

Grain P levels may not be as much of a concern for early season vigour for some parameters. We did not observe differences in germination speed, and many root and shoot parameters at 5 and 12 days after germination. However, a significant manure effect was detected for shoot biomass and height 12

days after germination, which would indicate that those plants would have a decrease their competitive ability.

The seed stock we sourced from was a result of grain P deficiency in prior field trials in 2020. Some argue that studies comparing seed reserve levels are from field trials, the poor performance from the low reserves' treatments are an artifact of general stress and not due to the nutrient in question. This can be overcome by using identified mutants with low grain P via low phosphorus harvest index and not due to environmental deficiency, similar to what Julia et al. (2018) demonstrated in rice.

Table C-8. Lsmeans and combined analysis of variance comparing early vigour parameters measured 5 days after germination among two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) from two different seed P levels.

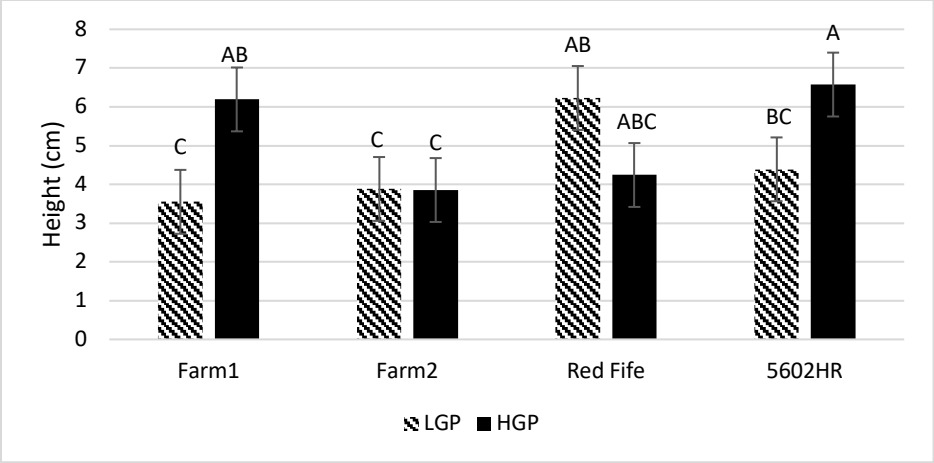
	Root Biomass	Shoot Biomass	Shoot Height	Root: Shoot Ratio	Root Length
Genotype (G)	mg	mg	cm		cm
Farm1	11.9	12.7	4.8	0.96	14.5
Farm2	14.1	12.4	3.8	1.1	15.1
Red Fife	16.2	12.6	5.2	1.3	15.2
5602HR	13.5	12.5	5.4	1.1	13.4
Genotype *P > F*	0.4612	0.9976	0.1322	0.2231	0.3502
Grain P Level (P)[β]					
(+)P	14.2	13.5	5.2	1.1	15.3
(-)P	13.7	11.6	4.5	1.2	13.8
Manure *P > F*	0.8178	0.1378	0.1573	0.487	0.063
G x P *P>F*	0.1408	0.0949	0.0149	0.6298	0.3004
Coefficient of Variation (%)	39	29	38	36	16
Farmer Genotypes Lsmeans	13	12.5	4.3	1.1	14.8
Parental Cultivars Lsmeans	14.8	12.6	5.3	1.2	14.3
Contrasts					
Farmer Genotypes vs. Parents *P>F*	0.3486	0.9747	0.1431	0.2561	0.5558
Estimate	-1.8	-0.004	-0.9	-0.1	0.49
Farmer Genotypes vs. 5602HR *P>F*	0.8218	1.0	0.1776	0.9252	0.1807
Estimate	-0.5	0	-1.1	-0.1	1.4
Farmer Genotypes vs. Red Fife *P>F*	0.1945	0.9587	0.2906	0.0829	0.6928
Estimate	-0.3	-0.008	-0.8	-0.2	-0.4

*Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.05$);
[β]LGP, low grain-P; HGP, high grain-P

Table C-9. Lsmeans and combined analysis of variance comparing early vigour parameters measured 12 days after germination among two spring wheat cultivars (Red Fife and 5602HR) and two spring wheat farmer genotypes (Farm1 and Farm2) from two different seed P levels.

	Root Biomass	Shoot Biomass	Shoot Height	Root: Shoot Ratio	Root Length
Genotype (G)	mg	mg	cm		cm
Farm1	20	24.6	20.2	0.88	21.5
Farm2	16.2	23.2	18.6	0.61	21.3
Red Fife	18.6	24.2	20.3	0.78	22.4
5602HR	20.3	26.3	20.3	0.85	22.2
Genotype *P > F*	0.5759	0.638	0.5007	0.5284	0.9326
Grain P Level (P)$^{\beta}$					
(+)P	20.4	27.5a	21.4a	0.74	22.3
(-)P	17.1	22.3b	18.3b	0.78	21.2
Manure *P > F*	0.1525	0.0053	0.0029	0.5129	0.3011
G x P *P>F*	0.8736	0.2981	0.2612	0.3557	0.3735
Coefficient of Variation (%)	33	23	15	24	17
Farmer Genotypes Lsmeans	18.1	23.9	19.4	0.76	21.4
Parental Cultivars Lsmeans	19.3	25.8	20.3	0.76	22.2
Contrasts					
Farmer Genotypes vs. Parents *P>F*	0.5532	0.3571	0.4080	0.9857	0.5989
Estimate	-1.3	-1.8	-0.9	0.001	-0.7
Farmer Genotypes vs. 5602HR *P>F*	0.4522	0.3381	0.488	0.8088	0.6493
Estimate	-2.0	-2.4	-0.9	-0.02	-0.7
Farmer Genotypes vs. Red Fife *P>F*	0.8292	0.5837	0.5091	0.7862	0.6882
Estimate	-0.05	-1.3	-0.9	0.02	-0.7

*Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.05$); $^{\beta}$LGP, low grain-P; HGP, high grain-P



1 **Figure C-8.** Genotype x seed P level interaction for per cent germination at 4 days (A); 5 days (B); and 6 days (C) after initiation of the experiment. LGP, low grain-P; HGP, high grain-P.
Means with the same letter are not significantly different within groups of treatments by an analysis of variance test (P≤0.05)

Other Tables

Table C-10. Lsmeans and combined analysis of variance comparing phosphorus parameters from 2020 collected under organic conditions in Libau, Manitoba among two spring wheat cultivars (Red Fife and 5602HR) and three spring wheat farmer genotypes (FarmA, FarmB, and FarmC) under limited soil test Olsen-phosphorus (3ppm) and amended with composted manure at 25 kg P ha^{-1}

	Straw P[a] Conc.[₮]	Straw P Acc.[¥]	Grain P Conc.	Grain P Acc.	Total P Acc.	PUptE[b]	PHI[c]	PYE[d]	PRE[e]	GrainN:P Ratio[f]
Genotype (G)	mg g^{-1}	kg ha^{-1}	mg g^{-1}	kg ha^{-1}	kg ha^{-1}	%	%	kg ha^{-1}	kg ha^{-1}	
FarmA	2.2	0.99	2.3bc	5.2	6.2	47	84	405bc	0.04	8.5
FarmB	2.6	0.99	2.4b	5.1	6.2	42	82	381cd	0.047	8.9
FarmC	2.5	1.1	2.2cd	5	6.1	44	82	420ab	0.042	8.7
AAC Scotia	2	0.94	2.1d	5.4	6.3	47	85	449a	0.034	8.6
Norwell	2.6	0.92	2.8a	5.6	6.6	48	85	346d	0.042	7.7
Genotype *P > F*	0.1664	0.9276	<.0001	0.4731	0.7934	0.6286	0.6813	0.0002	0.5914	0.4065
Manure (M)[β]										
(+)P	2.5	1.1	2.5a	6.3a	7.7a	24b	86a	385b	0.037	7.7b
(-)P	2.3	0.87	2.2b	3.9b	4.8b	67a	81b	415a	0.045	9.2b
Manure *P > F*	0.5134	0.0692	<.0001	<.0001	<.0001	<.0001	0.0283	0.0168	0.1246	0.0013
G x M *P>F*	0.0892	0.7017	0.0035	0.1871	0.4858	0.5052	0.3063	0.1258	0.3399	0.0487
Coefficient of Variation (%)	22	32	8	13	12.4	17	5	9	32	16
Farmer Genotypes Lsmeans	2.4	1.02	2.3	5.2	6.2	45	83	402	0.042	8.8
Parental Cultivars Lsmeans	2.3	0.93	2.4	5.5	6.4	47	85	397	0.038	8.2
Contrasts										
Farmer genotypes vs. Parents *P>F*	0.6236	0.4718	0.2068	0.5630	0.6913	0.7813	0.2913	0.7550	0.3867	0.35
Estimate	0.01	0.89	-0.13	-0.35	-0.26	-2.6	-1.98	4.5	0.004	0.59
Farmer genotypes vs. AAC Scotia *P>F*	0.1287	0.6141	0.1369	0.7993	0.8898	0.87	0.4494	0.0173	0.2003	0.8402
Estimate	0.04	0.079	0.2	-0.2	-0.11	-1.9	-1.8	-47	0.008	0.2
Farmer genotypes vs. Norwell *P>F*	0.4384	0.5251	0.0014	0.5090	0.6247	0.7830	0.3582	0.0054	0.9387	0.2047
Estimate	-0.02	0.1	-0.4	-0.5	-0.4	-3.3	-2.2	56	0.0005	1.02

*Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.05$); [β](-)P, limited P treatment; (+)P, P-amended treatment; [a]P, phosphorus; [b]PUptE, Phosphorus Uptake Efficiency; [c]PHI, Phosphorus Harvest Index; [d]PUE, Phosphorus Utilization Efficiency; [e]PRE, phosphorus return efficiency; [f]GrainN:P Ratio, Ratio of Grain N Concentration to Grain P Concentration

[₮]Conc., concentration; [¥]Acc. accumulation

Table C-11. Lsmeans and combined analysis of variance comparing phosphorus parameters from 2020 collected under organic conditions in Libau, Manitoba among two spring wheat cultivars (Red Fife and 5602HR) and three spring wheat farmer genotypes (FarmA, FarmB, and FarmC) under limited soil test Olsen-phosphorus (3ppm) and amended with composted manure at 25 kg P ha^{-1}

	Straw P[a] Conc.[₮]	Straw P Acc.[¥]	Grain P Conc.	Grain P Acc.	Total P Acc.	PUptE[b]	PHI[c]	PYE[d]	PRE[e]	GrainN:P Ratio[f]
Genotype (G)	mg g^{-1}	kg ha^{-1}	mg g^{-1}	kg ha^{-1}	kg ha^{-1}	%	%	kg ha^{-1}	kg ha^{-1}	
FarmA	1.1	0.29	1.6ab	2.7	3	31	90	596bc	0.016	12
FarmB	1.5	0.42	1.7a	2.7	3.1	31	85	557c	0.025	13
FarmC	1	0.38	1.6b	2.6	3	34	87	609b	0.021	12
AAC Scotia	1.3	0.34	1.4c	2.8	3.1	38	88	671a	0.018	13
Norwell	1	0.32	1.67ab	2.7	3	30	89	589bc	0.018	14
Genotype *P > F*	0.0825	0.2556	0.0007	0.9975	0.9950	0.7464	0.1925	0.0003	0.1198	0.0779
Manure (M)[β]										
(+)P	1.1	0.38	1.62	3a	3.4a	11b	88	599	0.012	12.6
(-)P	1.2	0.32	1.59	2.4b	2.7b	50a	88	609	0.2	13.1
Manure *P > F*	0.04597	0.1694	0.5085	0.0206	0.0171	<.0001	0.7695	0.4816	0.7468	0.1484
G x M *P>F*	0.47	0.2192	0.3665	0.4166	0.3940	0.2521	0.2169	0.6716	0.2208	0.2212
Coefficient of Variation (%)	37	34	9	31	28	25	5	7	41	10
Farmer Genotypes Lsmeans	1.2	0.367	1.6	2.7	3.1	33	88	587b	0.021	12.4b
Parental Cultivars Lsmeans	1.18	0.337	1.5	2.7	3.1	29	89	630a	0.018	13.5a
Contrasts										
Farmer genotypes vs. Parents *P>F*	0.8840	0.4854	0.0567	0.9344	0.9867	0.6620	0.5123	0.0027	0.2899	0.0178
Estimate	0.002	0.03	0.08	-0.02	0.005	3.6	-0.99	-43	0.002	-1.05
Farmer genotypes vs. AAC Scotia *P>F*	0.3589	0.7211	0.0007	0.9080	0.9509	0.6314	0.8936	<.0007	0.4104	0.0947
Estimate	-0.016	0.018	0.2	-0.042	-0.02	4.4	-0.2	-84	0.002	-0.9
Farmer genotypes vs. Norwell *P>F*	0.2531	0.4529	0.5471	0.9881	0.9298	0.7620	0.3661	0.9084	0.3891	0.0335
Estimate	0.02	0.039	-0.03	-0.005	0.034	2.8	-1.7	-1.9	0.003	-1.2

*Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.05$); [β](-)P, limited P treatment; (+)P, P-amended treatment; [a]P, phosphorus; [b]PUptE, Phosphorus Uptake Efficiency; [c]PHI, Phosphorus Harvest Index; [d]PUE, Phosphorus Utilization Efficiency; [e]PRE, phosphorus return efficiency; [f]GrainN:P Ratio, Ratio of Grain N Concentration to Grain P Concentration

[₮]Conc., concentration; [¥]Acc. accumulation

Table C-12. Lsmeans and combined analysis of variance comparing phosphorus parameters from 2020 collected under organic conditions in Libau, Manitoba among two spring wheat cultivars (Red Fife and 5602HR) and three spring wheat farmer genotypes (FarmA, FarmB, and FarmC) under limited soil test Olsen-phosphorus (3ppm) and amended with composted manure at 25 kg P ha^{-1}

	Straw P[a] Conc.[₮]	Straw P Acc.[¥]	Grain P Conc.	Grain P Acc.	Total P Acc.	PUptE[b]	PHI[c]	PYE[d]	PRE[e]	GrainN:P Ratio[f]
Genotype (G)	mg g^{-1}	kg ha^{-1}	mg g^{-1}	kg ha^{-1}	kg ha^{-1}	%	%	kg ha^{-1}	kg ha^{-1}	
FarmA	4.3	2.6	3.1b	8.2	10.8	79	76	250abc	0.097	7.9
FarmB	4.5	2.9	3.4a	8.9	12.1	85	74	220c	0.11	7.1
FarmC	4.4	2.8	3bc	8.5	11.5	75	77	259ab	0.095	7.7
AAC Scotia	4.1	3	2.7c	8.2	10.9	74	72	269a	0.11	8.4
Norwell	5.2	2.3	3.3a	8.2	10.5	74	77	327bc	0.096	8
Genotype *P > F*	0.4421	0.6841	0.0035	0.9319	0.6709	0.5397	0.7448	0.0449	0.8303	0.0519
Manure (M)[β]										
(+)P	4.9a	3.6a	3.3a	10.9a	14.5a	45b	74	222b	0.12a	6.9b
(-)P	4.1b	1.8b	2.8b	5.9b	7.8b	109a	76	272a	0.087b	8.8a
Manure *P > F*	0.0293	<.0001	0.0001	<.0001	<.0001	<.0001	0.4764	<.0001	0.0351	<.0001
G x M *P>F*	0.08577	0.3394	0.5885	0.7238	0.4668	0.363	0.6377	0.5185	0.5983	0.6036
Coefficient of Variation (%)	31	38	11	22	19	18	10	12	43	10
Farmer Genotypes Lsmeans	4.4	2.8	3.2	8.6	12	76	76	241	0.103	7.5
Parental Cultivars Lsmeans	4.5	2.6	3	8.2	11	76	75	256	0.103	8.3
Contrasts										
Farmer genotypes vs. Parents *P>F*	0.7987	0.5512	0.2259	0.6824	0.3526	0.9590	0.8407	0.2644	0.9955	0.1198
Estimate	-0.011	0.28	0.2	0.43	1.3	0.64	0.5	-14	0	-0.66
Farmer genotypes vs. AAC Scotia *P>F*	0.3438	.9630	0.0143	0.7551	0.4576	0.9377	0.4019	0.0571	0.7329	0.1011
Estimate	-0.05	0.028	0.4	0.42	1.3	-1.2	2.6	-33	-0.006	-0.87
Farmer genotypes vs. Norwell *P>F*	0.1631	0.3611	0.5365	0.7356	0.4632	0.8685	0.5768	0.8207	0.7266	0.4031
Estimate	-0.081	0.54	-0.1	0.45	1.2	2.5	-1.6	3.6	0.006	-0.44

*Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.05$); [β](-)P, limited P treatment; (+)P, P-amended treatment; [a]P, phosphorus; [b]PUptE, Phosphorus Uptake Efficiency; [c]PHI, Phosphorus Harvest Index; [d]PUE, Phosphorus Utilization Efficiency; [e]PRE, phosphorus return efficiency; [f]GrainN:P Ratio, Ratio of Grain N Concentration to Grain P Concentration

[₮]Conc., concentration; [¥]Acc. accumulation

Table C-13. Lsmeans and combined analysis of variance comparing belowground microbial activity in 2020 cllectedd under organic conditions in Libau, Manitoba among two spring wheat cultivars (Red Fife and 5602HR) and three spring wheat farmer genotypes (Farm1, Farm2, and Farm3) under limited soil test Olsen-phosphorus (3ppm) and amended with composted manure at 25 kg P ha^{-1}

	Rhizosphere Acid Phosphatase	Rhizosphere WEP[ŦΑ]	Arbuscule Infection	Hyphae	Arbuscules + Hyphae
Genotype (-P,+P)[β](G)	pmol MUF g^{-1} soil h^{-1}	mg P kg^{-1} soil	%	%	%
FarmA	758	8	6.5	5.8	12
FarmB	1938	7	8.6	6.3	15
FarmC	521	12	3.8	5.2	9
AAC Scotia	430	10	8.3	7.2	15
Norwell	1001	12	10.9	4.5	15
Genotype *P > F*	0.1269	0.9207	0.1384	0.5769	0.2902
Manure (M)[β]					
(+)P	806	12	7.7	4.3b	12
(-)P	1053	8	7.5	7.3a	15
Manure *P > F*	0.6339	0.2931	0.8792	0.0137	0.1863
G x M *P>F*	0.4740	0.7084	0.7437	0.8109	0.5926
Coefficient of Variation (%)	11.7	51	52	55	39
Farmer Genotypes Lsmeans	1072	9	6.2b	5.6	12b
Parental Cultivars Lsmeans	716	10	9.6a	5.8	16a
Contrasts					
Farmer genotypes vs. Parents *P>F*	0.2868	0.7921	0.0354	0.8407	0.0870
Estimate	356	-0.95	-3.3	-0.24	-3.63
Farmer genotypes vs. AAC Scotia *P>F*	0.1032	0.9689	0.2859	0.2930	0.1593
Estimate	641	-0.3	-2.1	-1.63	-3.7
Farmer genotypes vs. Norwell *P>F*	0.9767	0.6605	0.0219	0.4592	0.1795
Estimate	70	-1.5	-4.6	1.1	-3.5

*Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.10$)

[Ŧ]WEP, water extractable phosphate; [α]data natural log-transformed; [β](-)P, Limited P treatment; (+)P, P-amended treatment

Table C-14. Lsmeans and combined analysis of belowground microbial activity in 2021 conducted under organic conditions in Libau, Manitoba among two spring wheat cultivars and three spring wheat farmer genotypes under limited soil test Olsen-phosphorus (3ppm) and amended with composted manure at 25 kg P ha^{-1}

	Rhizosphere Acid Phosphatase	Rhizosphere WEP[Ŧα]	Arbuscule Infection	Hyphae	Arbuscules + Hyphae
Genotype (-P,+P)[β](G)	pmol MUF g^{-1} soil h^{-1}	mg P kg^{-1} soil	%	%	%
FarmA	483	1.4	10.2	17a	27
FarmB	363	2.9	5.9	9c	15
FarmC	290	0	6.6	14ab	20
AAC Scotia	1606	3	7.2	12bc	19
Norwell	851	8	9.3	13ab	23
Genotype *P > F*	0.7315	0.5019	0.3810	0.0501	0.1162
Manure (M)[β]					
(+)P	558	3.2	5.7b	12.2	18b
(-)P	879	2.4	10a	13.8	24a
Manure *P > F*	0.9619	0.5627	0.0172	0.3324	0.0600
G x M *P>F*	0.8266	0.2675	0.0541	0.0092	0.0140
Coefficient of Variation (%)	18	178	73	44	50
Farmer Genotypes Lsmeans	361	1	7.6	13.1	20.7
Parental Cultivars Lsmeans	1229	5	8.3	12.7	21
Contrasts					
Farmer genotypes vs. Parents *P>F*	0.1855	0.2976	0.7325	0.8297	0.9436
Estimate	-867	-4.2	-0.6	0.4	-0.25
Farmer genotypes vs. AAC Scotia *P>F*	0.1878	0.6922	0.8735	0.6198	0.7193
Estimate	-1245	-2.5	0.4	1.3	1.7
Farmer genotypes vs. Norwell *P>F*	0.4193	0.1879	0.4580	0.8424	0.6041
Estimate	-489	-5.8	-1.78	-0.4	-2.2

*Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test ($P \leq 0.10$)

[Ŧ]WEP, water extractable phosphate; [α]data natural log-transformed; [β](-)P, Deficient P treatment; (+)P, Amended P treatment

Table C-15. Lsmeans and combined analysis of belowground microbial activity in 2022 conducted under organic conditions in Libau, Manitoba among two spring wheat cultivars and three spring wheat farmer genotypes under limited soil test Olsen-phosphorus (3ppm) and amended with composted manure at 25 kg P ha^{-1}

	Rhizosphere Acid Phosphatase	Rhizosphere WEP[Ŧα]	Arbuscule Infection	Hyphae	Arbuscules + Hyphae
Genotype (-P,+P)[β](G)	pmol MUF g^{-1} soil h^{-1}	mg P kg^{-1} soil	%	%	%
FarmA	301	6.9	17	10a	27.2
FarmB	341	12.2	12	6b	18.5
FarmC	546	12.3	13	5b	18.5
AAC Scotia	338	9.3	14	6b	20.3
Norwell	309	8.9	16	7ab	22.8
Genotype *P > F*	0.6104	0.5437	0.6979	0.0912	0.3762
Manure (M)[β]					
(+)P	369	10.4	14.9	6.7	21.6
(-)P	365	9.3	14.2	7	21.3
Manure *P > F*	0.2284	0.3662	0.7957	0.7661	0.9242
G x M *P>F*	0.4064	0.3451	0.6156	0.6454	0.8075
Coefficient of Variation (%)	8.7	40	49	50	43
Farmer Genotypes Lsmeans	393	11.6	14.2	7.2	21.4
Parental Cultivars Lsmeans	323	9.2	15.2	6.4	21.5
Contrasts					
Farmer genotypes vs. Parents *P>F*	0.6884	0.2996	0.7183	0.5001	0.9653
Estimate	69	2.3	-0.9	0.76	-0.13
Farmer genotypes vs. AAC Scotia *P>F*	0.7942	0.3571	0.8877	0.4108	0.6892
Estimate	54	2.3	0.4	1.2	1.6
Farmer genotypes vs. Norwell *P>F*	0.7194	0.4543	0.4987	0.8066	0.6585
Estimate	84	2.4	-2.2	0.3	-1.8

*Means within the same column followed by the same letter are not significantly different within groups of treatments by an analysis of variance test (P ≤ 0.10)

[Ŧ]WEP, water extractable phosphate; [α]data natural log-transformed; [β](-)P, Limited P treatment; (+)P, P-amended treatment

LIST OF ABBREVIATIONS

(-)P	Deficient P treatment
(+)P	Amended P treatment
AAFC	Agriculture and Agri-Food Canada
AEA	AEC abscissa
Al	Aluminum
AMF	Arbuscular mycorrhizal fungi
AMMI	Additive main effects and multiplicative interaction
ANOVA	Analysis of Variance
Apase	Acid phosphatase enzyme activity
β_i	G x E regression coefficient
b_i	Environmental regression coefficient
C	Carbon
Ca	Calcium
CAR	Carman
CV	Coefficient of variation
CWRS	Canadian Western Red Spring
DAG	Days after germination
DAI	Days after initiation
E	Environment
EDM	Edmonton
EU	European Union
FHB	Fusarium head blight
Fe	Iron
G	Genotype
g	grams
GEI	Genotype x environment interactions
GGE	Genotype plus genotype by environment
HGP	High grain P
HI	Harvest index
K	Potassium
KNO:Dma	Kernel production efficiency at anthesis
LGP	Low grain P
LIB	Libau
LSD	Least significant difference
LSMeans	Least squares means

M	Manure
MAFRD	Manitoba Agriculture, Food and Rural Development
Mg	Magnesium
Mn	Manganese
N	Nitrogen
NO_3-N	Nitrate
NUE	Nitrogen use efficiency
OXB	Oxbow
P	Phosphorus
PC	Principle component
PHI	Phosphorus harvest index
P_i	Inorganic phosphate
PLS	Partial least squares
PPB	Participatory Plant Breeding
ppm	parts per million
PRE	Phosphorus return efficiency
PUE	Phosphorus use efficiency
PUptE	Phosphorus uptake efficiency
PYE	Phosphorus yield efficiency
QTL	Quantitative trait loci
Rh	Rhizosphere
ROB	Roblin
S^2d_i	Deviations from the regression line
SE	Standard Error
SO_4	Sulfate
SOM	Soil organic matter
STP	Soil test phosphorus (Olsen-P)
σ_i^2	Stability variance
WEP	Water extractable phosphate
Wi^2	Wricke's ecovalence