Identifying Quantitative Trait Loci (QTL) Associated with Lodging Resistance in *Brassica napus* L.

By

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A Thesis Submitted to the Faculty of Graduate Studies of

The University of Manitoba

In Partial Fulfillment of the Requirements of the Degree of

MASTER OF SCIENCE

Department of Plant Science

University of Manitoba

Winnipeg, Manitoba

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ACKNOWLEDGEMENTS

First and foremost, I would like to express my deepest gratitude to my parents, Dương and Xuân, for their unwavering love and support throughout my master's degree. Their profound influence and encouragement, along with that of my siblings Đức and Minh, have been instrumental in my academic pursuits. Additionally, I extend my heartfelt appreciation to my girlfriend, Thanh, for her tremendous support and encouragement. Without their belief in me, I might not have pursued studies at the University of Manitoba, nor would I have found the courage to envision it as a possibility.

My sincere gratitude goes to my advisor, Dr. Robert W. Duncan, for mentoring and support during my master's journey, and for allowing me to gain invaluable plant breeding experience. I also thank to my committee members, Dr. Curt McCartney and Dr. Jason Morrison, for their invaluable time and guidance, which have played a crucial role in my research process.

I want to thank Dr. Harmeet Chawla, who provided support with a Python script that helped with filter genetic data and bioinformatics knowledge and guided me throughout the linkage map construction. I am also thankful to Dr. Robert Gulden, who advised me on the statistics analysis.

I am also grateful to our field and lab technicians, Ms. Judith Nugent-Rigby, Mr. Rob Visser, Mr. Mike Erb, and Mr. Ralph Kowatsch, for their help in my research experiments. Special thanks to

Dr. Isobel Parkin and Ms. Erin Higgins from Agriculture and Agri-Food Canada in Saskatoon for their help in genotyping my samples.

I want to acknowledge the help of all my peers and friends from the Department of Plant Science, particularly Kenny So, Yong Liu, Sonia Wilson, and Lauren Gislason for their help and encouragement during my studies.

Finally, I would like to thank Nutrien Ag Solution, Bunge, NSERC, Agriculture and Agri-Food Canada, and all the scholarships available within the Faculty of Agricultural and Food Sciences for their support. Without this assistance, completing this degree would not have been possible.

"Bác học không có nghĩa là ngừng học"

This quote is originally from the dialogue between Charles Robert Darwin and his father. It can loosely be translated as

"Learning never stops, even when you are an expert in your field of study."

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ABSTRACT

Brassica napus L. (canola and rapeseed) is the world's second-largest oilseed crop, and it has significant economic importance to Canadian agriculture. However, lodging in canola is a significant agronomic issue, making maintaining high yield and oil quality in *B. napus* difficult. Discovering genetic regions associated with lodging resistance is essential to understand the mechanism controlling lodging. In this study, quantitative trait loci (QTL) analysis using the composite interval mapping method (ICIM) was conducted using two different doubled haploid (DH) populations. Genomic regions that govern lodging resistance, flowering date, plant height, and maturity date were successfully identified. Within the two populations (Z/B and L/R, which correspond to chapters three and four), QTLs associated with lodging explained 3.34 to 17.31 % and 5.6 to 11.52 % of phenotypic variation, respectively. QTL for lodging were identified on A01, A03, A05, A07, A10, and C01 in the Z/B population, and on A05, C02, C04, C05, and C07 in L/R population. Co-localization of QTL for lodging (LODG), day-to-flower (DTF), plant height (HT), and day-to-maturity (DTM) were found on A01 in Z/B, while no co-localization of QTL between different traits were observed in L/R. Most QTL identified for lodging were aligned with QTL for stem lignin, stem hemicellulose, branch number, sclerotinia stem rot, plant height, stem diameter, and stem breaking force in previous studies. Several of these QTL identified in this study appeared novel, which could be useful for future breeding programs to enhance lodging resistance in *B. napus,* resulting in improved yield and oil quality. QTL for lodging identified in the current study with phenotypic variation explained over 10 % were utilized to screen for potential candidate transcripts. Transcripts involved in plant cell wall biosynthesis/ modification, root development, cellulose activities, ethylene, auxin gibberellic acid and brassinosteroid signaling are promising candidate genes.

1. General Introduction

Brassica napus L. (canola or rapeseed) is the world's second-largest oilseed crop after soybean (Maheshwari et al., 2011). The estimated global production of this crop in 2023 reached approximately 86.9 million metric tons (USDA, 2023). Canada is one the world's largest producers, accounting for 1/6 of the global production (FAOSTAT, 2022). In Canada, the canola industry contributes an average of 29.9 billion Canadian dollars annually to the Canadian economy (LMC International, 2020). These statistics demonstrate the importance of this crop, especially in Canada. In addition, the demand for oil from this crop is anticipated to increase in the coming decades, indicating a need for increased production (Scheben et al., 2019). However, poor lodging resistance can limit canola seed yield.

Crop lodging is defined as the permanent displacement of the stem from its vertical growth habit and commonly occurs in cereal and canola crops (Pinthus, 1974; Wu et al., 2021a). Lodging can be further categorized into stem and root lodging. Stem lodging is described as the breaking or bending of the stem due to excessive bending (Berry et al., 2004), while root lodging refers to the anchorage failure of the root system in the soil (Baker et al., 2014). Not only does lodging affect canola yield, but it also affects canola seed quality by altering its fatty acid composition while increasing erucic acid and glucosinolate content (Khan et al., 2018). Therefore, maintaining lodging resistance in *B. napus* is essential to optimize yield while retaining high-quality canola oil.

Many stem- and root-lodging-related traits have been studied to understand the genetics controlling lodging resistance. For stem lodging, characteristics such as plant height (Xue et al., 2017), stem strength (Lemloh et al., 2014; Miller et al., 2018), mass density (Shah et al., 2017),

stem diameter (Wu & Ma, 2022), and stem wall thickness (Tripathi et al., 2003) were found to be related to lodging and can be utilized as criteria for lodging resistance selection. Characteristics related to root lodging have also been explored. Root lodging-related traits include root anchorage strength (Sposaro et al., 2008), root depth (Dreccer et al., 2020), root plate spread (Piñera-Chavez et al., 2016), root diameter (Kuai et al., 2017), root-soil cone (Li et al., 2023), root length (Sposaro et al., 2008) and root growth angle (Bian et al., 2016).

Apart from understanding the phenotypic variation, understanding the genetic control for lodging is also essential for improving lodging resistance and the value of *B. napus*. Presently, quantitative trait loci (QTL) for plant height (Miller et al., 2018; Shen et al., 2018), stem diameter (Li et al., 2018) and stem strength (Miller et al., 2018) have been explored, as these traits are known to be associated with lodging. Reports have shown that candidate genes involved in lodging resistance included gibberellin, brassinosteroid, auxin, flower development, phosphate ion transmembrane, gibberellin, cellulose biosynthesis, pectin methylesterase and lignin biosynthesis (Li et al., 2016; Li et al., 2018; Shen et al., 2018; Wei et al., 2017). Despite that, the genetic control of lodging resistance in *B. napus* remains limited. This demonstrates that lodging is a complex trait that involves the interaction between many genes and is highly influenced by environmental conditions and agronomic practices (Baker et al., 2014; Canola Council of Canada, 2020). Thus, further research to validate and explore new genomic loci associated with lodging resistance in *B. napus* is critical for breeding efforts.

The overall objective of this research is to identify genomic regions that are associated with lodging resistance in *B. napus* by conducting a QTL analysis. It is hypothesized that additional

QTL associated with lodging can be determined using *B. napus* genotypes that have not been utilized for lodging screening. Once QTL are found, they can help further validate QTL associated with lodging previously identified in other research. Moreover, the novel QTL detected in this study could aid future breeding programs to improve lodging resistance in *B. napus,* ultimately leading to improved yield and quality in canola production. To test this hypothesis, the following objectives were targeted:

(1) Phenotype lodging (by visual rating using a 1 to 5 scale) using two different and unrelated doubled-haploid (DH) populations derived from a cross between lodging susceptible and lodging resistant parents.
(2) Genotype these DH genotypes and their respective parents using the *Brassica* 60K SNP array.
(3) Identify quantitative trait loci (QTL) for lodging resistance in *B. napus*.

2. Literature Review

2.1. Background information

2.1.1. Introduction of *Brassica* genus/species

The mustard family (*Brassica*ceae or Cruciferae) consists of 338 genera and 3709 species, covering many horticulture crops, some of which are economically essential and consumed worldwide (Cartea et al., 2011; Warwick et al., 2006). Within the family, the *Brassica* genus is considered one of the most financially significant crop species in the mustard family, as they can be classified into oilseeds, vegetables, condiments, and forage crops (Cartea et al., 2011). Six major *Brassica* species are cultivated worldwide; these species can either exist as diploids (*Brassica rapa* L. (AA), *Brassica nigra* (L.) W.D.J. Koch (BB), and *Brassica oleracea* L. (CC)), or allotetraploids (*Brassica napus* L. (AACC), *Brassica juncea* (L.) Czern. (AABB), and *Brassica carinata* A. Braun (BBCC)) forming the "Triangle of U" (Hayward et al., 2012) (**Figure 2.1**). The primary vegetable species is *B. oleracea*, which includes vegetables like kale, brussel sprout, cabbage, broccoli, and cauliflower; *B. rapa* consists of vegetables like turnips, pak choi, Chinese cabbage, and oilseed types; *B. napus* is mainly used as oilseed and accounts for 14% of oilseed production globally. Lastly, *B. carinata*, *B. nigra*, and *B. juncea* are widely used as condiments, though some regions of the world consume *B. juncea's* leaves as vegetables (Cartea et al., 2011; Hayward et al., 2012).

2.1.2. History of Rapeseed and Canola (*B. napus* and *B. rapa*)

While *B. rapa* is a diploid crop species (AA, 2n=2x=20), *B. napus* is an alloploid species with 2n=4x=38 that formed approximately 7500 years ago from natural hybridization between *B. rapa* and *B. oleracea* (CC, 2n=2x=18) (Chalhoub et al., 2014; Wang et al., 2011). It was well-established

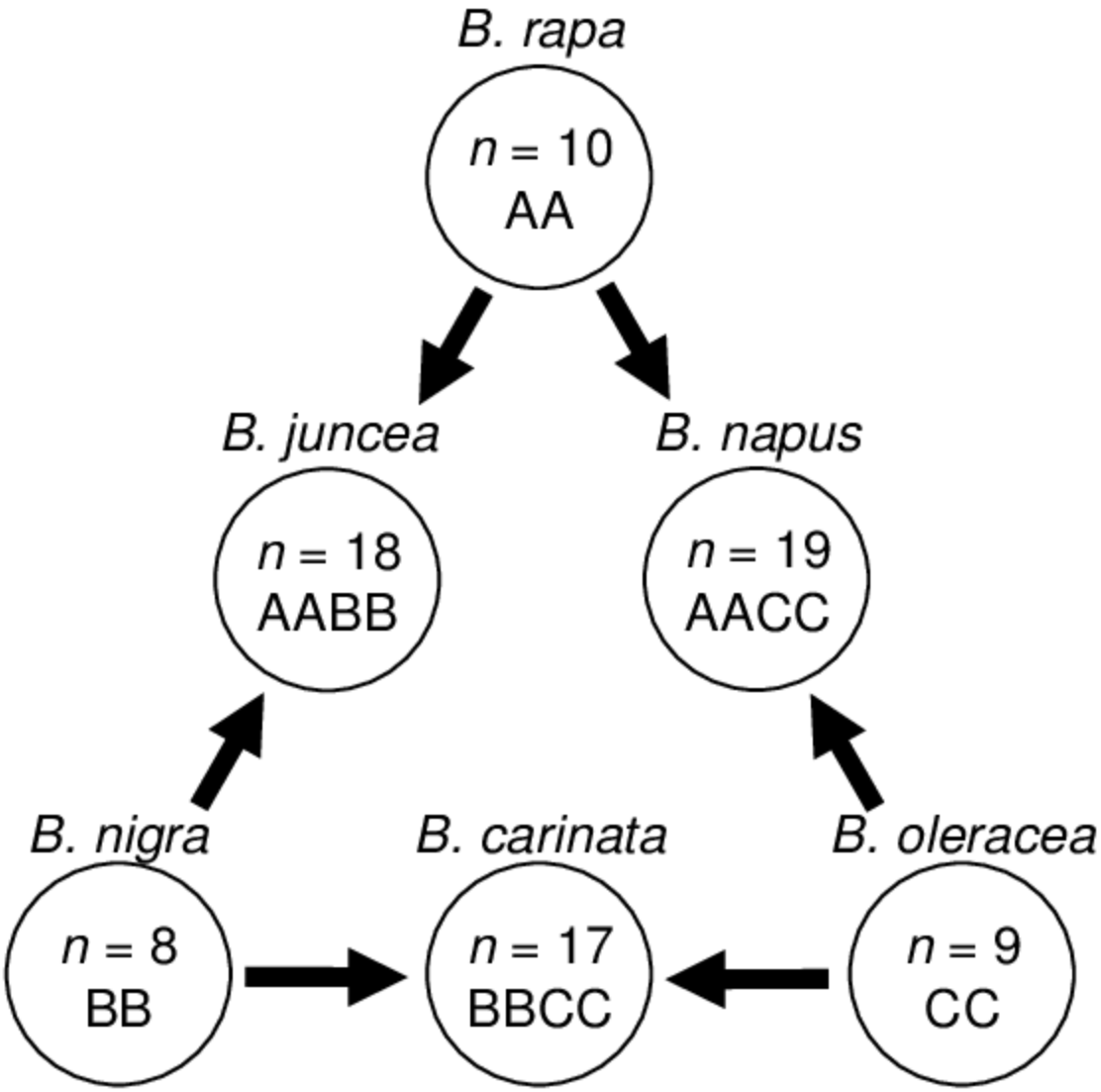

Figure 2.1: The "Triangle of U", a representation of the genetic relationships between the six cultivated species of Brassica species. Adapted from (U, 1935).

that *Brassica* oilseed (*B. rapa* and *B. napus)* has been extensively cultivated throughout Asia, Europe, and Northwestern Africa as edible oil for food and industrial applications (Government of Canada, 2017). However, *Brassica* oilseed was unknown in Canada and other areas of North America until World War II. The first *Brassica* oilseed grown in Canada was *B. rapa* brought to Canada by an immigrant farm family from Poland in 1928 (Daun, 2011). The invention of the steam engine further expanded the usage of this seed oil for water-washed components (Gulden et al., 2008). As a result, *B. napus* and *B. rapa* were first commercially grown in Canada in the 1940s as lubricant oil for naval and merchant ships' engines to serve the war effort in Europe (Craig, 1971; Luc Casséus, 2009). At the same time, because of the war, *Brassica* oilseed could not be sourced from Europe, so oilseed (*B. napus)* was obtained from Argentina instead (Daun, 2011). Hence, the early Canadian *Brassica* oilseed cultivars were descended from two seed sources: *B. rapa* from Poland and *B. napus* from Argentina (Gulden et al., 2008). The wide range of usages and applications of *B. rapa* and *B. napus* worldwide have shown that these two *Brassica spp.* are the most valuable crops within the mustard family.

When World War II ended, Western Canada had produced significant amounts of oil; however, the demand for oilseed as a lubricant had dramatically declined as steam engines were gradually replaced with diesel engines (Craig, 1971; Government of Canada, 2017). This resulted in an economic loss from oilseed production. By 1950, the Canadian government had taken the initiative to adopt *Brassica* oilseed as vegetable oil. The versatile properties of *Brassica* oilseed have mitigated its impact on the Canadian economy by transitioning from its initial use as a lubricant oil to becoming a pivotal source of edible oil. The oil from this crop has a low level of saturated fat, rapid maturation, relative ease of harvest without specialized equipment, and well adapted to

the Canadian Prairies (Daun, 2011). During this time frame, especially before 1970, *Brassica* oilseed cultivars had high levels of erucic acid in the oil, ranging from 20 – 40 % (Daun, 1983). This high-level erucic acid rapeseed raised concern when studies demonstrated a negative effect on rats' health, especially on heart health (Bell, 1982; Sauer & Kramer, 1983). As a result, the Food and Drug Directorate of the Department of National Health and Welfare decided to prohibit the sale of rapeseed oil as edible oil (Bell, 1982). However, due to the importance of the rapeseed industry to the Canadian economy, immediate actions were taken to breed rapeseed for reduced low erucic acid (Bell, 1982). Significant breeding efforts were made between 1960 and 1975 to lower erucic acid content to less than 5 % in the oil (Daun, 1983; Sauer & Kramer, 1983). The first *B. napus* with low erucic acid, called Oro was released in 1969. However, this variety was not extensively grown due to poor agronomic and low yield performance (Canola Council of Canada, 2023c; Daun, 1983). In 1971, breeding programs from the Agriculture and Agri-Food Canada Research Station in Saskatoon and the University of Manitoba started to develop two new varieties, 'Zephyr' and 'Span' using genotypes with low erucic content; the release of these new varieties was to replace low-yielding varieties of 'Midas' and 'Torch' (Daun, 1983).

The decrease in erucic acid resulted in superior oil quality, but the utilization of the meal component of oilseed rape remained a challenge. The glucosinolate content in oilseed rape meal was adequately high, which limited its usage as a high-protein supplement in animal diets, particularly in poultry and monogastric animals (Fenwick, 1982). Hence, efforts to breed for low glucosinolate began. The low-glucosinolate gene was integrated into Canadian breeding lines thanks to Dr. Jam Krzymanski, who detected low-glucosinolate genotypes in his collections of *B. napus* germplasm in 1967 (Krzymanski, 1974). Using these Canadian breeding lines with low

glucosinolate, Dr. Baldur Stefenasson from the University of Manitoba successfully developed and released the *B. napus* cultivar "Tower" in 1974. Tower was the first registered *B. napus* cultivar carrying low erucic acid in its oil profile and low glucosinate in its meal, also known as "double low" (Stefansson & Kondra, 1975). Today, "double low" oilseed rape cultivars are referred to as canola in many parts of the world. To be considered canola, oilseed rape needs to come from the *Brassica* genus (*B. napus*, *B. rapa* or *B. juncea*), in which the extracted oil should have less than 2 % of erucic acid in its fatty acid composition and have less than 30 micromoles of glucosinolate per gram of air-dry, oil-free solid (Canola Council of Canada, 2023c). Modification of fatty acid compositions has also been conducted for the last few decades to improve the functional and nutritional values of canola oil. For instance, a demand for the reduction of linolenic acids and an increase in linoleic (to shorten hydrogenation time and increase oil stability) led to the development of the canola cultivar "Stellar" (Scarth et al., 1988). Decades of breeding have further improved oilseed rape quality and increased the demand for this crop as an edible oil and protein supplement.

Apart from improving the quality of canola as an edible oil, efforts have been made to increase its erucic acid composition for industrial purposes. High erucic acid rapeseed (HEAR) is a term used to describe rapeseed cultivars that have high erucic acid in their fatty acids profile (>50%) and low glucosinolate contents in their meal (McVetty et al., 2016). Erucic acid is primarily used to produce erucamide, which is used as a slip agent for plastic films (Raboanatahiry et al., 2021). Derivatives from erucic acid can also be used for other purposes, such as in detergent production, hair products, food emulsifiers, the production of photographic material, and ingredients in soap making (Raboanatahiry et al., 2021). Reston was the first HEAR cultivar developed and released in 1982.

Reston has between 40 % - 45 % erucic acids in its oil profile and low glucosinolate content in its meal (Zelmer & McVetty, 2009). Diverse breeding targets have further expanded *Brassica* oilseed usage to more varied applications, as discussed below.

2.2. Canola Properties

Canola's oil properties make it ideal for various industrial applications and products. Triacylglycerols/triglycerides (94-99%) are the primary components of canola oil; they also contain high levels of plant sterols, which have been shown to decrease cholesterol (Eskin & Przybylski, 2003). The fatty acids found in canola include palmitic acid (C16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (C18:2), alpha-linolenic acid (C18:3) and erucic acid (22:1) (Eskin & Przybylski, 2003; Siemens & Daun, 2005). Its fatty acid profile of approximately 60 % oleic and 10 % linoleic acid gives it high nutritional and dietary value (Obermeier & Friedt, 2015). Regarding oleic acid concentration, canola oil ranks second to olive oil (Eskin & Przybylski, 2003). Vital fatty acids such as linoleic and linolenic acids are critical nutrients in controlling cardiovascular disease (Eskin & Przybylski, 2003). Canola oil is also suitable for producing high-quality biodiesel as it contains a relatively high level of mono-unsaturated fatty acids (oleic acid) and low quantities of polyunsaturated fatty acids (linoleic acid, alpha-linolenic acid) (Daun, 2011; Obermeier & Friedt, 2015).

2.3. Canola Meal

After canola oil extraction, the remaining product is known as the meal. Canola meal contains a small amount of oil, glucosinolates, phenolics, phytates, and a significant level of fibre, lowering the quality of the meal (Tan et al., 2011). The primary phenolic compounds in canola seed are

sinapate ester, which contributes to the dark colour, bitter taste, and astringency in the meal and the extracted proteins (Kozlowska et al., 1990; Tan et al., 2011). Despite containing anti-nutritional compounds, canola meal has an excellent amino acid profile and the highest protein efficiency ratio among all plant-based proteins, making it a desirable source of proteins for both animals and humans (Khattab & Arntfield, 2009).

2.4. *Brassica* Oilseed-End-Use Product

Today, oilseed rape is used in a wide range of industries to provide edible oils for humans, feeding proteins for both humans and livestock, biodiesel production, and renewable material (e.g. hydraulic fluid or lubricant oil) (PNW Canola Association, 2022). In human food, it is commonly used as a cooking fat and is a common ingredient in many food products such as margarine, mayonnaise and baked goods (Canola Council of Canada, 2023b). In addition, canola oil can also be used in salad dressings and processed foods due to its mild flavour and a good source of vitamin E (Canola Council of Canada, 2023a). Canola meal's nutritional content also provides several health benefits as an animal feed. Canola meal has beneficial functional qualities such as emulsifying, foaming, and gelling; hence, canola protein isolate has been proposed as an alternative to other proteins for human consumption (Tan et al., 2011). The seed also contains dietary fibre and several vitamins and minerals, including potassium, copper, iron, manganese, and zinc (Wickramasuriya et al., 2015). For industrial applications, canola oil is also used to produce biofuels to lower the use of fossil fuels (Canola Council of Canada, 2023a). By replacing petroleum-based fuels with plant-based alternatives, the consumption of canola oil can help reduce greenhouse gas emissions and address climate change.

2.5. Brassica Oilseed Production

Since the first canola-quality *Brassica* was released, it has significantly contributed to the edible oil industry. *Brassica* oilseed has become the world's second-largest oilseed crop after soybean (Maheshwari et al., 2011). The estimated global production of this crop in 2023 was around 86.9 million metric tons (USDA, 2023). Today, canola is one of the primary crops grown in Canada and the European Union. Canada contributes 1/6 of the global production, contributing an average of 29.9 billion Canadian dollars annually to the Canadian economy (FAOSTAT, 2022; LMC International, 2020).

2.6. Brassica Oilseed Production Issues

The demand for oil from this crop is expected to rise over the next few decades, implying that increased production per land unit is needed (Scheben et al., 2019). The rise in the demand for canola production is likely due to the increased world population and the widespread use of canola in many commercial products and industrial applications (Scheben et al., 2019; Wu et al., 2022). However, canola is susceptible to potential damage caused by weather conditions, pests, and diseases annually (Government of Manitoba, 2023). These production challenges can influence plant lodging and negatively affect the crop's potential yield and quality. Further details on how these factors impact lodging is discussed below.

2.7. Introduction of Lodging

Crop lodging is defined as the permanent displacement of the stem from its vertical growth habit and commonly occurs in cereal and canola crops (Pinthus, 1974; Wu et al., 2021a). It has been categorized into stem and root lodging from a breeding perspective. Stem lodging refers to the

breaking or bending at any point along the stem due to excessive forces (Wu & Ma, 2016). Stem lodging is caused primarily by previous tissue damage from insects and diseases or by physical force from storms and hail that break the stem's basal internodes (Pinthus, 1974; Wu et al., 2022). On the other hand, root lodging refers to the failure of the root-soil anchorage system (Wu et al., 2022). Root lodging risk is determined by several factors, including (1) physical forces applied to the plant, (2) the resistance of the root from bending, and (3) the resistance of soil to lateral compression caused by the rotation of the root-soil plate when external forces applied (Wu et al., 2022; Wu & Ma, 2018). It is worth noting that there have been reports suggesting that canola plants are more prone to root lodging as heat stress becomes more frequent during the summer (Wu et al., 2020; Wu & Ma, 2018).

2.8. The Impact of Lodging on *Brassica napus*

Lodging is one of the main constraints in oilseed rape production under favourable conditions as it leads to economic losses due to yield reduction, deterioration of the grain's quality, increased pod shattering and disease susceptibility, and mechanical harvesting difficulty, which leads to the increase of production costs (Hua et al., 2014; Kendall et al., 2017; Khan et al., 2018). The possible mechanisms of how lodging impacts yield, grain quality, and diseases is further expanded below.

Lodging that leads to a loss in yield has been studied in multiple crops (Hwang & Lee, 2019; Liu et al., 2018; Zhang et al., 2022). In canola, yield loss is due to the reduction of photosynthetic capacity and nutrient transport within the plant (Wu & Ma, 2022). As previously observed in cereal crops, lodging could deteriorate carbohydrate assimilation and negatively impact seed filling (Berry et al., 2004). Yield loss due to lodging depends on the lodging angle and timing. Yield loss

in canola was less intensive when plants lodged less than a 45-degree angle from the vertical growth habit, but yield loss could reach as high as 46 % when plants lodged closer to the 90-degree angle (Kendall et al., 2017). Plant lodging occurring during the flowering stage has a more significant negative impact on yield than any other stage after flowering (Kendall et al., 2017; Wu et al., 2021a). Likewise, lodging at the early seed-filling could lead to a higher yield loss than lodging at the late seed-filling stage (Baker et al., 2014; Khan et al., 2018). It is worth noting that lodging at late seed-filling has less effect on yield because of harvest difficulties (Wu et al., 2022).

Lodging deteriorates grain quality by reducing the oil content by 10 - 30 % and shifts the fatty acid composition by increasing arachidic, palmitic, and stearic acid and reducing linoleic and linolenic fatty acids (Khan et al., 2018; Li et al., 2022). In addition, lodging can also reduce oil content by promoting the production of protein, erucic acid, and glucosinolate biochemical compounds (Kendall et al., 2017; Khan et al., 2018).

A fallen canopy could also cause the stem to bend to the ground, making the plant more susceptible to diseases (Wu et al., 2022). The increased levels of diseases following lodging are likely due to restricted airflow, ultimately leading to more disease transmission (Kendall et al., 2017). Unfortunately, little research has investigated levels of disease when rapeseed plants are under lodging stress. However, deoxynivalenol and other mycotoxin levels in cereal crops were significantly higher in lodged canopies than non-lodged canopies (Wu et al., 2022).

2.9. Factors affect lodging

Crop lodging can be significantly influenced by the complex interactions between environmental forces (E), crop management practices (M), and genetics (G) (Baker et al., 2014; Canola Council of Canada, 2020; Wu & Ma, 2018). These factors are discussed below.

2.9.1. Environmental Forces

Plant lodging resistance is the ability of the plant to stay upright when forces are applied to the plant. Lodging resistance is dependent on the plant's bending moment. The bending moment of the plant is induced by wind and the plant's weight (Stubbs et al., 2020). When the bending moments exceed the maximum the plant can support, stem breaking/bending is expected to occur. Heavy rain can also increase the risk of lodging by adding to the plant's weight and bending moment while decreasing soil strength (Chauhan et al., 2019). Heavy rain with strong wind can further amplify lodging risk (Niu et al., 2022).

Besides wind and rain affecting plant lodging, sunlight can also indirectly influence lodging. Increasing light interception below the plant canopy can improve the mechanical strength of the basal internode by increasing structural carbohydrate content and stem thickness, thereby improving lodging resistance in maize (Xue et al., 2017). In contrast, the low light intensity can enhance stem elongation and reduce stem wall thickness, stem diameter, and lignification process (Shah et al., 2017; Wu et al., 2022). This phenomenon was also observed in soybeans, where plants attempted to escape shading by boosting their plant height and internode length, reducing stem diameter and dry matter accumulation, ultimately leading to higher lodging risk (Hussain et al., 2020).

Drought can increase root and stem lodging susceptibility by reducing nitrogen and phosphate uptake, thereby significantly stalling the root and stem growth rates in canola (Biswas et al., 2019). Studies have demonstrated that lower stem moisture during drought can increase stem lodging susceptibility. In an *Arabidopsis thaliana* (L.) Heynh., turgor pressure has been shown to play a vital role in stem mechanical resistance (Paul-Victor & Rowe, 2011). In particular, it was observed that *Arabidopsis* stems lost 10 % of their stem stiffness after 15 minutes, but when the stem was sealed with wax at both ends, stem stiffness loss was extended to 25 minutes (Paul-Victor & Rowe, 2011). A similar result was also found in woody plants in which moisture content within the stem strongly affected the stem's mechanical strength (Shah et al., 2017).

Recently, heat stress was shown to be associated with stem and root lodging in canola. Heat stress increases stem lodging due to a lower bending strength (Wu et al., 2021b). Specifically, when canola plants were exposed to high temperatures in a controlled environment, the maximum bending strength was reduced by as much as 14 % compared to typical temperatures (Wu et al., 2021b). Elferjani & Soolanayakanahally (2018) also demonstrated that yield loss in canola could reach as high as 80 % due to the increased lodging risk under severe heat stress. For stem lodging, it was reasoned that heat stress hastened cell development and proliferation, resulting in larger cells with thinner cell walls and weaker vascular bundles and leading to reduced stem elasticity (measured via Young's modulus) (Wu & Ma, 2018). In the roots, heat stress influenced root lodging by decreasing root biomass, length, and volume, ultimately affecting root anchorage strength (Wu & Ma, 2018). It was further discovered that the taproot morphology remains relatively constant under heat stress at all growth stages (Wu et al., 2021), which suggests that high temperatures increase root lodging risks by directly impacting the development of the lateral roots. Tripathi et

al. (2015) reasoned that heat stress increasing root lodging is likely caused by a rise in crop leaves' surface temperature, thereby suppressing plant photosynthesis and root development processes.

Soil properties and composition may also affect crops' lodging resistance (Erndwein et al., 2020). Soil compaction reportedly increased lodging resistance in canola because lodging severity was significantly lowered compared to no soil compaction (Zuo et al., 2017). In contrast, soil compaction leads to poor root system development, increasing in root lodging risk observed in maize (Bian et al., 2016). In addition to soil compaction, the pore water suction within the soil can also impact lodging risk. Mickovski et al. (2007) showed that plants in wet sand require much greater force to pull out the root than in dry sand. Berry et al. (2004) further described the importance of soil texture in determining root lodging resistance. Clay soil has a stronger cohesive force than sandy soil because individual clay particles are bound together when moist, while there is no significant attraction between grains of sand (Berry et al., 2004). In conclusion, environmental variation due to rain, wind, rain, sunlight, drought, temperature, and soil conditions all impact stem mechanical strength and root anchorage strength.

2.9.2. Agronomic Management Affecting Lodging

Alongside environmental effects, agronomic practices such as fertilizer quantity, seeding date, plant regulators, and plant density can also affect crop lodging. Nitrogen applications benefit crop growers by increasing seed yield; however, extensive use can lead to higher lodging risk in wheat and rice (Li et al., 2022; Zhang et al., 2016). In canola, seed yield and lodging were significantly higher when nitrogen fertilizer input increased from 50 to 200 kg N per ha (Wu & Ma, 2016). A correlation between high rate of nitrogen usage and plant height was established in many studies

(Khan et al., 2018; Öztürk, 2010; Wang et al., 2005). It was reported that the increase in nitrogen content could significantly improve plant height in canola and maize, with the maximum plant height achieved at 150 kg nitrogen per ha (Khan et al., 2018; Öztürk, 2010; Wang et al., 2005). From a mechanical perspective, lower plant height has a lower lodging risk due to the lower center of gravity and lower above-ground fresh weight (Wu et al., 2022). Likewise, it was reported that the increase in nitrogen concentration leads to long stem, weak stem breaking strength, reduced root biomass, and low lignin content in the sclerenchyma and vascular bundles area (Chauhan et al., 2019; Zhang et al., 2016). These are all characteristics that can be associated with stem and root lodging. Wu et al. (2022) found that abundant nitrogen fertilizer could promote crop canopy development and reduce light transmittance, thereby altering the microenvironment within the canopy and weakening the basal stem. Kuai et al. (2016) discovered that increasing nitrogen application from 120 to 360 kg/ha reduces PAL gene expression and lignin content, ultimately increasing lodging risk in rapeseed. Wu & Ma (2016) observed that high nitrogen rates could also restrict the growth of taproots and lateral roots of canola. Hence, these findings illustrate that increased nitrogen application may have a trade-off effect between increased yield and lodging risk. However, it is difficult to maintain high-yield crops without using such fertilizer. Knowing the ideal nitrogen concentration may aid in alleviating lodging risk while achieving high yield. Li et al. (2023) have attempted to determine this rate and found that 240 kg/ha to be the optimum rate. Of course, this rate can also be impacted by other environmental factors, such as plant genotype and soil conditions.

The timing of applying N has also been shown to influence lodging resistance in canola. It was reported that split application (50 kg/ha at pre-plant plus 150 kg/ha) significantly improved

bending strength and lodging resistance compared to pre-plant-only nitrogen (Wu & Ma, 2016). It was believed that applying nitrogen before to stem elongation resulted in excessive growth during the vegetative stage and lowered lignin content in sclerenchyma and cell wall thickness (Wu et al., 2022). Khan et al. (2018) further showed that nitrogen application timing could impact yield and lodging depending on the lodging angle at the time of the application. In non-lodged plants, the increase in nitrogen rate led to an increase in seed yield, reaching a maximum of 360 kg nitrogen per ha and showing no further increase in seed yield when additional nitrogen was added (Khan et al., 2018). In lodged plants, the yield loss was generally amplified when increasing nitrogen rate was applied (Khan et al., 2018). Thus, the nitrogen fertilizer rate and timing both play a role in impacting lodging risk.

Unlike nitrogen fertilizer, which increased plant height and yield, other types of fertilizers, such as organic carbon and silicon, were also reported to impact lodging capacities (Hu et al., 2022; Kuai et al., 2017). These two fertilizers have been shown to enhance plant height, stem thickness, stem diameter, vascular bundle area, xylem area, and bending strength (Hu et al., 2022). Besides plant height, increasing all stem-related traits listed above is associated with reduced lodging. The relation of how these stem characteristics affect lodging will be discussed in detail in the next section. Hu et al. (2022) also found that organic carbon and silicon fertilizer can enhance stem lignin accumulation. Enzymatic activity analysis revealed an upregulation of PAL, 4CL, CAD, and POD, which are lignin-related enzymes. Likewise, Kuai et al. (2017) also found that the applications of these fertilizers could improve stem strength and root diameter, which ultimately increased the resistance to lodging. From these studies, it is likely that organic carbon and silicon fertilizer lower lodging risk by enhancing stem mechanical strength and root anchorage while

targeting the lignin biosynthetic pathway to increase plant's lignin accumulation (Hu et al., 2022; Kuai et al., 2017).

Besides fertilizer application, sowing date can also influence lodging risk. In wheat, early sowing can increase crop lodging as it increases the nitrogen uptake efficiency (NUE) and promotes vegetative growth, resulting in higher crop self-weight moments while having lower stem bending and root anchorage strength (Berry et al., 2004; Chauhan et al., 2019). Conversely, a delayed sowing date was reported to reduce NUE by up to 9.9 % compared to typical sowing date due to the reduced root density and dry matter accumulation before anthesis, thereby increasing lodging resistance by as much as 49.6 % (Dai et al., 2017). In barley, delayed sowing was reported to lower lodging risk (Shah et al., 2017). These results suggest that delayed planting dates positively affect lodging resistance in crops such as wheat and barley. However, in canola, delayed plant dates showed a decrease in seed yield due to the increase in lodging risk, which also resulted from the reduction of root anchorage strength and stem bending strength (Wu & Ma, 2016). It is also worth noting that the increase in the sowing depth from 2 to 3 cm in canola significantly lowered lodging risk (Zuo et al., 2017).

Plant regulators are also utilized based on lower plant height to reduce lodging risk. The application of paclobutrazol (up to 300 mg/L) showed an increase in yield in different canola cultivars (Kuai et al., 2015). This is because plant height significantly decreased with the rise of plant regulator applications (Kuai et al., 2015). In addition, the application of plant growth regulators also increased the root system's thickness and dry biomass (Kuai et al., 2015). Miller et al. (2018) found similar results regarding the positive effect of plant regulators in controlling lodging. However, the

excessive usage of a plant regulator can have some negative consequences. Plant regulators such as paclobutrazol are known to regulate plant height by inhibiting the gibberellin signaling pathway (Rubinovich and Weiss, 2010). Inhibition of the gibberellin transduction pathway using paclobutrazol has been shown to suppress seed germination (Rubinovich and Weiss, 2010). A previous study in wheat demonstrated that excessive use of paclobutrazol significantly reduced grain yield (Peng et al., 2014). Due to this complex interaction of paclobutrazol in yield, plant height, and the root system, it is difficult to determine the impact of plant regulators on lodging.

Chauhan et al. (2019) summarized that plant density also affects lodging in many crops, such as wheat, barley, and maize. As plant density increased from 7.5 to 9 plants/m^2 in maize, plants were more sensitive to lodging (Huang et al., 2021). A high lodging risk was due to increased height while having a thinner basal internode (Huang et al., 2021). Gene expression study showed that lignin-related enzymes were downregulated as density increased from 6 to 9 plants/m^2 (Li et al., 2021). Similarly, research showed that increasing plant density between 30 to 60 plants/m^2 in canola resulted in elongated stem length and reduced stem diameter, making the plant more lodging susceptible (Khan et al., 2017). It was reasoned that denser planting increases lodging risk due to the fierce competition for nutrients and light among plants (Chauhan et al., 2019; Kuai et al., 2016). On the contrary, it was also observed that increased plant density from 10 to 30 plants/m^2 in canola resulted in a significantly higher seed yield and enhanced lodging resistance due to reduced plant height (Khan et al., 2018). Concurrently, erucic and arachidic acids also reduced considerably as plant density increased (Khan et al., 2018). Lignin-associated enzymes such as PAL, CCR1, and 4CL were upregulated as plant density increased from 15 to 45 plants/m^2 in canola (Kuai et al., 2016). However, Khan et al. (2018) also observed that elevated plant density also caused a

reduction in root diameter and dry biomass, thereby decreasing the plant's root anchorage strength. These findings suggest that plant densities influence lodging in canola by affecting both the stem and root-related traits, with a more substantial effect on the stem. In addition, these results suggest that plant densities between 30 and 45 plants/m^2 are appropriate density range that growers should use to minimize lodging risk and improve yield in canola. Unfortunately, the optimum density will vary based on the year (variable environmental conditions) and the soil type/conditions.

2.9.3. Influence of Genetics

The plant's genetic composition, which affects its root and stem characteristics, also impacts lodging (Wei et al., 2017). For instance, Invigor 5440, a previous commercial hybrid, illustrated an improved lodging resistance compared to the 13C204 canola genotype (Wu et al., 2020). Further analysis between lodging resistance and susceptible genotypes showed significant differences in the root characteristics. In Invigor 5440 lodging resistant canola, root traits such as length, surface area, volume, and biomass were significantly improved (Wu et al., 2020). Lateral roots that influence lodging resistance have also been demonstrated in another study. The presence/absence and position of lateral roots were shown to affect the pull-out resistance of the root system (Mickovski et al., 2007). The deeper the lateral root expands into the soil, the greater the plant's ability to resist being pulled out (Mickovski et al., 2007).

Similar to the root system, genetic factors that govern plant height, stem diameter, canopy shape, stem bending/breaking resistance, stem fresh weight, and stem biochemical composition would also influence resistant to stem lodging in crops.

It has been reported that lower plant height lowers the risk of lodging (Li et al., 2021; Niu et al., 2022; Wu et al., 2022; Xue et al., 2017). The positive correlation between lodging and plant height was demonstrated in canola (Hu et al., 2022). In cereal crops, plant height is known to be associated with stem lodging and regulated by genes controlling brassinosteroid (BR) and gibberellin (GA) signaling pathways (Niu et al., 2021). Analysis of plant height illustrated that the lower stem section played a more critical role in stem lodging than the middle and upper stem sections (Berry et al., 2004; Wu & Ma, 2016). Stubbs et al. (2020) explained that the basal internode has the highest lodging risk because it experiences the largest bending moments induced by external forces and the plant's weight. This was further supported by studies in which basal internode length showed a positive correlation with lodging, suggesting that a longer internode at the base increases plant's lodging sensitivity (Huang et al., 2021; Li et al., 2021; Li et al., 2022).

Stem diameter is also a critical factor in lodging resistance in many crops (Kashiwagi et al., 2008; Shen et al., 2018; Xiao et al., 2015; Zhang et al., 2022). In rice, lodging resistant genotypes have a larger stem and higher culm wall thickness (Kashiwagi et al., 2008). Stem diameter was significantly correlated with breaking strength in barley (Zhang et al., 2022). A negative correlation between stem diameter and lodging was found in peas and wheat (Gawłowska et al., 2022; Xiao et al., 2015). A mathematical lodging model in wheat showed that increased stem diameter would delay the lodging return period (years) (Piñera-Chavez et al., 2016). For example, a wheat genotype with a height of 0.7 m, yielding 6 tons per ha with materials strength of 35 MPa and a stem diameter of 4.04 mm, would have a lodging return period of 5 years (Piñera-Chavez et al., 2016). However, when this stem diameter was increased to 4.76 mm, the model predicted the lodging return period was extended to 25 years (Piñera-Chavez et al., 2016).

Similar to stem diameter, the canopy structure of the plant can also play a role in lodging. Canopy shapes that help to reduce air movement can improve the basal structure and minimize the impact of wind, thereby alleviating lodging risk (Huang et al., 2021). Canopy structures that allow more light transmission means more light being intercepted by other plant structures (Xue et al., 2016). When more light is captured, the plant's photosynthetic capacity increases, improving dry matter accumulation and stem strength (Xue et al., 2016).

Increased stem strength can enhance the maximum total stress the stem can bear (Li et al., 2022). Stem bending strength is the maximum bending force the plant can support before stem breakage, while stem breaking is the force required to break the stem (Pinthus, 1974; Stubbs et al., 2020). As a result, strong stem bending and breaking strength were explored to improve lodging resistance. A negative correlation between lodging and breaking strength/pushing resistance was established in the rice (Kashiwagi et al., 2008; Zhang et al., 2016). Stem bending strength was significantly positively correlated with lodging resistance in canola (Hu et al., 2022). Elastic modulus, or Young's modulus, is a ratio of stress applied to a stem material to the linear strain it exhibits; it reflects the combination of properties given by the different stem materials (Shah et al., 2017). Stem-breaking strength was reported to correlate positively with high elastic modulus in flax and rice (Bourmaud et al., 2015; Chuanren et al., 2004). This suggests that stems with high elastic modulus values would have a stiffer stem material and a higher resistance to applied forces, which could be used to predict lodging resistance.

Stem characteristics such as fresh weight and stiffness could also predict lodging resistance. In soybeans, a positive correlation between stem breaking strength and above-ground fresh weight and stem fresh weight per unit length was found (Hussain et al., 2020). In addition, greater weight at the lower stem internode has shown a better lodging resistance in rice (Kashiwagi et al., 2008). These results indicate that increasing stem fresh weight, especially at the lower internodes, could make plants more resistant to lodging. Stem stiffness was also shown to relate to lodging. Stiffness is the ratio of force and displacement and is known as a property of structure, with stiffer structures deflecting less for a given load (Shah et al., 2017). Plant stem stiffness can contribute to lodging resistance, as stem stiffness can increase plant resistance from deformation (Li et al., 2022).

Moreover, stem biochemical composition can also affect crop lodging resistance. For instance, smaller secondary xylem and phloem areas were previously demonstrated to increase lodging risk (Wu et al., 2021b), and these small secondary areas were found to be associated with a lower level of lignin in the cell wall (Li et al., 2022). Lignin, cellulose, and hemicellulose are structural carbohydrates in plants and primary components of the cell wall and secondary cell wall of the stem (Li et al., 2022). Further research found that not only lignin but also cellulose, hemicelluloses, chitin, silica, and soluble sugar contents were also involved in plant lodging resistance (Niu et al., 2021; Wu et al., 2022). These structural carbohydrates have been reported to have the ability to enhance stem mechanical strength and rigidity (Hu et al., 2022; Niu et al., 2021; Zhang et al., 2016). For instance, a significant negative correlation between stem lignin, cellulose, hemicellulose, and lodging rate was demonstrated in maize and canola (Li et al., 2021; Wei et al., 2017; Xue et al., 2017). Wider layers of sclerenchyma that have high levels of lignin and cellulose content have also been reported to improve lodging risk in canola (Wu & Ma, 2018). Furthermore,

bending strength has been reported to be significantly positively correlated with lignin content in the stem (Hu et al., 2022). Transcriptomic analysis revealed that methonine/S-adenosylmethionine cycle (Met/SAM cycle), phenylpropanoid/lignin biosynthesis-related genes such as *PAL, 4CL, CCR, CAD, PER, COMT,* and *CCoAOMT* were highly expressed in high stem strength canola plants (Tian et al., 2024), suggesting the possible mechanism for stem lodging resistance. Similar results have also been found in maize and rice, in which the overexpression of key enzymes (PAL, CAD, 4CL, POD, and COMT) elevated lignin content and lodging resistance (Li et al., 2021; Liu et al., 2018; Ookawa et al., 2014). Silicon was reported as an essential structural component of stem strength (Wu & Ma, 2022). This may further support why silicon fertilizer could enhance stem and root anchorage strength, thereby enhancing lodging resistance in cereal crops (Kuai et al., 2017) and canola (Hu et al., 2022).

In summary, many roots and stem characteristics have been shown to affect lodging resistance in many crops. Some research groups have even demonstrated genes and biosynthetic pathways that govern lodging resistance. Despite these findings, additional findings are still needed to explore and deepen the knowledge of the bio-mechanism that controls lodging, especially knowing that a complex interaction of many genes regulate lodging.

2.10. Selection for Lodging Resistance

2.10.1. Visual Rating

Conventionally, lodging has been visually evaluated on a scale between 1 to 5, based on the degree of inclination of the plants from their upright growth habit, with one for a plant or group of plants standing completely vertical and a rating of five for a plant or group of plants that are lying flat on

the ground (**Figure 2.2**). Since lodging is a complex trait influenced by environment and genetics, different lodging assessment methods have also been deployed for plant lodging selection.

2.10.2. Characteristics for Lodging Evaluation

Stem morphological, anatomical, and mechanical parameters such as plant height, stem breaking strength, stem bending strength, stem diameter, flexural rigidity (a measurement of the material's stiffness), Young's modulus, and mass density have been utilized for crop lodging resistance (Berry et al., 2004; Wu & Ma, 2016, 2018).

In these traits, plant height has been extensively used to select lodging resistance in crops as lower plant height is equivalent to lower lodging risk (Li et al., 2021; Niu et al., 2022; Wu et al., 2022; Xue et al., 2017). Different dwarfing genes from other related species have been introduced to improve lodging resistance in canola and reduce plant height. One such gene was the introgression of the Brrga1-d gene from mutant *B. rapa* into *B. napus* to lower plant height and effectively improve lodging (Miller et al., 2018). However, based on studies from other crop species, such as rice and wheat, improving lodging resistance using dwarfing genes is only possible up to a limit (Yano et al., 2015). This is because plants with reduced height would have more weight at the top, resulting in uneven weight distribution between the canopy and the total body weight (Dreccer et al., 2020; Yano et al., 2015). As a result, a further decrease in plant height is likely counterproductive to reduce lodging and increase yield (Dreccer et al., 2020). In addition, the adverse pleiotropic effects of dwarfing genes include a delay in flowering time, which was reported in canola, making this gene unsuitable for certain growth regions (Miller et al., 2018).



1	2	3	4	5
Standing straight at approximately 90-degree angle from the ground.	**All plants are leaning slightly, or a few plants down.**	**All plants are leaning more than 45-degrees from the ground or 25-50% down.**	**All plants are leaning less than 45-degrees from the ground or 50-80% down.**	**All or most plants completely lay flat on the ground**

Figure 2.2: Lodging rating scale.

Lodging rating system adapted from Holshouser, 2015 and Quijada et al., 2004.

Similarly, the dwarfing gene has reduced rice fertility, grain size, stem strength, diameter and thickness (Long et al., 2020; Yano et al., 2015). These reports suggest that more goals than plant height alone must be elected to be influential in selecting for lodging resistance.

The bending test is another approach for lodging resistance selection. To evaluate stem bending in quantitative units, the three-point bending test is often used to measure basal stem-breaking strength in crops (Lemloh et al., 2014; Wu & Ma, 2018). In the bending test, the basal stem, which is a critical region where buckling generally occurs, is balanced between two supporting blocks, and a testing probe with a pre-set loading capacity is pushed down on the stem at a constant speed until the stem breaks (Lemloh et al., 2014; Miller et al., 2018).

Mass density (mg cm^{-1}), dry weight per unit length of the basal stem, borrowed from textile engineering, can also indicate plant resistance to applied force (Shah et al., 2017). Mass density was indirectly used in canola studies to determine lodging resistance due to its close correlation with stem bending strength, stem diameter, and wall thickness (Li et al., 2023; Wu et al., 2022).

Root anchorage strength and root-soil cone have also been used for root lodging assessment (Li et al., 2023; Wu & Ma, 2016). Root anchorage strength is measured using a prostrate tester machine. In this type of assessment, eight different plants from each plot are selected one week before physiological maturity (Wu & Ma, 2016). The chosen plants were pruned at a height of 20 cm and measured using a prostrate tester machine attached to an adjustable mounting plate at the 10 cm point of the pruned stem (Wu & Ma, 2016). The stem's resistance from bending supplied by the root system is recorded when it is pushed to bend at a 45-degree angle from its upright position

(Wu & Ma, 2016). Other studies have also explored a less time-consuming, labour-intensive, and non-destructive method to measure root lodging. In these studies, the researchers used an LCR meter with two electrodes, one in the plant base and the other in the soil medium, to measure electrical signals such as electrical capacitance and electrical impedance; this is mainly because electrical measurement has a direct correlation, both positively and negatively, to root surface area (Wu et al., 2017, 2020; Wu & Ma, 2016). Nevertheless, electrical measurements depend on voltage, current frequency, electrode position, soil moisture, temperature, and salinity (Wu et al., 2020).

The root system is another parameter to determine root lodging resistance because visual taproot and lateral root differences between lodging-susceptible and lodging-resistant genotypes have been observed in cereal crops (Wu & Ma, 2020). Likewise, a positive correlation between root diameter and lodging resistance has been reported (Wu & Ma, 2020), indicating that root diameter can also be used to select lodging resistance. It was reported that tap and lateral roots of lodging-resistant genotypes have greater anchorage strength, thicker cells, and more extensive root diameters when compared to susceptible genotypes (Wu et al., 2022). The taproot is well anchored to the ground due to multiple lateral roots, and the network between these roots forms a large elliptical root-soil cone volume (Wu et al., 2022). The larger the root-soil cone, the greater the ability of the root system to resist rotation (Wu et al., 2022). The root-soil cone can be used to measure root lodging as the Pearson correlation coefficient shows a positive correlation between root-anchorage strength (Sr) and root-soil cone (Li et al., 2023).

2.10.3. Remote Sensing Applications

Direct selection for lodging by visual rating and indirect selection based on other plant characteristics related to lodging are labour-intensive, expensive, and time-consuming (Beissinger et al., 2013). In the past decade, remote sensing (RS) to estimate crop lodging has been extensively used to overcome the constraints of high labour consumption and unfavourable environmental conditions (Chauhan et al., 2019). For instance, unmanned aerial vehicles, commonly known as drones, have been deployed to assess lodging by taking multiple spectral images between different lodging levels (Sun et al., 2019, Wu & Ma, 2022). These images allow researchers to establish indices to report vegetation growth status quantitatively (Sun et al., 2019). In those indexes, the normalized differential vegetation index (NDVI) is the most widely used index, with the smaller NDVI number equivalent to increased lodging severity (Sun et al., 2019). This specific index has also been performed to determine lodging susceptibility in canola (Wu & Ma, 2022). Wu & Ma (2022) found that NDVI is closely related to lodging resistance, which suggests that NDVI could be used to assess lodging in canola. Despite the capability to provide consistent and continuous data in the spatial and temporal domains, utilizing remote sensing to evaluate lodging yet to be widely adopted (Chauhan et al., 2019). This is due to a need for more scientific consensus on the methodologies used for lodging assessment, and lodging using RS systems can sometimes be underestimated due to the heterogeneous distribution of lodging (Chauhan et al., 2019). Another challenge is that RS uses spectral and textural indices from optical imagery to assess crop lodging, and the dynamic changes of these indices could be caused by various factors other than lodging, such as soil condition, leaf pigments concentrations, canopy structure, diseases, etc. (Chauhan et al., 2019).

2.10.4. Molecular Markers

Environmental factors such as wind, rain, and temperature vary yearly and from location to location, possibly influencing lodging phenotypically. Hence, it is more efficient and sustainable as a long-term strategy to select for lodging resistance genetically. In the late nineteenth century, molecular markers were extensively used to study the genetic mechanism of quantitative traits at the molecular level (Su et al., 2015). Quantitative loci have been reported in both animal and plant species. For instance, more than 2000 loci have been discovered in soybeans up to 2010 (Su et al., 2015). Currently, the use of molecular markers to identify loci associated with its phenotypic characteristic has successfully identified genes that give rise to its phenotype and eliminate the influence of other external factors. With advances in genome sequences in recent decades, trait selection using molecular markers has been widely adopted in breeding programs. A few examples of molecular markers that were and have been used in the past include restriction fragment length polymorphism (RFLPs), amplified fragment length polymorphism (AFLPs), simple sequence repeats (SSR), and single nucleotide polymorphisms (SNP). SSR and SNP markers are the most common that have been used in lodging research (Hwang & Lee, 2019; Shao et al., 2022; Wang et al., 2016; Yamaguchi et al., 2015). The following paragraphs briefly summarize how SSR and SNP are utilized in genetic studies.

Polymerase chain reaction (PCR) was invented to eliminate the need for large quantities and high-quality genomic DNA (Blears et al., 1998). PCR-based SSR markers, also known as microsatellite markers, have been utilized in plant breeding due to their high abundance and fast mutation rate (Mason et al., 2017). SSRs are tandem repeat motifs of 1-6 nucleotides in length, and they are highly abundant in eukaryote and prokaryote genomes (Agarwal et al., 2008). SSR markers can be

mononucleotides (T), dinucleotides (GA), trinucleotides (TAA), tetranucleotides (TAGC), pentanucleotides (ATACT), and hexanucleotides (TGTACA) (Nadeem et al., 2018). The main drawback of SSR markers is that they are less common in plant genomes than in animal genomes, along with their poor linkage to genes (Mason et al., 2017; Patel et al., 2015). Although SSR markers have been extensively used for linkage mapping and identification of potential major genes within a 10-20 cM spanning interval, they have not been compelling enough to detect many small effect genes/loci (Mir & Varshney, 2013).

Single nucleotide polymorphisms (SNPs) are single nucleotide variations in the genome sequences of an individual in a population (Agarwal et al., 2008). SNPs can be transitions (G/A or C/T), transversions (A/T, C/G, T/G or C/A), or insertions/deletions (indels) (Mason et al., 2017). Since SNPs are the most basic unit of inheritance, they can provide a maximum number of polymorphic markers between individuals (Nadeem et al., 2018). SNP markers are highly desirable for marker development to study complex traits due to the following: (1) their high abundance (Mir & Varshney, 2013), (2) their uniform distribution in the genome (usually 1 SNP in every 100-300 base pair in plants) in both coding and non-coding regions (Mir & Varshney, 2013), and (3) their relative stability over generations (Mir & Varshney, 2013). In addition, SNPs have the potential for solid linkage to traits of interest, making them a suitable marker of choice for applications such as high-density map construction, QTL mapping, marker-assisted selection, and genetic diversity studies (Hayward et al., 2012).

Molecular marker breeding can save significant time in the breeding process. It allows the selection to occur at the early phases of plant development cycles and removes unsuitable genotypes from

further evaluation (Beissinger et al., 2013). Most plant characteristics cannot be visually assessed or measured in the early development stages (e.g., grain/seed quality can only be evaluated after flowering) (Moose & Mumm, 2008). Traits like diseases and lodging can also be subjected to environmental conditions (Moose & Mumm, 2008). In contrast, molecular markers are not subject to environmental conditions, allowing trait selection regardless of the environmental factors. Molecular markers genetically linked to a trait of interest can speed up the selection process as DNA can be extracted from a young plant before traits are phenotypically expressed (Beissinger et al., 2013). Molecular-assisted selection can also save time, especially for species with long generations. Molecular markers can also help to understand the genetics of the trait. For instance, when markers are linked to a trait of interest, the markers can be used to clone genes. When selecting desirable traits, markers can help reduce the number of genotypes that need to be screened by eliminating inferior genotypes in the early selection phase (Beissinger et al., 2013).

2.11. Development of *Brassica* Reference Genome

A reference genome has become an indispensable tool for research communities in plant breeding, genome revolution, gene function, and organism adaptation. The first plant genome to be sequenced was *Arabidopsis thaliana,* which has been used as the 'model' plant in plant genetics research (Arabidopsis Genome Initiative, 2000). Since its publication, the number of plant species sequenced has steadily increased. Due to the economic importance of *Brassica* species, a collaboration between researchers around the world was initiated to sequence *B. rapa* (Wang et al., 2011) and followed by *B. oleracea* (Liu et al., 2014; Parkin et al., 2014). In 2014, with the collaboration efforts from multiple research institutes around the globe, the *B. napus* genome was assembled (Chalhoub et al., 2014). This project, a French homozygous *B. napus* winter genotype

'*Darmor-bzh'* was selected as the reference genome to be sequenced (Chalhoub et al., 2014). Several sequencing technique were utilized, including GS FLX Titatimum 454, 0.1x Sanger BES, and 53.9 Illumina HiSeq (Chalhoub et al., 2014). *B. napus* ancestor's genomes, which are *B. rapa* (Wang et al., 2011) and *B. oleracea* (Liu et al., 2014), were also utilized to deduce the most probable orientation when more than one location was available on the same scaffold (Chalhoub et al., 2014).

Since the release of the genome sequence of winter-type oilseed rape '*Darmor-bzh*', other rapeseed accessions, which include winter-type 'Tapidor' (Bayer et al., 2017), semi-winter-type 'ZS11' (Sun et al., 2017) and 'NY7' (Zou et al., 2019), have also been sequenced. Despite having multiple reference genomes sequenced for *B. napus*, they have not been sufficient in capturing all genetic variation within *B. napus* or satisfying the needs of subsequent functional genomics research and molecular breeding in *B. napus* (Song et al., 2020). Hence, there is a demand for a reference genome that represents the genomic sequences of different *B. napus* ecotypes are required. The pan-genome assembly was developed using eight well-assembled genomes from winter-, semi-winter-, and spring ecotypes and the previously sequenced '*Darmor-bzh*' genome (Song et al., 2020). This pan-reference-genome can be exploited to identify candidate genes for traits of interest, accelerate the development of better genetic markers for marker-assisted selection, and, more recently, genomic selection.

2.12. The Development and Use of The Multiplex Platform *Brassica* 60K Illumina Infinium

The development and release of *Brassica* genome sequences led to the development of the high-density 60K *Brassica* Illumina Infinium SNP array that contains 52,157 SNP markers (Clarke et al., 2016). Extensive filtering steps were applied to produce the high-density array to ensure that most of these SNP probes only anneal to a single locus in the alloploid and homologous sub-genomes (Clarke et al., 2016). Later, approximately 30K SNP markers from the B genome were added to the array, increasing the SNPs from 60K to 90K probe. The 90K chip array allows researchers to genotype *B. napus*, *B. oleracea, and B. rapa,* and the rest *of Brassica* species from the U's triangle as the array carrying SNP arrays from A, B, and C sub-genomes. The effectiveness of this array has been extensively tested in *B. napus* germplasm from three different ecotypes (winter, semi-winter, and spring), and the results showed that SNPs are not only locus-specific but also hybridize to both A and C sub-genomes (Mason et al., 2017).

Since its development, the array has seen massive international adoption in the rapeseed community for multiple reasons (Mason et al., 2017). First, it can genotype thousands to a million SNP loci per sample, with numerous samples simultaneously in a single experiment (Mason et al., 2017). Second, utilization of the array requires a straightforward sample preparation technique and quick data collection (within 2-3 days). Third, the data produced is relatively easy to analyze and requires minimum computer knowledge. Fourth, data reproductivity is high as the same SNPs can be genotyped across many individuals, which allows a straightforward comparison.

The array has been widely used for numerous applications, which include quantitative trait loci (QTL) analyses and genome-wide association studies (GWAS) for traits such as seed germination and vigour (Hatzig et al., 2015), plant height, and primary branch number (Li et al., 2016), sclerotinia stem rot (*Sclerotinia sclerotiorum* (Lib). de Bary) disease resistance (Wei et al., 2016), and flowering time (Xu et al., 2015).

2.13. QTL Mapping

Many agronomic traits like yield, plant height and days to flowering are quantitative and governed by many genes on the same and different chromosomes (Mohan et al., 1997). Chromosomal regions containing genes linked to these quantitative traits are known as quantitative trait loci (QTL) (Collard et al., 2005). The purpose of QTL mapping is to identify the location of the genes that control these traits of interest (Mohan et al., 1997). Several steps are involved in QTL mapping, which is described in detail below and in (**Figure 2.3**).

The first key step of QTL mapping is to select two diverse parents with allele variations controlling one or more traits of interest (Collard et al., 2005). Backcrosses (BCs) doubled haploid (DH), recombinant inbred lines (RILs), near-isogenic lines (NILs), and F_2 populations can all be employed for mapping (Nadeem et al., 2018). For preliminary genetic mapping studies, population size usually ranges between 50-250 individuals (Collard et al., 2005; Nadeem et al., 2018); however, a larger population is required for a higher mapping resolution (Collard et al., 2005). The

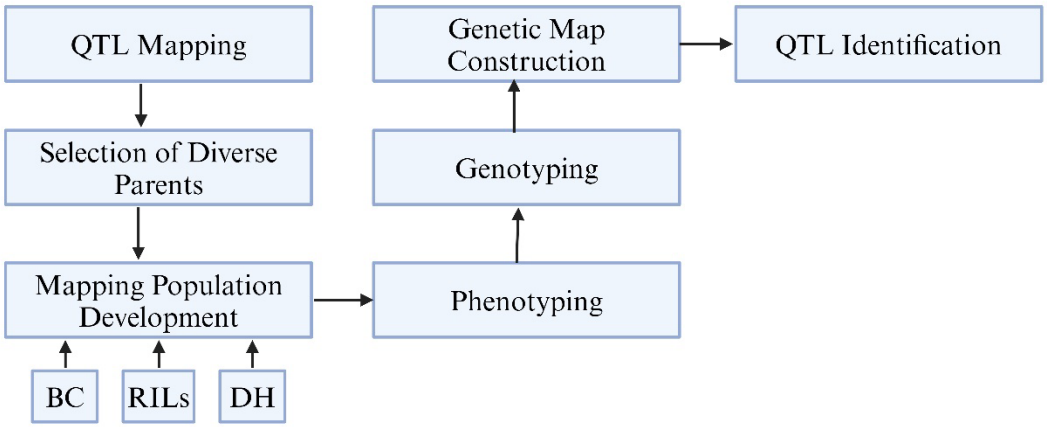

Figure 2.3: Quantitative trait loci mapping steps.

Adapted from Nadeem et al., 2018.

next step is to select molecular markers that show polymorphisms between the two parents for QTL mapping (Collard et al., 2005). Various marker types, such as SSR and SNP, to name a few, have been utilized for the construction of linkage in several different plant species (Semagn et al., 2006). In the recent past, 100-200 markers are often used for mapping studies (Nadeem et al., 2018); however, with the advent of NGS technologies, thousands of markers can be deployed for high-resolution genetic mappings (Beissinger et al., 2013). The third step in QTL mapping is constructing the genetic/linkage map. The linkage map is the road map that illustrates the locations and relative genetic distance between markers (Nadeem et al., 2018). Marker positions and their relative distance are determined based on marker segregation via chromosome recombination during meiosis. Markers that are closely linked will move together during crossing over as opposed to markers that are far away from each other (Nadeem et al., 2018). Through this recombination frequency, the actual distance and the relative orders of these markers can be calculated using odds ratios (i.e the ratio of linkage vs no linkage (Collard et al., 2005). This ratio is often expressed in the logarithm of odds value (LOD). A LOD above three is considered ideal for linkage map construction (Collard et al., 2005). For instance, a LOD equal to three means that the linkage between two markers is 1000 times more likely than no linkage. Once the linkage map is constructed, different methods, such as single-marker analysis, simple interval mapping, and composite interval mapping, can identify QTL related to the trait of interest (Collard et al., 2005). After the QTL identification, an additional step can be taken to determine the validity of that particular QTL (Collard et al., 2005; Nadeem et al., 2018). This involves the development of diverse populations by crossing parents with different genetic backgrounds to test for the presence of the particular QTL detected earlier (Nadeem et al., 2018).

2.14. Mapping Studies for Lodging-Associated Traits in Wheat

As previously stated, stem strength, root anchorage strength, and plant height are lodging-related traits. Numerous studies have identified QTL/genes associated with lodging resistance in wheat, barley, rice and canola (Long et al., 2020; Piñera-Chavez et al., 2021; Sameri et al., 2009). In wheat, QTL linked to stem strength traits (diameter, cell wall thickness, material strength), root anchorage strength characters (root plate spread and root plate depth), and plant height have been identified (Piñera-Chavez et al., 2021). When QTL for stem strength was aligned with the reference genome in wheat, the researchers detected the TraesCS3B02G607725 gene, which codes for protein P-loop NTPase (P-loop containing nucleoside triphosphate hydrolase) domain superfamily (Piñera-Chavez et al., 2021). According to Leipe et al. (2004), this gene likely functions in various biological processes, such as disease and stress responses in plants. Markers linked to QTL for root plate spread are located near the TraesCS5B02G255000 gene, which codes for a protein kinase-like superfamily (Piñera-Chavez et al., 2021). Plant protein kinases are known to be components of signaling networks in response to biotic agents, plant hormones, and environmental stresses. This protein family has functions in diurnal and circadian regulation, cell cycle regulations and development processes (Lehti-Shiu & Shiu, 2012).

2.15. Mapping Studies for Lodging-Associated Traits in Barley

Investigating QTL for lodging resistance in barley focused on the mechanisms controlling crop lodging. Multiple QTL governing culm lengths have been identified using sequence-tagged sites (STS) and simple sequence repeats (SSR) markers (Sameri et al., 2009). Several genes in barley, such as Uzu (reduced height, dense bases of spikes, and upright growth habit), sdw (semi-dwarf), and dwf (extreme dwarf), have been determined. The Uzu1 gene encodes a protein kinase that acts

as a brassinosteroid receptor and is orthologous to the BRASSINOSTEROID-INSENSITIVE1 (BRI1) gene in *Arabidopsis* (Li & Chory, 1997). Sdw1 is involved in gibberellin biosynthesis and has been found to contribute to improved lodging resistance and a higher harvest index in semi-dwarf genotypes (Kuczyńska et al., 2013). Dwf2, on the other hand, encodes a dominant dwarfing gene insensitive to gibberellic acid and causes a very short growth habit in the barley mutant (Niu et al., 2022).

2.16. Mapping Studies for Lodging-Associated Traits in Rice

Rice has also been subjected to QTL analysis to understand genetic factors underlying lodging resistance better. Major QTL for lodging resistance have been identified in wild rice (*Oryza longistaminata* A.Chev. & Roehr.) as this QTL increases stem diameter and breaking strength (Long et al., 2020). Within these QTL, gene annotation shows that UDP-glucuronate xylan alpha-glucuronosyltransferase could enhance stem mechanical strength and CHD3-type chromatin-remodeling factor PICKLE, which is associated with growth (Long et al., 2020). The homolog *HEADINGDATE3a (Hd3a)* has been reported to affect plant height and flowering (Zheng et al., 2017). *Ghd7,* encoded for a CTT protein domain, has been demonstrated to affect plant height, grain per panicle, and heading date (Shen et al., 2018).

2.17. Mapping Studies for Lodging-Related Traits in *Brassica napus*

Quantitative trait loci analysis for lodging-related have also been conducted in canola. QTL responsible for plant height has been identified across numerous studies. Miller et al. (2018) identified significant SNP markers associated with plant height on chromosome A03/C07. Miller et al. (2018) demonstrated that this marker resides close to a gene orthologous to *Arabidopsis*

BR11, which is responsible for plant height in *Arabidopsis*. Shen et al. (2018) identified five significant QTL that affect plant height. Two QTL reside on A02, while three QTL reside on A07. The candidate genes identified from these QTL include genes related to gibberellin, brassinosteroid, auxin, flower development, and phosphate ion transmembrane. Other researchers also used an association mapping study to identify 7 SNP markers related to plant height (Li et al., 2016). In this study, two significant SNP markers were detected on chromosome A03, at position 7333 and 26561 kb of the genome. Two other markers were identified on A05 at position 2025 and 21465 kb of the genome. The last three markers were found on A07 at positions 10109, 17704, and 22928 kb of the genome. When the authors performed a Basic Local Alignment Search Tool (BLAST) to align and compare DNA sequences against all the genes in *A. thaliana*, they found three orthologous genes involved in gibberellin synthesis (Li et al., 2016).

For stem diameter, GWAS research was conducted using the 60K array to identify multiple QTL for stem diameter located on A04, A08, A10, C01, C03, C06, and C08, and candidate genes involved in cellulose biosynthesis were identified (Li et al., 2018). Studies have shown a strong correlation between stem absolute strength and stem diameter ($R^2 = 0.69$), suggesting that SNPs for stem diameter can be inferred indirectly using SNPs responsible for stem absolute strength (Miller et al., 2018). Significant SNPs for stem absolute strength were found on A2/C2, and A5/C5 (Miller et al., 2018). Markers found on A2/C2 and A5/C5 are near *Arabidopsis* orthologs and are expected to have pectin methylesterase (PME) or pectin methylesterase inhibitor (PMEI) activity. However, the actual function of the candidate gene is still unclear. Mutation of the candidate gene in *Arabidopsis* shows a decrease in stem strength and a reduction of demethylesterification activity, suggesting the candidate gene functions as a pectin methylesterase (Miller et al., 2018). The role

of the candidate gene is further validated since methylesterase is shown to play a role in stem mechanical strength and cell wall expansion (Wolf et al., 2009; Hongo et al., 2012). Other significant SNPs for stem absolute strength were also identified on A3/C3. No candidate genes were identified for these markers (Miller et al., 2018). Researchers used QTL meta-analyses in another study to identify five significant QTL corresponding to stem diameter (Shen et al., 2018). One of these QTL resides on A02, while the other four reside on A07. The detected candidate genes included genes that participated in cellular developmental processes, the gibberellic acid-mediated signaling pathway, and plant-type cell wall organization.

In addition to plant height and stem diameter, other stem-related traits, such as branch initiation height, has also been shown to influence *B. napus* lodging resistance characteristic (Cai et al., 2016; Shen et al., 2018). Five QTL were detected for branch initiation height; three of these QTL resided on A02 and the other two on A07, respectively (Shen et al., 2018). Candidate genes identified included genes involved in cell differentiation, flower development, and the gibberellic acid-mediated pathway (Shen et al., 2018). An overlap between QTL responsible for height, stem diameter, and branch initiation height was evidenced, as these QTL clustered into two main regions of the genome (Shen et al., 2018). One cluster is localized on A02 with a physical interval of 0.1-8.2 Mb. The other cluster is localized on A07 with a physical interval of 15.1-22.0 Mb. This suggests that these QTL involve several genes that have pleiotropic effects on *B. napus* development. For instance, BnaA02g02560, and BnaA0208490 were constantly detected in multiple traits, and these genes are orthologs of *A. thaliana* GASA4 and GNL, respectively. Studies have reported that overexpression of GASA4 could promote gibberellin response, such as seed germination and flowering, and play different roles in cell wall elongation (Irshad et al., 2008;

Rubinovich & Weiss, 2010). GNL codes for a transcription factor that targets the gibberellin signaling pathway and regulates flowering time (Bi et al., 2005; Richter et al., 2013).

Lignin accumulation within the stem has been found to lower lodging risk by increasing stem mechanical strength (Hu et al., 2022; Khan et al., 2018; Kuai et al., 2017). QTL for lignin biochemical constituents were discovered on A03, A06, and A07, and four candidate genes regulating lignin biosynthesis, including glycosyl hydrolase, *CYT1, SHINE1, and DAR6* were identified (Wei et al., 2017).

2.18. Objectives

Reducing lodging is a key strategy to maintain high yield and seed quality in *B. napus*. The main goal of breeding programs is to increase yield, which ultimately increases the above-ground biomass, facilitating an increase in lodging potential. Thus, it is necessary to understand the genomic regions associated with lodging and lodging resistance. QTL for lodging resistance using visual field rating in soybean has been successfully utilized by Hwang & Lee (2019), but QTL for lodging resistance in *B. napus* using a visual scale has yet to be explored. As a result, the main focus of this research study is to identify potential QTL associated with lodging resistance in *B. napus* using a visual rating system.

3. Discovering Quantitative Trait Loci (QTL) Associated with Lodging Resistance in A *Brassica napus* L.

3.1. Abstract

Lodging is a major disadvantage in canola production as it causes yield loss and alters several biochemical properties of the seed. Breeders need to introduce lodging resistance into current cultivars to increase lodging resistance in canola and ultimately mitigate yield loss. Herein, a doubled-haploid population derived from a cross between lodging susceptible (268-2 ZSDH6550/ LLHR10282-B-9-2) and lodging resistant (CB131037-6-8) parents were used to construct a high-density SNP map covering a genetic distance of 1000 cM, with an average marker interval of 0.21 cM. Phenotypic assessment for lodging using a lodging scale between one and five was conducted across four environments. Quantitative trait loci (QTL) analysis was performed using inclusive composite interval mapping (ICIM) to determine QTL for lodging resistance. Nine QTL were detected and distributed on chromosomes A01, A03, A05, A07, A10, and C01. Of these, six QTL (qLODG.Z/B-A01, qLODG.Z/B-A03.1, qLODG.Z/B-A03.3, qLODG.Z/B-A05, qLODG.Z/B-A07.1, and qLODG.Z/B-C01) were considered as stable QTL as they were consistently detected in more than one environment. Co-localization between QTL for lodging and QTL for plant height, days-to-flower, and days-to-maturity were also highlighted in this study, indicating that QTL for lodging may have a potential pleiotropic effect or linkage with QTL controlling other traits.

3.2. Introduction

Canola (*Brassica napus* L., 2n=38, genome AACC*)* is the world's second-largest oilseed crop after soybean, and it is utilized as protein for animal feed, vegetable oil for humans, as well as for biodiesel (Wang et al., 2011). Plant lodging is a primary concern for canola production, causing a loss in yield, impacting seed quality and inducing complications during mechanical harvesting (Hua et al., 2014; Kendall et al., 2017; Kuai et al., 2015). Depending on the severity of the lodging, yield loss may vary. For instance, when lodging was less than 40 degrees from vertical growth habit, the yield loss was less significant, ranging from 6 to 13 %. In comparison, when plants were lodged greater than 80 degrees from vertical growth, yield loss could be as much as 30 % (Khan et al., 2018). Lodging also impacts seed quality by promoting the accumulation of glucosinolates in the meal, while reducing oil content in the seed, mainly when lodging occurs during the pod-fill stage (Kendall et al., 2017).

Lodging is often categorized into stem and root lodging (Baker et al., 2014; Berry et al., 2004). Stem lodging occurs at any point along the stem, when the bending force exceeds the stem strength (Baker et al., 2014). Root lodging occurs when the bending force exceeds root/soil strength (Sterling et al., 2018). Many factors influence crop lodging, including abiotic (topography, precipitation, wind) (Chauhan et al., 2019; Niu et al., 2022; Stubbs et al., 2020; Wu & Ma, 2018), genetics (Li et al., 2022) and agronomic practices (Dai et al., 2017; Erndwein et al., 2020; Hu et al., 2022). As lodging depends on stem and root characteristics, various stem and root-related parameters have been screened for lodging resistance.

Breeders have associated several stem-related traits with lodging resistance. For example, plant

height was associated with lodging resistance due to its negative correlation in many crops (Li et al., 2021; Niu et al., 2022; Xue et al., 2017). Developing lodging-resistant cultivars by reducing plant height was widely explored in rice, barley, and other crops (Hwang & Lee, 2019; Long et al., 2020; Shao et al., 2022; Zhang et al., 2022). In canola, the integration of dwarf and semi-dwarf genes to lower the stem length was achieved (Wei et al., 2017). However, improving lodging resistance by reducing plant height could result in yield loss and susceptibility to diseases (Wei et al., 2017). The basal internode often plays a more significant role in lodging resistance than the middle and upper internodes, as the basal internode experiences the most critical internal forces (i.e., plant biomass) and external forces (i.e., winds and precipitation). Improved basal internode quality (i.e., dry weight per unit length, vascular area, and reduced length) has increased lodging resistance in maize (Huang et al., 2021).

Thicker stem diameter was also associated with lodging resistance (Miller et al., 2018; Shen et al., 2018), suggesting that stem diameter is a promising trait to screen for lodging resistance. Mechanical stem bending and breaking strength were also recommended for stem lodging resistance selection due to their strong positive correlations (Hu et al., 2022; Hussain et al., 2020). These findings illustrate that selecting stem lodging based on various stem characteristics, including its length, dry matter accumulation, and diameter, can be effective secondary traits for lodging resistance selection.

Various root characteristics were also studied to better understand factors that could affect crop root lodging. Phenotypic differences between the taproot and lateral root systems were observed in lodging-susceptible and lodging-resistant canola and wheat (Shah et al., 2019; Wu et al., 2020).

A positive correlation between root diameter and lodging resistance was found by Wu et al. (2021a). Other mechanical factors related to root lodging resistance were also explored. Kamara et al. (2003) suggested that vertical-pulling resistance could be used as an indicator to select root lodging resistance due to its correlation with lodging. Soil shear strength (a measurement of the anchorage strength of the root) was also reported to have a significant effect on lodging (Sposaro et al., 2008). Likewise, the root and soil interaction could also play a role in lodging resistance. Zuo et al. (2017) demonstrated that improving the root/shoot ratio by increasing sowing depth or soil compaction lowered lodging risk and eliminated weed infestation. These studies provide evidence that root-related traits such as root diameter, vertical pulling resistance, anchorage strength of the root, and increased root-to-shoot ratio could all be used to select against root lodging.

Lodging has become a more immediate concern as heat stress significantly impacts crop lodging frequency. Wu & Ma (2018) found that stem and root anchorage strength were reduced considerably when canola was exposed to high temperatures above 27 °C in a controlled environment (Wu & Ma, 2018). It was further discovered that heat stress increased the risk of lodging in *B. napus* at all developmental stages, but especially during early flowering, as it dramatically decreased stem parameters such as the number of vascular bundles, diameter, flexural rigidity, and root parameters such as lateral root growth, root-soil cone and root shear strength (Wu et al., 2021a, 2021b). The alteration of crop lodging under high-temperature regimes could be due to the following reasons: (1) heat stress could alter plant resource allocation, leading to adverse effects on root structure, which then alters the root anchorage strength; (2) heat stress may also accelerate cell division and growth, leading to thinner stem cell walls and a low number of vascular

bundles that may affect stem breaking strength (Wu et al., 2020). Yield loss due to lodging is concerning as rising global temperatures will likely continue.

Selecting quantitative trait loci (QTL) associated with lodging resistance is crucial to meet the increasing demand for oil and protein. Although much attention has been focused on mapping stem lodging-related traits such as plant height and branch initiation height (Shen et al., 2018; Wang et al., 2016), QTL identification for lodging has remained limited. This is likely because lodging is a complex, quantitative trait controlled by multiple genes and highly influenced by environmental conditions (Baker et al., 2014; Canola Council of Canada, 2020). Despite that, further studies could be conducted to explore additional QTL conferring lodging resistance. Once the QTL influencing lodging are identified, they could be utilized to elucidate lodging resistance mechanisms. Additionally, markers associated with QTL for lodging resistance can be used as marker-assisted selection in breeding programs.

In recent years, single nucleotide polymorphism (SNP) markers have become the marker of choice because of their prevalence in the genome and strong linkage to traits (Hayward et al., 2012). With the advancement of technology and the quick evolution of high throughput sequencing/genotyping, QTL analysis using SNP makers has become more economical and efficient in determining the genomic regions associated with quantitative traits in *Brassica napus* (Shen et al., 2018). Since the commercial release of the Illumina Infinium *Brassica* 60K SNP array (Clarke et al., 2016), and the completed sequencing of *Brassica napus* (Chalhoub et al., 2014), this SNP array has been successfully used in diverse applications, including molecular karyotypes, germplasm collection characterization, genome-wide association study, and bi-parental QTL

mapping (Fujimoto et al., 2019; Li et al., 2018; Mason et al., 2017; Shao et al., 2022). However, to our knowledge, the 60K SNP array has yet to be utilized for QTL mapping for lodging resistance in canola.

The objectives of this study were to (1) phenotype a doubled haploid (DH) population for lodging using a visual lodging scale (2) genotype these DH lines and their parents using the *Brassica* 60K SNP array, and (3) identify quantitative trait loci (QTL) for lodging resistance in *B. napus*. To conduct the research, high erucic acid rapeseed genotypes were utilized to determine QTL for lodging. A DH population was derived from a cross between a lodging-susceptible and lodging-resistant genotype. A high-density genetic map was constructed to identify the QTL associated with lodging.

3.3. Materials and Methods

3.3.1. Plant Material

The plant material was composed of 183 DH lines derived from a cross of 268-2 ZSDH6550/ LLHR10282-B-9-2 (lodging susceptible female parent) and CB131037-6-8 (pedigree: BC_1F_3 selection Castor*/UM500) (lodging resistant male parent), henceforth referred to as Z/B population. The DH population was developed from the microspore culture of F_1 plants of a cross between the two parents. These parents were selected in the field nursery in 2016, where lodging was highly prevalent. In that year, the 268-2 ZSDH6550/ LLHR10282-B-9-2 parent was highly susceptible to lodging and was assigned a lodging score of 5 on a 1-5 scale. The male CB131037-6-8 parent was lodging resistant, with a lodging score of 1. 268-2 ZSDH6550, LLHR10282-B-9-2 and UM500 are all other high erucic acid genotypes previously selected based on agronomic

performance and seed quality. The female parent, 268-2 ZSDH6550/ LLHR10282-B-9-2 was acquired through single plant selection (F_2 and F_3) from a cross between the 268-2 ZSDH6550 doubled haploid line and LLHR10282-B-9-2 (an F_4 generation cross between 06C421 and LLHR499-1-49). Castor is a high erucic acid and low glucosinolate summer rape (*Brassica napus* L.*)* that was well adapted to the southern regions of Western Canada (McVetty et al., 1998).

3.3.2. Seed Increase for Field Experiments

The Z/B DH lodging population was grown in the greenhouse for seed increase. The DH lines were sown in a cell pack and allowed to germinate until they reached the 2-4 true leaf stage (GS 12-14). These seedlings were then transplanted into a 14 cm diameter pot for growth. Water-soluble fertilizer (20 % nitrogen, 20 % phosphorus, 20 % potassium) was mixed with 15 mL in 3.8 L of water to feed the plants at bolting (GS 30). Orthene® soluble granule (4 mL) (Arysta LifeScience North America, Carry, NC) was mixed in 25 L of water and applied as a soil drench when aphids became evident. Selfing plastic bags were used to cover the plants during flowering (GS 60) to prevent cross-pollination. The bags were shaken every other day to ensure pollination. They were removed once plants reached the pod-filling stage (GS 70). Plants were hand-harvested after reaching maturity (GS 99). The collected seeds were later used for the field trials.

3.3.3. Field Experiments

The DH lines and their respective parents were phenotypically evaluated in four environments within Manitoba, Canada, in 2020, 2021, and 2022. Agriculture land in Carman (Latitude 49.50, Longitude -98.03), Portage la Prairie (Latitude 50.06, Longitude -98.27), and Winnipeg (Latitude 49.81, Longitude -97.12) was used to conduct the field experiments. Location-year combinations

were treated as environment/site-year, which were coded as CM20 (Carman, Manitoba, Canada, 2020), CM21 (Carman, Manitoba, 2021), PT21 (Portage la Prairie, Manitoba, Canda, 2021), and UM22 (Winnipeg, Manitoba, 2022). Site-years CM20 and CM21 were planted at the University of Manitoba Ian N. Morrison Research Farm in Carman. Soil properties were described as loamy lacustrine (Mills & Haluschak, 1993). Site-year PT21 was conducted near Portage la Prairie, and the soil texture was classified as clay lacustrine (Michalyna & Smith, 1972). UM22 was conducted at The University of Manitoba Field Research Laboratory in Winnipeg. The soil in this region was categorized as Riverdale Silty Clay (Michalyna et al., 1975). Entries were grown in a randomized complete block design (RCBD) with three blocks per environment, and each block represents a replicate. Before propagating, the seed of each genotype was treated with Helix, Lumiderm, and Buteo (2.66 mL/100 g). An experimental unit was a single row 3 m in length with a row spacing of 25 cm. For each genotype, 0.5 g of seed was planted for each row. The spring rapeseed cultivar MillenniUM 03 (McVetty et al., 2000) was utilized as check and guard entries for the experiments.

3.3.4. Field Management

The seedbed was prepared with a disc harrow with two passes in the fall or spring. Edge® Granular Herbicide (Gowan Canada, Winnipeg, MB) was applied at 27.9 kg/ha. Following a soil test, granular fertilizer (33 % of nitrogen, 12 % of phosphorus, 0 % of potassium, and 5 % of sulphur) was used at a rate between 444-642 kg/ha. Before sowing, an additional pass was made with a vibra-shank cultivator and circular harrows.

In Carman 2020, planting occurred on May 15 and 16 when the soil temperature was approximately 10 °C. Matador® (Syngenta Canada Inc., Guelph, ON) at the rate of 1.48 L/ha was

applied twice on May 29 and June 11 when the plants were at first to second leaf stage (GS 11-12) to control flea beetles (*Phyllotreta spp*). On June 11, a tank mixture of 1 % Merge® (BASF Canada Inc., Mississauga, ON) with the volume of water and 0.56 L/ha Faucet® L (BASF Canada Inc., Mississauga, ON) was applied to control green foxtail (*Setaria viridis* L.). On the same date, a tank of a mixture of 0.56 L/ha Lontrel™ (Corteva Agriscience Canada Company, Calgary, AB) and 0.67 L/ha Poast Ultra (BASF Canada Inc., Mississauga, ON) and 0.030 kg/ha Muster® Toss-N-Go® (FMC of Canada Limited, Mississauga, ON) was utilized to control broadleaf and other grassy weeds. Hand weeding for green foxtail occurred on at least two dates during the season when it became necessary. On July 6, Proline® Fungicide (Bayer CropScience Inc., Calgary, AB) was applied at 0.37 L/ha to control sclerotinia stem rot at the 40 % flowering stage (GS 64). Each row was individually harvested on August 27. Plants were harvested at maturity (GS 92) by straight cutting with a Wintersteiger plot combine (Wintersteiger Inc., Salt Lake City, UT).

In Carman 2021, planting occurred on May 11, with soil temperatures at approximately 12 °C. Matador® (Syngenta Canada Inc., Guelph, ON) at the rate of 1.48 L/ha was applied on June 3 and June 15, when seedlings were at the cotyledon to second leaf stage (GS 10-12) and when plants were in between the second to fourth leaf stages (GS 12-14) to control flea beetles. On June 3, a tank mixture of 0.54 L/ha Lontrel™ (Corteva Agriscience Canada Company, Calgary, AB), 0.67 L/ha Poast® Ultra (BASF Canada Inc., Mississauga, ON), and 0.03 kg/ha Muster® Toss-N-Go® (FMC of Canada Limited, Mississauga, ON) was applied at the second to third leaf stage (GS 12-13) to control broadleaf and grassy weeds. Hand weeding for green foxtail occurred on at least two dates during the season when it became necessary. Inter-row cultivation was also conducted on

June 2. No fungicide applications were applied due to extreme weather conditions. All rows were individually harvested on September 6 and 7 using the abovementioned method.

In Portage 2021, seed was sown on May 14 when the soil temperature was approximately 13 °C. Matador® (Syngenta Canada Inc., Guelph, ON) was applied at the rate of 1.48 L/ha on June 1 and June 8, when seedlings were at the cotyledon to second leaf stage (GS 10-12) and when plants were in between the second to fourth leaf stages (GS 12-14) to control flea beetles, respectively. On June 14, a tank of a mixture of 0.54 L/ha Lontrel™ (Corteva Agriscience Canada Company, Calgary, AB) and 0.67 L/ha Poast® Ultra (BASF Canada Inc., Mississauga, ON) and 0.03 kg/ha Muster® Toss-N-Go® (FMC of Canada Limited, Mississauga, ON) were applied when plants were at the second to third leaf stage (GS 12-13) to control broadleaf and other grassy weeds. Hand weeding for volunteer canola and wheat occurred on at least two dates during the season when necessary. Inter-row cultivation was conducted on June 26 and 29. No fungicides were sprayed at this location due to extreme dry weather conditions. Rows were harvested on August 26 and 27.

In Winnipeg 2022, seed was sown on May 27 on block 23 when the soil temperature was approximately 15 °C. Minor changes were applied to this environment. DH genotypes were planted in single rows of 4 m with row spacings of 35 cm instead of 3 m in length with row spacings of 25 cm. Matador® (Syngenta Canada Inc., Guelph, ON) was applied at the rate of 1.48 L/ha on June 9 and June 20, when seedlings were at the first to second leaf stage (GS 11-12) and when plants were in between the third to fourth leaf stage (GS 13-14) to control of flea beetles, respectively. On June 20, a tank mix of 1 % Merge® (BASF Canada Inc., Mississauga, ON) per volume of water, 0.5 L/ha Lontrel™ (Corteva Agriscience Canada Company, Calgary, AB) and

0.67 L/ha Poast® Ultra (BASF Canada Inc., Mississauga, ON), and 0.03 kg/ha Muster® Toss-N-Go® (FMC of Canada Limited, Mississauga, ON) were applied to control broadleaf and grassy weeds. Hand weeding for round leaf mallow (*Malva pusilla* Sm.), Canada thistle (*Cirsium arvense* L.), green foxtail, and other significant weeds occurred at least three dates during the season when it became necessary. All rows were individually harvested on September 14.

3.3.5. Phenotypic Evaluation

Lodging (LODG), days-to-flower (DTF), plant height (HT), and days-to-maturity (DTM) data were recorded throughout the growing season. The degree of lodging susceptibility was defined as the angle between a completely vertical growth habit and the ground. A visual lodging score between 1 and 5 was utilized (Holshouser, 2015; Quijada et al., 2006). A lodging rating of one was given to rows that stood erect, and five to rows that touched or were near the ground (**Figure 2.2**). The rating for lodging was conducted when the plants were at the maturing stage (GS 83-85) (Wu et al., 2019). To avoid bias during lodging evaluation, the same person invesigated lodging score across all environments. The days-to-flower was recorded for each plot when 50 % of the plants commenced flowering (GS 63) (Wu et al., 2019). For plant height, a few plants within the row were used to measure from the ground to the top of the plant during the pod-filling stage (GS 70-72) (Shen et al., 2018). Days-to-maturity was collected when 70 – 80 % of the pods changed colour (GS 87-89).

3.3.6. Statistical analysis

Analysis of variance (ANOVA) was conducted to determine the statistical significance of genotype, site-year, and genotype by site-year interaction for lodging data. When the data could

not be combined across site years, data was analyzed as a single site. The statistical analyses followed the generalized linear mixed model (GLMM) using PROC GLIMMIX in the Statistical Analysis Software (SAS) version 9.4 (SAS Institute, Cary, NC). The GLMM equation is as follows:

$\eta_{ij} = \eta + \tau i + bj$ (Bowley, 2015, p. 47)

Where η (eta) is the intercept, τ (tau) is the fixed effect, and b is the random effect. The GLMM is a recommended model for the non-normal distribution of proportion data (Hernández et al., 2017). Before the analysis, lodging ratings were converted to correspond to the proportion range between 0 and 1, excluding 0 and 1 (**Table 3.1**) (Bowley, 2015, p. 120; Brand et al., 2022). As a result, the lodging rating of five was converted to a proportional of 0.999. Due to the nature of the data (i.e., a proportion between 0 and 1), the model statement in PROC GLIMMIX was specified as a beta distribution with a logit link (Bowley, 2015, p. 115). In this study, genotype, site-year, and the genotype by site-year interaction (written as genotype*site-year) were considered fixed effects, while block nested in site-year (written as (block(site-year))) was treated as random.

Since the mean estimate and standard error associated with the mean estimate were computed on the model scale, the ilink option was used to convert the model scale to the data scale (Bowley, 2015, p. 83; Brand et al., 2022). The maximum likelihood with Laplace approximation was utilized as it gave the lowest chi/degree of freedom. The mean separation between the parental and the check genotypes was computed using the ESTIMATE statement, with significant differences declared at $p < 0.05$.

Table 3.1: Lodging rating scale and its corresponding proportion for beta distribution analysis.

Rating	Lodging description	Proportion[a]
1	Standing straight at about 90-degree angle from the ground	0.2
2	All plants were leaning slightly, or a few plants were on the ground	0.4
3	All plants were leaning more than a 45-degree angle from the ground, or 25-50 % of the plants were down	0.6
4	All plants are leaning less than a 45-degree angle from the ground, or 50-80 % of the plants were down	0.8
5	All or most plants completely laid flat on the ground	0.999

[a] For a given lodging rating, severity proportion = (rating value * 0.2), except rating of 5 was set to 0.999 as beta distribution data can only be analyzed with a range value greater than 0 but less than 1.

The correlation analysis was done separately by individual site year. Correlation coefficients were computed using PROC CORR in SAS version 9.4. Spearman correlation coefficients (Bowley, 2015, p. 63) were determined between LODG and DTF, HT, and DTM, while Pearson correlation coefficients (Bowley, 2015, p. 61) were computed between DTF, HT, and DTM.

The software multi-environment trial analysis with R (META-R) for Microsoft Windows (Alvarado et al., 2020) was used to calculate broad sense heritability (h^2). According to the formula:

$$h^2 = {\sigma^2}_G/({\sigma^2}_G + {\sigma^2}_e/nreps)$$

where ${\sigma^2}_G$ is the genotypic variation, ${\sigma^2}_e$ is error variance, and nreps is the number of replicates.

3.3.7. DNA Extraction and Genotyping

Each genotype was sown into 4 × 3 cell packs containing Sunshine Metro Mix potting soil (Sungro® Horticulture, MA, USA), watered daily, and kept inside the growth chamber (day temperature 22 °C; night temperature 18 °C; light cycle 16-hour light, 8 hours dark) for germination. Seedlings were allowed to grow until the second to fourth true leaf stage (GS 12-14), and leaf tissue was collected and stored at -80 C until DNA extraction could occur. Genomic DNA was extracted using the DNeasy Plant mini kit (Qiagen, Toronto, Canada). The extraction steps followed the Qiagen extraction manual with slight modifications to optimize DNA yield. The following paragraph describes in detail how DNA was extracted using the kit.

Young leaf tissue was ground using a plastic pestle in a 1.5 mL microcentrifuge tube. A volume of 400 µL AP1 solution was added to the previously ground tissue, and the liquid mixture was gently

ground again until they were homogenized, then 3 µL of RNase A was added. To lyse the cells, the samples were incubated in a water bath at 65 °C for 25 min while samples were vortexed at 5 min intervals. To precipitate unwanted chemicals such as detergents, proteins, and polysaccharides, 130 µL of P3 was added to the lysate, mixed, and incubated on ice for 5 min. The lysate was centrifuged at 16,500 g for 6 min. A volume of 500 µL lysate was transferred to the QIA shredder Mini spin column, placed in a 2 mL collection tube, and centrifuged at 16,500 g for 3 min. A volume of 400 µL of aqueous flow through was transferred to a new 1.5 mL microcentrifuge tube. For precipitation, 1.5 volumes of Buffer AW1 was added to the aqueous flow through and pipetted up and down to mix. The solution was incubated at room temperature for 5 min. For DNA binding, 650 µL of the mixture was transferred onto the DNeasy Mini spin column, placed in a 2 mL collection tube, centrifuged for 1 min, and discarded the flow through. This step was repeated to the remaining precipitated DNA solution. The DNeasy Mini spin column was then placed into a new 2 mL collection tube, and 500 µL of AW2 was added for washing. The samples were centrifuged at 6,500 g for 1 min and discarded to flow through. This washing step was repeated and centrifuged at 16,500 g for 3 min. The DNeasy Mini spin column was transferred to a new 1.5 mL microcentrifuge tube, 50 µL of AE solution was added, incubated at room temperature for 10 min, and centrifuged at 6,500 g for 1 min to release DNA from the column. The flow through was re-added to the column and the previous step was repeated to further extract DNA from the DNeasy Mini spin column.

The extracted DNA was quantified using a Nanodrop™ 2000 UV-Vis Spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA). To evaluate the quality and purity of the DNA samples, the absorbance measurements at 260 nm and 280 nm were obtained, yielding the A260:A280 ratio.

This ratio served as an indicator of the sample's quality and purity, and all recorded results were utilized to measure DNA quantity and quality for SNP array analysis. After that, the genomic DNA was genotyped using the Illumina Infinium™ 60K *Brassica* SNP BeadChip Array containing 52,157 SNPs (Illumina Inc., CA, USA) (Clarke et al., 2016), conducted in the lab of Dr. Isobel Parkin at Agriculture and Agrifood Canada (Saskatoon, Saskatchewan, Canada).

3.3.8. SNP Filtering

The raw genotyping data were analyzed with the genotyping module in Genome-Studio V2.0.5 software package (Illumina Inc., San Diego, CA, USA) using the default clustering parameter. The *Brassica* 60K final cluster file (Clarke et al., 2016) was added. The cluster file removed SNP markers that exhibited minimal amplification and displayed unsolved cluster patterns in a wide range of *B. napus* genotypes, typically caused by hybridizing of the A and C genomes (Mason et al., 2017). The options of "Calculated Samples and SNP Statistic" and "Calculate Heritability" were used. The "Gene Score Threshold" was changed to 0.05 from 0.15. Samples with a call rate < 0.8 were filtered out. The raw data was exported from Genome Studio to "Include Zeroed SNPs in the report and intensity only SNPs" with the option to "use top strand" while excluding "GenCall score".

A Python script file provided by Dr. Chawla Harmeet (Department of Plant Science, University of Manitoba, Winnipeg, Canada, personal communication) was utilized to filter the data further. The script file assigned first parent (i.e. female) and second parent (i.e. male) alleles to a single letter code of A and B, respectively. The ratio method was selected as the filtering option. The ratio filtering method allowed skewed markers from 1:1 to be filtered through. Specifically, SNP

markers with skewness up to 3:2 at each locus were retained. This slight deviation was utilized as segregation distortion and anticipated in DH settings as viability varies between genotypes (Ferrie & Möllers, 2011; Niemirowicz-Szczytt, 1997; Starosta et al., 2023). The proposed explanation of occurrences for this phenomenon is its linkage with genes involved in the embryonic potential of the microspores (Ferrie & Möllers, 2011). In other words, some individuals without specific gene combinations were culled, leading to the observed skewness ratio. Further filtering steps were applied using Microsoft Office Excel to remove loci in which the parents were genotyped as missing data. After these filtering steps, 5,153 out of 77,970 SNP makers were left in the Z/B DH population.

3.3.9. Genetic Linkage Map Construction

One hundred eighty-three individuals from the Z/B DH population were used to calculate the genetic linkage maps. Genetic linkage maps were generated using MSTMap software (Wu et al., 2008), applying the Kosambi map and maximum likelihood objective functions. Markers with more than 15 % missing observations were also removed from the map. The option "detect_bad_data" was turned on to detect bad data during the map construction process. To determine SNP markers' physical location on chromosomes, markers were anchored to *Brassica. napus* cv. 'Darmor-*bzh*' reference v4.1 genome (Chalhoub et al., 2014) using the basic local alignment search tool (BLAST) (http://blast.ncbi.nlm.nih.gov). The "cut_off_p_value" threshold used for clustering markers into linkage groups (LG) was manually adjusted until constructed linkage groups comprised at least 90 % of makers of the same chromosome.

3.3.10. QTL Mapping

Prior to QTL mapping, the phenotypic means for LODG, DTF, HT, and DTM were adjusted based on Best Linear Unbiased Estimates (BLUEs) (Bourke et al., 2019; Muqaddasi et al., 2019; Samayoa et al., 2014) using META-R software previously mentioned. According to Alvarado et al. (2020), genotypes were considered fixed when calculating BLUES for individual environments, while other terms were considered random. Similarly, when computing the BLUEs in combined across environments, genotype and environment effect were considered fixed, whereas other terms were treated as random.

QTL detection for lodging resistance and other traits was conducted using inclusive composite interval mapping (ICIM-ADD) functions(Shao et al., 2022; Shen et al., 2018; Zhao et al., 2019) in the IciMapping v4.2.53 software package (Meng et al., 2015). Analysis of additive effect QTL was carried out using a 0.1 cM scanning interval, and the logarithm of the odds (LOD) significance threshold was calculated with 1000 permutations at a 95 % confidence level (Churchill & Doerge, 1994). Based on the premutation test, the LOD threshold for additive QTL was 2.8. Detected additive-QTL were assigned the letter “q” (abbreviated for QTL), followed by trait abbreviation, population name, and chromosome number (Thambugala et al., 2020). If multiple QTL were detected on the same chromosome, additional numerical values were used to distinguish these QTL (e.g., qLODG.Z/B-A03.1**)**. QTL on the same chromosomes were considered separate QTL when they were on different linkage groups or separated by more than 15 cM in genetic distance.

3.3.11. Candidate Genes/Transcripts Analysis

To identify candidate transcripts for lodging, physical intervals of QTL for lodging that had phenotypic value explained (PVE) above 10 % were utilized to screen for possible mRNAs and their corresponding protein-accessions using genome assembly Darmor-*bzh* version 10 annotation (.gff) file from (Rousseau-Gueutin et al., 2020) (http://www.genoscope.cns.fr/plants). Once the protein accessions were acquired, the (supplementary table S2: Functional gene annotation of the reference genome assembly Darmor-*bzh* version 10 (AHRD-Version 3.11)) from Vollrath et al. (2021) was used to determine its corresponding functions and gene-ontology (GO). Candidate transcripts were selected based on GO terms associated with plant-type cell wall, cellulose, lignin, root development, and ethylene, gibberellin, auxin and brassinosteroid-related activities (Li et al., 2022; Liu et al., 2022; Niu et al., 2021; Shah et al., 2019).

3.4. Results

3.4.1. Phenotypic Analysis

The analysis of variance (ANOVA) was conducted on combined site-years to evaluate the effect of genotype, site-year, and their interaction on the phenotypic expression of the agronomic traits. In the combined site-years, genotypes, site-years, and genotype-by-site-year interactions were all found to affect all traits significantly ($p < 0.001$) (**Table 3.2**). Since site-year was shown to be significantly different in the combined site-years, ANOVA of individual site-year was further investigated to determine the effect of genotype on the phenotypic expression of all traits in each site-year. After the statistical analysis, genotype was revealed to influence all traits' phenotypic expression significantly **(Table 3.3).** These results indicated that genetic and environmental factors significantly influenced LODG, DTF, HT, and DTM.

Table 3.2: Analysis of variance for lodging (LODG), days-to-flower (DTF), height (HT), and days-to-maturity (DTM) in the DH Z/B population grown in four environments in 2020, 2021, and 2022.

Source of variation	LODG	DTF	HT	DTM
Genotype	6.20***	23.17***	9.83***	10.49***
Site-year	515.88***	4297.31***	78.81***	730.87***
Genotype*Site-year	1.70***	3.06***	2.29***	3.43***

$***p < 0.001$; $**p < 0.01$; $*p < 0.05$

The data was analyzed using a generalized linear mixed model and adjusted using a beta distribution. Genotype, site-year, and genotype by site-year interaction were considered fixed, while blocks nested in site-year were considered random. F-test values are presented, with an asterisk(s) representing significant levels.

Table 3.3: Analysis of variance for Lodging (LODG), Days-to-flower (DTF), Height (HT), and Days-to-maturity (DTM) in the DH Z/B Population grown in CM20, CM21, PT21, and UM22.

	Source of variation	LODG	DTF	HT	DTM
20CM	**Genotype**	4.74***	10.58***	3.36***	7.26***
21CM		1.50***	6.50***	3.59***	4.33***
21PT		3.01***	9.37***	4.41***	7.40***
22UM		4.16***	8.55***	5.10***	3.63***

****p < 0.001; **p < 0.01; *p < 0.05*

CM20: 2020, Carman, Manitoba, Canada

CM21: 2021, Carman, Manitoba, Canada

PT21: 2021, Portage la Prairie, Manitoba, Canada

UM22: 2022, Winnipeg, Manitoba, Canada

The data was analyzed using a generalized linear mixed model with adjustments made using a beta distribution. Genotype, site-year, and genotype by site-year interaction were considered fixed effects, while block nested in site-year was treated as a random effect. The F-test values are reported, with asterisks denoting the levels of significance.

3.4.2. Evaluation of Lodging in The Parents, Check, and DH Genotypes

The check cultivar (MillenniUM 03), the lodging-resistant male parent (CB131037-6-8), and the lodging-susceptible female parent (268-2 ZSDH6550/ LLHR10282-B-9-2) showed a lodging score ranging between 2.66-4.64, 1.06-2.02, and 2.66- 4.50, respectively, among four site-years (**Table 3.4**). A significant difference ($p < 0.05$) for lodging was detected in all environments when comparing lodging-resistant and lodging-sensitive parents. No comparison between the parents was made for UM22 due to missing data. Lodging-resistant parents at all site-years were significantly different from the check cultivar. In contrast, lodging susceptible parents at all site-years was not significantly different from the check cultivar. These results suggest that the male parent is likely to harbour lodging-resistant alleles. The maximum lodging score difference between the female and the male parent in all site-years was 2.82 ($p < 0.05$, CM21).

The DH genotypes among four site-years showed a considerable variation in lodging score, ranging from 1.06 to 4.93. The largest and the most minor lodging score variation was observed in PT21 and CM20, respectively. The population grand means ranged between 1.69 and 3.23 across site-years, with the lowest and highest lodging grand mean illustrated in CM20 and UM22, respectively (**Table 3.4**). The broad sense heritabilities (h^2) for lodging in CM20, CM21, PT21, and UM22 were computed as 0.72, 0.49, 0.63, and 0.76, respectively.

3.4.3. Correlations Between Lodging and Other Traits

Given the significant effect of site-year ($p < 0.001$) (**Table 3.2**) on LODG, the correlation analysis was conducted at individual site-year levels rather than combined site-years. The Spearman's correlation coefficient between LODG and other critical agronomic traits,

Table 3.4: Lodging score for the DH Z/B genotypes, a check cultivar, resistant parent, and susceptible parent in CM20, CM21, PT21, and UM22.

Environment	Check (MillenniUM 03)	Male (Lodging Resistant)	Female (Lodging susceptible)	DH Genotypes Range	Grand Mean	Heritability
CM20	2.66 B	1.06 A	2.66 B	1.06-3.66	1.69	0.72
CM21	4.30 B	1.68 A	4.50 B	1.68-4.57	2.66	0.49
PT21	2.97 B	1.35 A	2.66 B	1.08-4.93	2.23	0.63
UM22	4.64 A	2.02 B	.	1.34-4.82	3.23	0.76

The male and female were initially selected with lodging scores of 2 and 5, respectively.

CM20: 2020, Carman, Manitoba, Canada

CM21: 2021, Carman, Manitoba, Canada

PT21: 2021, Portage la Prairie, Manitoba, Canada

UM22: 2022, Winnipeg, Manitoba, Canada

Each value represents the least square mean of lodging response from three replicates using a scale of 1 to 5, where 1 represents complete lodging resistance, and 5 represents lodging susceptibility. Means with the same letter(s) are not significantly different (α=0.05). UM22 with a "." added to its column space was not evaluated.

including DTF, HT and DTM were detected across four environments (**Table 3.5**). A significant negative correlation ($p < 0.001$) was calculated for DTF and LODG ($r_s = -0.37$, UM22). Interestingly, HT also exhibited a significant negative correlation ($p < 0.001$) with LODG ($r_s = -0.15$, UM22). Conversely, the DTM was significantly positively correlated ($p < 0.001$) with LODG ($r_s = 0.36$, CM21).

The Pearson's correlation coefficients between DTF, HT, and DTM traits were also detected across four environments. The correlation coefficient between these traits ranged from 0.00 to 0.52 (**Table 3.5**). The correlation between DTF and DTM was significantly positively correlated with the highest coefficient ($r = 0.52$, PT21). This significant positive correlation was also observed between DTF and HT ($r = 0.36$, CM20). The correlation between DTM and HT was significantly positively correlated in only three environments, and no correlation was observed in CM20.

3.4.4. Linkage Map

A high-density genetic linkage map was developed using the 60K *Brassica napus* Infinium SNPs chip. A total of 4,668 SNP markers were mapped in the Z/B population. These SNP makers were then constructed into 21 distinct linkage groups anchored to 15 physical chromosomes of *B. napus* Darmor-*bzh*, excluding A02, A04, A09, and C09 (**Table 3.6**). The total coverage of these makers was 1000 cM on the genetic map, and the average marker interval was 0.21 cM. When these markers were anchored to the physical chromosome, they showed alignment with most of the B. napus Darmor-bzh chromosomes. The number of markers varied across the constructed linkage groups, with linkage group 22 comprising 11 markers and being anchored to C05, while

Table 3.5: Correlation coefficient (r) among traits calculated in Z/B DH population grown in Carman 2020 (CM20), Carman 2021 (CM21), Portage la Prairie 2021 (PT21), and Winnipeg 2022 (UM21) using Spearman ranking for lodging (LODG), and Pearson method for days-to-flower (DTF), height (HT), and days-to-maturity (DTM).

		Spearman	**Pearson**	**Pearson**
	Trait	**LODG**	**DTF**	**HT**
CM20	DTF	-0.24***		
	HT	-0.14***	0.36***	
	DTM	0.25***	0.26***	0.00
CM21	DTF	-0.11*		
	HT	-0.04	0.31***	
	DTM	0.36***	0.47***	0.25***
PT21	DTF	-0.17***		
	HT	-0.10*	0.30***	
	DTM	-0.09*	0.52***	0.31***
UM22	DTF	-0.37***		
	HT	-0.15**	0.18***	
	DTM	0.13**	0.06	0.36***

****p < 0.001; **p < 0.01; *p < 0.05.* Significant levels are indicated using asterisks.

Table 3.6: Chromosome location on the *Brassica napus*-Damor reference genome and map coverage in cM of linkage groups constructed in MSTmap using Z/B DH *Brassica napus* population of 183 individuals.

Linkage Group	Number of markers	Total Map Coverage (cM)	Chromosome (*B. napus*-Damor)
LG-2	95	16.59	A01
LG-8	194	61.19	A03
LG-9	135	35.59	A03
LG-10	608	90.43	A05
LG-12	50	15.58	A05
LG-13	283	137.25	A06
LG-3	205	82.40	A07
LG-15	14	13.73	A07
LG-18	13	1.88	A08
LG-19	131	53.37	A10
LG-0	260	40.93	C01
LG-4	1528	81.61	C02
LG-7	76	11.47	C03
LG-26	80	18.18	C03
LG-6	93	50.34	C04
LG-21	404	97.70	C04
LG-14	215	98.07	C05
LG-22	11	0.00	C05
LG-16	23	16.42	C06

Table 3.6: Continued

Linkage Group	Number of markers	Total Map Coverage (cM)	Chromosome (*B. napus*-Damor)
LG-23	75	16.20	C07
LG-25	122	61.17	C08
Total	4615	1000.10	

Linkage groups with less than ten markers and markers mapped into a genetic map of 0.00 cM are not shown. This results in the exclusion of 53 markers from the table.

linkage group 04 consisted of 1528 markers. In several instances, multiple linkage groups were anchored to the same physical chromosome (e.g., LG-8 and LG-9 on A03, LG-10 and LG-12 on A05, LG-3 and LG-15 on A07).

3.4.5. QTL Analysis (Z/B Population)

3.4.5.1. Lodging

In the Z/B DH population, applying the ICIM method with the additive effect module from IciMapping led to the identification of nine additive-QTL associated with LODG **(Table 3.7)**. These QTL located on A01, A03, A05, A07, A10, and C01, and were named qLODG.Z/B-A01, qLODG.Z/B-A03.1, qLODG.Z/B-A03.2, qLODG.Z/B-A03.3 qLODG.Z/B-A05, qLODG.Z/B-A07.1, qLODG.Z/B-A10, and qLODG.Z/B-C01, respectively. The male alleles of CB131037-6-8 increased lodging resistance at six of these QTL, which were expected as the male was the resistant parent. The phenotypic variation explained ranged from 3.34 to 17.31 %. QTL on chromosomes A03 and A07 illustrated the highest phenotypic variation explained among all the QTL. The qLODG.Z/B-A03.3 and qLODG.Z/B-A03.1 explained 13.07 % and 17.31 % of the observed phenotypic variation. Similarly, the qLODG-A07.1 explained 16.00 % of the observed phenotypic variation.

Six lodging QTL (qLODG.Z/B-A01, qLODG.Z/B-A03.1, qLODG.Z/B-A03.3, qLODG.Z/B-A05, qLODG.Z/B-A07.1, and qLODG.Z/B-C01) were detected in more than one environment **(Table 3.7)**. Two QTL (qLODG.Z/B-A3.1 and qLODG.Z/B-A7.1) had a phenotypic variation that explained over 15 %, suggesting that these QTL could have a major contribution to lodging resistance in the Z/B DH population. LODG QTL (qLODG.Z/B-A01) co-localized with QTL for

Table 3.7: Detection of additive-effect QTL for Lodging, Days-to-Flower, Height and Days-to-Maturity in a bi-parental Z/B DH population grown in CM20, CM21, PT21, and UM22. ICIM mapping method was utilized.

Trait /QTL	Environment	LG	Chr.	Genetic Position	LOD	PVE(%)	Add	CI (cM)	PI (Mb)
Lodging									
qLODG.Z/B-A01	CMB	2	A01	5.90	4.41	7.95	0.11	5.85-8.05	3.29-3.72
	UM22	2	A01	13.40	4.26	8.75	0.18	13.35-15.85	5.05-5.17
qLODG.Z/B-A03.1	UM22	8	A03	30.40	8.03	17.31	-0.25	29.65-30.45	23.45-23.49
	CMB	8	A03	38.60	3.54	6.32	-0.10	37.25-39.45	22.92-22.97
qLODG.Z/B-A03.2	CM20	9	A03	21.20	7.03	7.20	-0.23	15.95-25.85	3.11-3.76
qLODG.Z/B-A03.3	CM20	9	A03	35.50	11.47	13.07	0.31	35.35-35.5	0.20-2.44
	CMB	9	A03	35.50	3.46	6.64	0.11	35.35-35.5	0.20-2.44
qLODG.Z/B-A05	CM20	10	A05	59.30	5.55	5.57	0.20	58.25-59.35	6.59-6.74
	CMB	10	A05	70.70	2.89	5.12	0.09	68.55-70.75	12.10-26.40
qLODG.Z/B-A07.1	PT21	3	A07	23.90	5.07	16.00	0.20	22.55-23.95	15.60-15.70
	CM21	3	A07	39.70	6.54	15.63	0.19	39.65-40.95	16.92-23.93
	CMB	3	A07	39.70	4.52	9.54	0.13	39.65-40.85	16.92-23.93

Table 3.7: Continued

Trait /QTL	Environment	LG	Chr.	Genetic Position	LOD	PVE (%)	Add	CI (cM)	PI (Mb)
qLODG.Z/B-A07.2	CM20	3	A07	61.40	3.41	3.34	0.15	59.65-63.65	18.77-19.07
qLODG.Z/B-A10	UM22	19	A10	0.00	3.01	6.06	-0.15	0.00-0.05	18.56-18.80
qLODG.Z/B-C01	CM21	0	C01	22.00	3.13	7.17	0.13	20.95-22.05	38.26-41.27
	CM20	0	C01	33.10	6.41	7.86	0.24	32.55-33.65	8.62-21.32
	CMB	0	C01	33.80	4.45	8.20	0.12	33.75-36.15	8.62-19.44
Days-to-flower									
qDTF.Z/B-A01	PT21	2	A01	5.90	3.01	7.45	-0.44	5.85-8.05	3.29-3.72
	CM20	2	A01	13.40	3.39	10.75	-0.51	13.35-16.05	5.05-7.33
	CMB	2	A01	13.40	3.12	8.35	-0.40	13.35-15.95	5.05-5.17
qDTF.Z/B-C04	CMB	6	C04	22.40	2.91	7.86	0.40	20.25-22.75	3.00-3.65
	CM21	6	C04	22.70	3.75	7.33	0.72	20.25-22.75	3.00-3.65
Height									
qHT.Z/B-A01	PT21	2	A01	12.80	5.41	9.20	-2.06	11.75-13.35	4.27-5.05
	UM22	2	A01	13.40	5.13	9.69	-2.56	13.35-16.05	5.05-7.33

Table 3.7: Continued

Trait /QTL	Environment	LG	Chr.	Genetic Position	LOD	PVE (%)	Add	CI (cM)	PI (Mb)
	CM20	2	A01	13.40	4.60	9.25	-1.90	13.35-16.05	5.05-7.33
	CMB	2	A01	13.40	8.03	15.05	-2.00	13.35-14.95	5.05-5.17
qHT.Z/B-A03	UM22	8	A03	16.20	8.46	16.69	-3.38	14.35-16.75	25.51-48.7
qHT.Z/B-A06	CM21	13	A06	47.20	3.69	8.20	-1.53	39.35-47.25	38.66-39.93
qHT.Z/B-C03	PT21	7	C03	3.30	3.57	5.90	-1.67	3.25-6.05	24.64-25.11
qHT.Z/B-C06	CM20	16	C06	0.00	3.72	7.39	-1.71	0.00-0.05	46.62-47.06
	PT21	16	C06	0.00	5.08	8.59	-2.00	0.00-0.05	46.62-47.06
	CMB	16	C06	0.00	5.16	9.30	-1.58	0.00-0.05	46.62-47.06
Days-to-maturity									
qDTM.Z/B-A01	CMB	2	A01	11.80	2.90	6.93	-0.39	11.75-12.85	4.27-5.05
	UM22	2	A01	12.90	3.76	6.41	-0.66	11.75-12.85	4.27-5.05
qDTM.Z/B-A03	UM22	8	A03	28.90	17.18	35.32	-1.56	28.45-28.95	23.60-23.98
qDTM.Z/B-A06	CMB	13	A06	74.10	3.59	8.67	-0.45	72.95-74.15	6.94-6.99
qDTM.Z/B-C03	PT21	26	C03	1.60	2.86	7.01	-0.62	0.85-1.65	70.28-70.55

QTL, Quantitative Trait Loci; Env., Environment; LG, Linkage Group; Chr., Chromosome; Pos., Genetic position on the linkage group in cM; LOD, Logarithm of odds peak; LOD threshold for Inclusive Composite Interval Mapping (ICIM) is 2.8 resulted from a 1000 premutation test at the threshold of $\alpha = 0.05$; PVE, phenotypic variation explained (r^2; %); Add, Additive effect of allele substitution. A positive number indicated that the female (susceptible parent) allele increased the respective quantitative trait and vice-versa; CI, Genetic Confidence Interval (LOD ±1); PI, Physical Confidence Interval; DTF, Days-to-flower, DTM, Days-to-maturity, HT, Plant height (cm); CMB, combined data set; CM20, Carman 2020; CM21, Carman 2021; PT21, Portage la Prairie 2021; UM22, Winnipeg 2022. **Bolded QTL indicates that it was detected in more than one environment.**

DTF (qDTF.Z/B-A01), HT (qHT.Z/B-A01), and DTM (qDTM.Z/B-A01); the overlapping interval region for these traits was between 11.75 to 12.85 cM (**Figure 3.1**).

3.4.5.2. Days-to-Flower

Two additive QTL for DTF were revealed in the Z/B DH population. The QTL resided on A01 and C04 and were named qDTF.Z/B-A01 and qDTF.Z/B-C04 (**Table 3.7**). These two QTL were considered as stable QTL as they were detected in more than one environment. The female parent (268-2 ZSH6550/ LLHR10282-B-9-2) alleles delayed DTF at qDTF.Z/B-C04. Male parent (CB131037-6-8) alleles delayed DTF at qDTF.Z/B-A01. The phenotypic value explained ranged between 7.33 and 10.75 %.

3.4.5.3. Height

Five additive-QTL for HT were detected in the Z/B DH population. The QTL were located on A01, A03, A06, C03, and C06, and labelled as qHT.Z/B-A01, qHT.Z/B-A03, qHT.Z/B-A06, qHT.Z/B-C03, and qHT.Z/B-C06 (**Table 3.7**). The female (268-2 ZSH6550/ LLHR10282-B-9-2) alleles reduced HT in all QTL. qHT.Z/B-A01 and qHT.Z/B.C06 were considered as stable QTL. The female alleles from the lodging susceptible parent all reduced plant height, suggesting a possible inverse relationship between lodging and height. This inverse relationship was also evident in the significant negative correlation identified in (**Table 3.5**). The phenotypic value explained for HT ranged from 5.90 to 16.69 %.

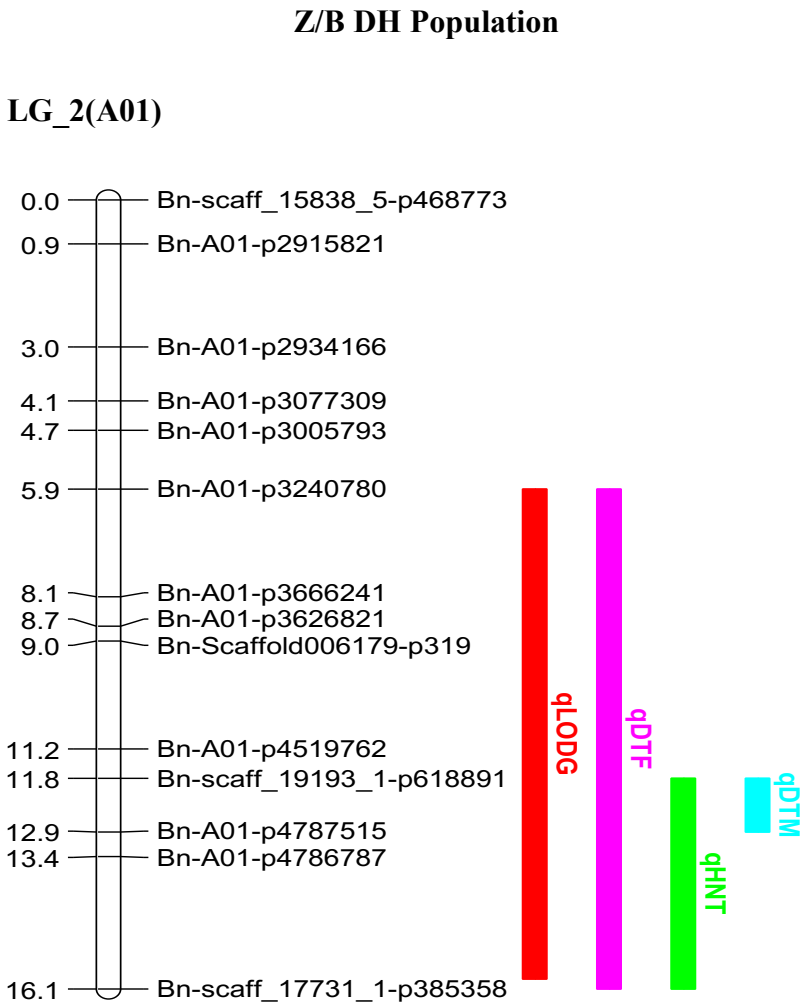

Figure 3.1: QTL mapping for lodging in biparental Z/B DH population at four individual site-years and a combined site-year using Inclusive Composite Interval Mapping (ICIM). Only QTL that overlapped between traits are shown.

3.4.5.4. Days-to-Maturity

Four additive-QTL for DTM were found in the Z/B DH population. The QTL were located on A01, A03, A06, and C03 and named as qDTM.Z/B-A01, qDTM.Z/B-A03, qDTM.Z/B-A06, qDTM.Z/B-C03 (**Table 3.7**). Only qDM.Z/B-A01 was considered as a stable QTL. The male (CB131037-6-8) alleles delayed maturity for all of these QTL. The phenotypic value explained for DTM ranged between 2.86 and 17.18 %.

3.4.6. Candidate Transcript Mining for Lodging

The physical confidence intervals of qLODG.Z/B-A03.1, qLODG.Z/B-A03.3, qLODG.Z/B-A7.1, and qLODG-A7.2 were utilized to screen for putative candidate protein transcripts as their phenotypic variation explained were above 10 %. Based on GO terms, forty-four potential candidate transcripts were identified for the LODG trait **(Table 3.8)**. Candidate transcripts mainly functioned in plant cell wall biosynthesis/modification, root development, and cellulose activities, such as root meristem growth factor 6, expansin, zinc finger family protein, cellulose synthase, and NGR2. The remaining transcripts were involved in several important signaling pathways associated with ethylene, auxin, gibberellic acid, and brassinosteroid, including ethylene-responsive transcription factor ERF115, auxin response factor, zinc finger protein GIS, zin finger protein 6, and protein BRASSINAZOLE-RESISTANT 1.

Table 3.8: Candidate transcripts for lodging in the DH Z/B population based on physical QTL interval detected with ICIM mapping methods and had phenotypic variation explained above 10 %.

QTL	Protein Accession	Gene Ontology	Description
qLODG.Z/B-A03.1			
	A03p49910.1_BnaDAR	root system development; lateral root development; regulation of auxin polar transport	Root meristem growth factor 6
qLODG.Z/B-A03.3			
	A03p00590.1_BnaDAR	plant-type cell wall organization	Expansin
	A03p01040.1_BnaDAR	regulation of meristem growth; regulation of epidermal cell differentiation; root development	Zinc finger family protein
	A03p01050.1_BnaDAR	plant-type secondary cell wall biogenesis	Fasciclin-like arabinogalactan protein 11
	A03p02200.1_BnaDAR	plant-type primary cell wall biogenesis; cellulose biosynthetic process	Cellulose synthase

Table 3.8: Continued

QTL	Protein Accession	Gene Ontology	Description
	A03p02660.1_BnaDAR	root hair cell development	Gb|AAF02129.1
	A03p02780.1_BnaDAR	xylem vessel member cell differentiation; regulation of plant-type cell wall cellulose biosynthetic process	Gb|AAD30234.1
	A03p03190.1_BnaDAR	ethylene-activated signaling pathway	Ethylene-responsive transcription factor ERF115
	A03p04300.1_BnaDAR	plant-type primary cell wall biogenesis; cellulose biosynthetic process	Cellulose synthase
	A03p04420.1_BnaDAR	ethylene-activated signaling pathway	Ethylene insensitive 3 family protein
	A03p04730.1_BnaDAR	abscisic acid-activated signaling pathway; ethylene-activated signaling pathway; root developments	Histidine kinase 5
	A03p05480.1_BnaDAR	maintenance of root meristem identity;	CLAVATA3/ESR (CLE)-related protein 22

Table 3.8: Continued

QTL	Protein Accession	Gene Ontology	Description
		phloem or xylem histogenesis; root development	
qLODG.Z/B-A07.1			
	A07p20940.1_BnaDAR	auxin-activated signaling pathway	Amino acid transporter, putative
	A07p23170.1_BnaDAR	plant-type cell wall modification	Phosphatidylinositol 4-phosphate 5-kinase 4
	A07p23330.1_BnaDAR	plant-type cell wall assembly	Protein FATTY ACID EXPORT 1, chloroplastic
	A07p23860.1_BnaDAR	gibberellic acid mediated signaling pathway;	Zinc finger protein GIS
	A07p25100.1_BnaDAR	ethylene-activated signaling pathway	Ethylene-responsive transcription factor ERF035
	A07p25790.1_BnaDAR	auxin-activated signaling pathway	Auxin response factor
	A07p25840.1_BnaDAR	auxin-activated signaling pathway	Auxin-responsive protein

Table 3.8: Continued

QTL	Protein Accession	Gene Ontology	Description
	A07p26180.1_BnaDAR	auxin-activated signaling pathway; ethylene-activated signaling pathway; lateral root formation	Protein TRANSPORT INHIBITOR RESPONSE 1
	A07p26190.1_BnaDAR	response to gibberellin; gibberellin mediated signaling pathway	Gibberellin receptor
	A07p26230.1_BnaDAR	plant-type cell wall organization	Expansin-A20
	A07p26410.1_BnaDAR	root development	Regulator of telomere elongation helicase 1 homolog
	A07p27050.1_BnaDAR	root cap development	NAC domain containing protein
	A07p27710.1_BnaDAR	ethylene-activated signaling pathway	Ethylene-responsive transcription factor RAP2-4
	A07p27800.1_BnaDAR	auxin-activated signaling pathway	Auxin response factor
	A07p28000.1_BnaDAR	cellulose synthase complex;	C2 domain-containing protein

Table 3.8: Continued

QTL	Protein Accession	Gene Ontology	Description
		plant-type cell wall cellulose biosynthetic process	
	A07p28110.1_BnaDAR	lateral root formation	F-box family protein
	A07p28460.1_BnaDAR	auxin-activated signaling pathway	Auxin efflux carrier family protein
	A07p28730.1_BnaDAR	gibberellic acid mediated signaling pathway	Gibberellin regulated protein
	A07p28780.1_BnaDAR	auxin biosynthetic process	Protein SHI RELATED SEQUENCE 5
	A07p29080.1_BnaDAR	brassinosteroid mediated signaling pathway; regulation of growth;	Protein BRASSINAZOLE-RESISTANT 1
	A07p29220.1_BnaDAR	root hair cell development	Galactosyltransferase family protein
	A07p29570.1_BnaDAR	plant-type cell wall	Strictosidine synthase family protein
	A07p29690.1_BnaDAR	ethylene-activated signaling pathway	ETHYLENE INSENSITIVE 3-like 3 protein
	A07p30200.1_BnaDAR	lateral root branching	NGR2

Table 3.8: Continued

QTL	Protein Accession	Gene Ontology	Description
	A07p30640.1_BnaDAR	plant-type cell wall	Peroxidase
	A07p30870.1_BnaDAR	auxin-activated signaling pathway	Auxin efflux carrier component
	A07p31500.1_BnaDAR	phloem development; regulation of auxin mediated signaling pathway	CLAVATA3/ESR (CLE)-related protein 45
	A07p31540.1_BnaDAR	cellulase activity; cellulose 1,4-beta-cellobiosidase activity; cellulose catabolic process; cellulose binding	Exoglucanase-2
	A07p32940.1_BnaDAR	auxin-activated signaling pathway	Auxin response factor
	A07p33300.1_BnaDAR	ethylene-activated signaling pathway	Ethylene receptor
	A07p33490.1_BnaDAR	regulation of root development	S-adenosyl-L-methionine:carboxyl methyltransferase family protein

Table 3.8: Continued

QTL	Protein Accession	Gene Ontology	Description
	A07p33740.1_BnaDAR	abscisic acid-activated signaling pathway; gibberellic acid mediated signaling pathway;	Zinc finger protein 6

Candidate transcripts were screened using the physical interval of QTL for lodging that has a phenotypic value explained above 10 %. Gene ontology terms associated with plant-type cell wall, cellulose, lignin, root development, and ethylene, gibberellin, auxin and brassinosteroid-related activities were utilized to select the transcripts.

3.5. Discussion

Lodging poses a significant challenge in *B. napus* production as it increases economic loss by affecting yield, seed quality, pod shatter resistance, disease severity and incidences, and mechanized harvesting capabilities (Hua et al., 2014; Kendall et al., 2017; Khan et al., 2018). It is essential to identify genetic loci that confer lodging resistance and utilize these loci in breeding programs.

Given the critical role of genetic factors on lodging resistance, this study employed a visual rating method for lodging, proven effective in soybeans, peas, and sorghum (Gawłowska et al., 2021; Wang et al., 2020; Yamaguchi et al., 2015), to identify QTL for lodging resistance in *B. napus*. Several of the detected QTL in this study aligned with QTL for lodging-related traits that were previously reported. For instance, qLODG.Z/B-A01 in the current study was identified as a QTL for stem lignin, branch number and branch angle by several researchers (Shao et al., 2022; Shen et al., 2018; Zheng et al., 2017). Similarly, qLODG.Z/B-A03.3 detected in this study was found to correlate with QTL for the multi-main stem trait by Zhao et al. (2019), while qLODG.Z/B-A05 was linked to QTL for *Sclerotinia sclerotiorum* resistance (Shao et al., 2022), and QTL for stem breaking force (Wei et al., 2017). The qLODGZ/B-A7.1 in the present study were found as QTL for plant height, branch initiation height, branch number, hemicellulose, S/G monolignol ratio, multi-main stem, stem diameter, breaking force, and breaking strength in multiple studies (Li et al., 2018; Shao et al., 2022; Shen et al., 2018; Wei et al., 2017; Zhao et al., 2019; Zheng et al., 2017). Additionally, the qLODG.Z/B-C01 in the current study coincided with QTL for *S. sclerotiorum* resistance by Shao et al. (2022). The alignment of several QTL for lodging identified here with QTL for other stem-related traits from previous studies has demonstrated that lodging is

a complex trait that can be influenced by stem architecture traits. Several QTL discovered in this study (i.e qLODG.Z/B-A03.1, qLODG.Z/B-A07.2, and qLODG.Z/B-A10) were not previously reported in the literature, making them novel and helpful in enhancing lodging resistance in *B. napus.*

Within the QTL identified for lodging, putative candidate transcripts were screened using GO annotations previously identified in the literature as associated with LODG (Li et al., 2022; Liu et al., 2022; Niu et al., 2021; Shah et al., 2019). The involvement of plant hormones such as gibberellin, auxin, and ethylene were expected as these plant hormones have regulatory roles in cell wall components (Hussain et al., 2020). The screening results also demonstrated that some candidate transcripts were repeatedly identified among the QTL for LODG, including expansin and ethylene response factors, auxin response factors, and zinc finger family proteins. Expansins, a group of cell wall proteins comprising various subfamilies, such as α-expansin and β-expansin (Marowa et al., 2016), are critical in this context. Over-expression and under-expression of *α-expansin* led to an increase and a decrease in lateral root formation, respectively (Xing et al., 2009). Similarly, overexpression *β-expansin* promotes internode elongation at earlier development stages (Xing et al., 2009). ETHYLENE RESPONSE FACTOR 115 (ERF115) regulates developmental growth in the absence of wounding or injury (Canher et al., 2022). ERF115 enhances auxin sensitivity, correlating with improved lateral root formation and xylem maturation, while the knockout of *ERF115* leads to reduced lateral root numbers (Canher et al., 2022). Auxin response factors (ARFs) are essential in in many plant growth and development aspects, including the root and stem (Li et al., 2023). In *A. thaliana*, a single mutation of ARF led to a reduction in lateral root numbers, while a double mutant displayed a massive decrease in lateral roots (Lee et al., 2009).

Over-expression of ARFs showed a reduced plant height in rice and wheat (Wang et al., 2019; Zhang et al., 2015). Zinc finger proteins are involved in root hair growth and development (Guoliang et al., 2020). It was revealed that over-expression of zinc finger protein such as *ZINC FINGER PROTEIN1* (*AtZP1*) restricts root hair elongation in *A. thaliana* (Guoliang et al., 2020). Similarly, mutation of *ZINC FINGER PROTEIN6* (*ZFP6*) showed a reduction in lateral branches and main inflorescence stems (Zhou et al., 2013). Other significant transcripts influencing plant characteristics, which affect lodging resistance, include root growth factor and NAC transcription factor. Root growth factor (RGF) is a 13-amino sulfated peptide hormone expressed explicitly in the root tip and functions to maintain root stem cell activity (Matsuzaki et al., 2010; Shinohara, 2021); the mutation of RGF mutation would result in a diminished root apical meristem size (Shinohara, 2021). NAC transcription factor was previously reported as a master switch for secondary cell wall biosynthesis and downstream regulation of cellulose and lignin (Wang & Dixon, 2012). In summary, these candidate transcripts demonstrated that the lodging resistance is controlled by complex regulatory signaling in the root and stem development.

Building upon the discovery of QTL for lodging resistance in this research, further analysis also revealed overlapping QTL for LODG with QTL associated with critical traits such as DTF and HT. This phenomenon was expected as co-localized QTL between traits has been identified in other crops, such as stem rot resistance and flowering time in canola (Wu et al., 2019) or flowering time and plant height in rice (Zhou et al., 2016). In this study, QTL for LODG co-localized with QTL for DTF, HT and DTM. Overlapping between these QTL suggests that they could be tightly linked or have a pleiotropic effect on traits (Quijada et al., 2006). These co-localized QTL may also aid in explaining the significant correlation between LODG, DTF, and HT.

Exploring the correlation among traits such as LODG, DTF, and HT may reveal insight into how these traits influence one another. This study's negative correlation between LODG and DTF demonstrates that delayed flowering increases plant lodging resistance. When the populations was conducted on the field across multiple locations, it was observed that late flowering genotypes had poor pod sets. Low seed yield decreases the weight and force exerted on the stem, reducing the risk of stem lodging. Similar patterns of negative correlation between LODG and DTF have also been documented in other crops (Kebede et al., 2001; Tripathi et al., 2003). A negative correlation was found between LODG and HT, which implies that taller plants are less susceptible to lodging. This finding contrasts with previous research (Xue et al., 2017; Zhang et al., 2016). Nevertheless, such a negative correlation between LODG and HT can be seen on the field, where taller genotypes tend to have thicker stems and low pod sets. Unfortunately, stem diameter and yield data are not available to further support this claim. However, the negative correlation between LODG and HT can be further supported by previous research where increased plant height does not necessarily indicate greater susceptibility to lodging. For instance, "Leaf Star", a variety of rice, possesses long stems while having superior resistance to lodging (Ookawa et al., 2014). Further examination of this rice showed a thick layer of cortical fiber tissue with a well-developed secondary cell wall (Ookawa et al., 2014). This finding in rice may suggest that a thick layer of cortical tissues and secondary cell wall is likely contributing to the negative correlation between LODG and HT. Furthermore, a positive correlation between DTF and HT suggests that late-flowering plants tend to have increased height. This result is consistent with a previous study in *B. napus,* which showed a significant positive correlation between DTF and HT (Mathur et al., 2023). However, a negative correlation of DTF with HT in *B. napus* was also reported by Wu et al. (2019). Overall, the

correlation analysis between traits demonstrated that these traits have a complex relationship, as one trait can influence the outcome of the other.

3.6. Conclusion

In conclusion, this study identified several novel and major QTL for lodging resistance and other critical agronomic traits. This research provides insight into the genetic basis controlling lodging resistance and has the potential to advance marker-assisted selection for lodging resistance in *B. napus* in breeding programs. Detailed genetic mechanisms on how lodging is regulated remain to be clarified.

4. Construction of A High-Density Linkage Map and Quantitative Trait Loci (QTL) Detection for Lodging Resistance Trait in a *Brassica napus* Doubled-Haploid Population

4.1. Abstract

Lodging presents a significant challenge in canola production as it causes yield loss and increases the production cost due to mechanical harvesting difficulty. To overcome this issue and ultimately mitigate yield loss and production cost, breeders must introduce new genetic materials with enhanced lodging resistance into existing cultivars to maximize yield potential. Herein, a doubled haploid population derived from a cross between lodging susceptible and lodging resistant parents was employed to construct a high-density SNP map covering a genetic distance of 1559 cM, with an average marker interval of 0.22 cM. Lodging phenotypes were evaluated using a system lodging scale between one and five across four environments. Quantitative trait loci (QTL) analysis was performed through inclusive composite interval mapping (ICIM) to determine QTL for lodging resistance. Seven QTL were detected and mainly distributed on chromosomes A05, C02, C04, C05 and C07. Of these, only qLODG.L/R-C07.2 was considered stable QTL as it was consistently detected in multiple environments. This finding could provide valuable information regarding lodging resistance QTL in *B. napus,* and genetic markers associated with these QTL could be used as marker-assisted selections to increase lodging resistance.

4.2. Introduction

After palm and soybean oils, canola (*Brassica napus* L., AACC, 2n=38) is the third most predominant oil crop globally. It is an amphiploid species that derived from the interspecific hybridization between turnip rape (*Brassica rapa* L. syn. Campestris, AA, 2n=20) and cabbage (*Brassica oleracea* L., CC, 2n=18) (Starosta et al., 2023). Despite its global economic significance, canola production faces multiple challenges, including diseases, excessive heat, drought, and lodging, which impact its yield. Considerable efforts have been conducted to tackle these challenges (Elferjani & Soolanayakanahally, 2018; Li et al., 2018; Li et al., 2023; Shen et al., 2018). However, despite these ongoing efforts, these concerns will likely persist and could bear additional research, particularly in lodging. Crop lodging is defined as the permanent deviation of the stem from its vertical growth habit (Pinthus, 1974). In canola, lodging can cause yield loss between 10-30%; in some severe cases, yield loss can reach higher than 50 % (Li et al., 2022). Lodging can also reduce oil content by 10-30 % (Li et al., 2022). Crop lodging is classified as stem or root lodging (Berry et al., 2004; Wu & Ma, 2016), and is known to be regulated by agronomic practices and genetic factors (Chauhan et al., 2019; Dai et al., 2017; Erndwein et al., 2020; Li et al., 2022). Stem lodging is described as the breakage or bending of the basal internodes of the stem due to previous tissue damage by insects and by external forces such as winds, storms or hails (Pinthus, 1974). Root lodging is mainly due to the failure of the root-soil anchorage system (Berry et al., 2004).

Agronomic practices such as fertilizer application and sowing density can influence lodging. The application of fertilizers, particularly nitrogen (N), has been reported to impact lodging. Specifically, increasing N application from 0 to 360 kg per ha could significantly increase plant

lodging risk (Li et al., 2022). In other studies, researchers found that elevated nitrogen application restricted the development of both taproot and lateral roots while promoting aboveground growth (Wu et al., 2022; Wu & Ma, 2016). Furthermore, a report showed that elevated N supply promoted stem elongation while constricting the basal internode's stem diameter and cell wall thickness (Khan et al., 2018). These findings indicate that escalating the use of N application may cause the imbalance between root and stem growth by promoting stem elongation, thereby increasing the shoot-to-root ratio and the likelihood of stem lodging. Besides the amount of N applied, the timing of N application is also important to manage lodging risk. According to Wu et al. (2022), elevated plant height due to stem elongation from excessive N fertilizer only affected canola plants when applied during the early development stage. Conversely, using top-dressing N after the onset of stem elongation had a minimal effect on plant height (Wu et al., 2022). These studies have illustrated that the impact on lodging depends on the quantity of nitrogen fertilizer and the timing of such applications.

To some extent, plant density could positively impact lodging resistance in canola (Kuai et al., 2016; Li et al., 2018). However, increased plant density could also have negative impacts on yield. Khan et al. (2018) showed that plant densities of up to 30 plants/m^2 could improve yield, while those exceeding 30 plants/m^2 negatively affected yield. These results demonstrate that planting density should be limited to levels that benefit yield while enhancing lodging resistance. The physiological mechanism for the positive correlation between plant density and lodging resistance is likely due to increased stem lignin content. It was reported that the increase of plant density from less than 30 plants/m^2 to 45 plants/m^2 resulted in the upregulation of phenylalanine ammonia-lygase (PAL), and cinnamoyl-CoA reductase 1 (CCR1) in the stem, leading to increased lignin

content in the stem, and lodging resistance (Kuai et al., 2016). The active involvement of lignin was further evidenced to have a positive correlation with stem breaking strength, leading to lodging resistance (Wei et al., 2017).

Other biochemical constituents, such as cellulose, hemicellulose, and chitin, could also be used to select for lodging resistance as they influence elastic modulus, which ultimately affects the flexural rigidity of the basal stem (Kuai et al., 2016; Shao et al., 2022). A thicker layer of sclerenchyma tissues containing high cellulose and lignin levels was reported to lower lodging risk in canola (Wu et al., 2022).

Many stem traits, partly due to plant genetics, were also identified as lodging-related traits due to their strong correlation with lodging. For instance, flexural rigidity, stem diameter, wall thickness, and basal stem bending strength were positively correlated to lodging resistance (Wu & Ma, 2016). Plant height is another trait associated with lodging as taller plant cultivars were more susceptible to lodging than shorter genotypes (Piñera-Chavez et al., 2016). A shorter plant height is often lead to more lodging resistance likely due to the lower height of the center of gravity and lower above-ground fresh weight (Wu et al., 2022).

Besides stem traits, root lodging-related traits could also affect plant lodging. Root length and root dry weight were reported to be closely related to lodging resistance (Sposaro et al., 2008). Other researchers also found that increased root density in subsoil, and root hair length might enhance lodging resistance (Siddique et al., 2015). It was also found that plants' lodging resistance depended on the cone of rigid coronal roots that emerged from the stem base (Shah et al., 2019).

Other root characteristics such as root mass, root volume, root number, and root growth angle were closely associated with root lodging resistance (Bian et al., 2016).

Selecting quantitative trait loci (QTL) associated with lodging resistance is imperative to meet the growing demand for protein and oil from canola seeds. Although much attention has focused on mapping genomic regions that are associated with stem lodging-related traits (plant height, branch initiation height, and stem strength) (Li et al., 2018; Shen et al., 2018; Wang et al., 2016), specific QTL identification for lodging has remained limited. Additional studies could be conducted to explore additional QTL conferring lodging resistance. Upon identifying QTL that influence lodging, these QTL could potentially be used to elucidate the mechanisms underlying lodging resistance. Moreover, these identified QTL could also serve as markers associated with lodging resistance, paving the way for effective marker-assisted selection.

In recent years, single nucleotide polymorphism (SNP) markers have become the marker of choice because of their prevalence in the genome and strong linkage to traits (Hayward et al., 2012). Since the commercial release of the Illumina Infinium *Brassica* 60K SNP array (Clarke et al., 2016), and the completed sequencing of *Brassica napus* (Chalhoub et al., 2014), this SNP array has been successfully used for numerous studies on lodging-related traits in *B. napus* (Li et al., 2018; Shao et al., 2022; Shen et al., 2018; Zhao et al., 2019). However, to our knowledge, the 60K SNP array has yet to be directly utilized for QTL mapping for the lodging trait.

The purpose of this study was to (1) phenotype a doubled haploid (DH) for lodging using a visual lodging scale, (2) genotype these DH genotypes and their respective parents utilizing the *Brassica*

60K SNP array, and (3) identify quantitative trait loci (QTL) for lodging resistance in *B. napus*. High erucic acid rapeseed genotypes were utilized to determine QTL for lodging to perform such an experiment. A DH population was derived from a cross between a genotype showing lodging susceptibility and a genotype demonstrating lodging-resistant characteristics. A high-density genetic map was constructed to identify the QTL associated with lodging. It is hypothesized that genetic regions related to lodging resistance will be identified, given that this genetic background has yet to be utilized for lodging studies.

4.3. Materials and Methods

4.3.1. Plant Material

The plant material was composed of 195 DH genotypes derived from a cross of 9-1 LLM03/ HR202-B-9-1 (lodging sensitive female parent) and CRB141047-3-9-3 (13331 BC_1F_4 selection from 08C344/30220-D8RR//*Red River 1861) (lodging resistant male parent), thereafter referred to as the L/R population. Red River 1861 was a summer rape (*Brassica napus* L.). It was the fourth Roundup Ready™ low glucosinolate, high erucic acid (HEAR) line and was well adapted to southern regions of Western Canada (McVetty et al., 2006). The DH parents were selected in the field nursery in 2016 and 2017, where lodging was prevalent. The 9-1 LLM03/ HR202-B-9-1 parent was selected in 2016 as plants were extremely lodging sensitive and had a lodging score of 5 on a 1-5 scale. CRB141047-3-9-3 was selected in 2017, in which plants were lodging resistant and were assigned to a lodging score of 1.

4.3.2. Seed Increase for Field Experiments

Seed was increased as described in **3.3.2 Seed Increase for Field Experiments.**

4.3.3. Field Experiments

The experiment was designed using section **3.3.3 Field Experiments.**

4.3.4. Field Management

Details of field management were described in section **3.3.4 Field Management** with minor changes applied to the Winnipeg 2022 environment (UM22). In this environment, seeds were sown on June 6 on blocks 23 for replication 1 and on block 14 for replication 2 and 3. Applying 1 % Merge® (BASF Canada Inc., Mississauga, ON) and other chemicals (i.e. Lontrel™, Poast Ultra, Muster® Toss-N-Go®) to control broadleaf and grass weeds was applied on June 28. In addition, prior to harvesting, the desiccant containing a mixture of 0.32 L/ha Heat® LQ (BASF Canada Inc., Mississauga, ON), 7.41 L/ha glyphosate, and 2.97 L/ha Merge® (BASF Canada Inc., Mississauga, ON) was applied on September 22. Plots were individually harvested on September 27, 2022.

4.3.5. Phenotypic Assessment

A phenotypic assessment was carried out as described in the section **3.3.5 Phenotypic Evaluation.**

4.3.6. Statistical Analysis

Statistical analysis was conducted following the section **3.3.6 Statistical analysis.**

4.3.7. DNA Extraction and Genotyping

The detailed steps for extracting and genotyping DNA is described in section **3.3.7 DNA Extraction and Genotyping**.

4.3.8. SNP Filtering

SNP markers were filtered according to the method described in **3.3.8 SNP Filtering**. Further filtering steps were applied using Microsoft Office Excel to remove loci where one of the parent's alleles was genotyped as missing data. Out of 77,970 SNPs, 7,559 SNP markers were left through these filtering steps for the L/R DH population.

4.3.9. Genetic Linkage Map Construction

One hundred ninety-five individuals from the L/R DH population were used to calculate the genetic linkage maps. Genetic linkage maps were constructed using the software *MSTMap* (Wu et al., 2008) as described in **3.3.9 Genetic Linkage Map Construction.**

4.3.10. QTL Mapping

The means adjusted for lodging (LODG), days-to-flower (DTF), height (HT), and days-to-maturity (DTM) were calculated as follows in **3.3.10 QTL Mapping.** QTL for lodging and other traits were conducted with ICIM-ADD functions in the IciMapping v4.2.53 software package (Meng et al.,

2015). The QTL analysis steps are described in **3.3.10 QTL Mapping**. Based upon the premutation test, the LOD threshold for additive QTL was 3.0. Detected QTL were assigned by the letter "q" (abbreviated for QTL), followed by trait abbreviation, population name, and chromosome number (Thambugala et al., 2020). If multiple QTL were detected on the same chromosome, additional numerical values were used to distinguish these QTL (e.g., qLODG.L/R-C07.1**)**. QTL on the same chromosomes were considered separate QTL when they were on a different linkage group or separated by more than 15 cM in genetic distance.

4.3.11. Candidate Genes/Transcripts Analysis

Candidate transcripts were identified according to **3.3.11 Candidate Genes/Transcripts Analysis.**

4.4. Results

4.4.1. Phenotypic Analysis

Agronomic traits (i.e LODG, DTF, HT, and DTM) on a combined site-years basis were statistically analyzed to determine the effect of genotype, site-years and their interaction on the phenotypic expression of these traits. The analysis of variance (ANOVA) revealed highly significant differences ($p < 0.001$) among genotypes, site-year, and genotype by site-year (G X E) interaction for all traits in the combined site-years (**Table 4.1**). Since the site-year factor significantly affected the phenotypes of all traits in the combined site-years analysis, individual site-year analysis was also performed to evaluate the effect of genotype on each environment. In individual site-year

Table 4.1: Analysis of variance for lodging (LODG), days-to-flower (DTF), height (HT), and days-to-maturity (DTM) in the DH L/R population grown in four environments in 2020, 2021, and 2022.

Source of variation	LODG	DTF	HT	DTM
Genotype	6.25***	10.25***	9.33***	7.00***
Site-year	20.58***	150.19***	124.39***	44.23***
Genotype x Site-year	2.25***	2.37***	2.07***	2.66***

****p < 0.001; **p < 0.01; *p < 0.05*

The data was analyzed using a generalized linear mixed model and adjusted using a beta distribution. Genotype, site-year, and genotype by site-year interaction were considered as fixed while blocks nested in site-year were considered random. F-test values are presented in the table, with an asterisk(s) representing for significance levels.

analysis, the ANOVA revealed that genotype significantly ($p < 0.001$) affected all agronomic traits tested (**Table 4.2**).

4.4.2. Lodging Assessment in The Parental, Check, DH Genotypes

At the maturity stage, lodging was evaluated in four environments using a scoring system ranging between 1 to 5, with 1 = completely erected plants and 5 = plants touching or entirely lying flat on the ground plants **(Figure 2.2)**. The check, the male parent (lodging resistant), and the female parent (lodging susceptible) showed a lodging score ranging from 2.34 to 4.57, 1.38 to 3.02, and 2.66 to 4.90, respectively, among the four site-years (**Table 4.3**). Significant lodging differences ($p < 0.05$) were found in all environments, except PT21, when lodging resistant and lodging sensitive parents were compared. The mean of lodging resistant parents at all site-years, except PT21, significantly differed from the check cultivar's mean. In contrast, lodging susceptible parents at all site-years, except UM22, was not substantially different from the check cultivar.

The DH lines among four site-years showed a considerable variation in lodging score, ranging from 1.09 to 4.94, with PT21 having the broadest variation and UM22 having the narrowest variation. The population lodging grand means ranged between 2.15 and 3.75 across the four environments, with the lowest and highest grand mean observed in CM20 and UM22, respectively **(Table 4.3)**. The broad sense heritabilities (h^2) of lodging for CM20, CM21, PT21, and UM22 were 0.44, 0.50, 0.65, and 0.55, respectively.

Table 4.2: Analysis of variance for Lodging (LODG), Days-to-Flower (DTF), Height (HT), and Days-to-Maturity (DTM) in the L/R DH Population grown in CM20, CM21, PT21, and UM22.

	Source of variation	LODG	DTF	HT	DTM
CM20	Genotype	2.41***	15.58***	4.66***	3.73***
CM21		2.71***	2.77***	3.80***	3.07***
PT21		3.87***	6.55***	6.16***	4.91***
UM22		2.93***	7.30***	2.71***	1.81***

****p < 0.001; **p < 0.01; *p < 0.05*

CM20: 2020, Carman, Manitoba, Canada

CM21: 2021, Carman, Manitoba, Canada

PT21: 2021, Portage la Prairie, Manitoba, Canada

UM22: 2022, Winnipeg, Manitoba, Canada

The data was analyzed using a generalized linear mixed model with adjustments made using a beta distribution. Genotype, site-year, and genotype by site-year interaction were considered as fixed effects, while block nested in site-year was treated as a random effect. The F-test values are reported, with asterisks denoting the levels of significance.

Table 4.3: Lodging response of the DH L/R genotypes, a check cultivar, resistant parent, and susceptible parent in CM20, CM21, PT21, and UM22.

Environment	Check (MillenniUM 03)	Male (Lodging resistant)	Female (Lodging susceptible)	DH Genotypes Range	Grand Mean	Heritability
CM20	2.34 A	1.38 B	2.66 A	1.09-3.64	2.15	0.44
CM21	2.67 A	3.02 B	3.61 A	1.61-4.62	2.98	0.50
PT21	3.66 A	2.98 A	3.35 A	1.65-4.94	2.84	0.65
UM22	4.57 A	2.97 B	4.90 C	2.65-4.91	3.75	0.55

The male and female were originally selected with lodging scores of 1 and 5, respectively.

CM20: 2020, Carman, Manitoba, Canada

CM21: 2021, Carman, Manitoba, Canada

PT21: 2021, Portage la Prairie, Manitoba, Canada

UM22: 2022, Winnipeg, Manitoba, Canada

Each value represents the least square mean of lodging response from three replicates on a scale of 1 to 5, where 1 represents complete lodging resistance and 5 represents lodging susceptibility. Means with the same letter(s) are not significantly different (α=0.05).

4.4.3. Correlation Between Lodging and Other Traits

Since the ANOVA showed site-year has a significant effect ($p < 0.001$) on lodging response **(Table 4.1)**, the correlation was determined on individual site-year levels rather than combined site-years. The Spearman's correlation coefficient between LODG and other critical agronomic traits, including DTF, HT, and DTM, was detected across four environments. The DTF was significantly positively correlated ($p < 0.01$) with LODG in CM21 and PT21 but negatively correlated ($p < 0.001$) in UM22. No correlation between DTF and LODG was detected in CM20 (**Table 4.4**). The HT was significantly negatively correlated ($p < 0.05$) with LODG in PT21, while no significant correlation was observed in the other three environments (CM20, CM21, and UM22). The DTM was significantly positively correlated ($p < 0.001$) with LODG in CM20, CM21, and PT21, with the highest correlation ($rs=0.53$, CM21).

The Pearson's correlation coefficient between DTF, HT, and LODG traits was also detected across four environments. The correlation coefficient between these traits ranged from 0.00 to 0.63 (**Table 4.4**). As expected, the correlation between DTF and DTM was significantly positively correlated, with the highest coefficient ($r= 0.63$, CM21). A positive correlation was also shown between HT and DTM ($r =0.25$, UM22). The relationships between DTF and HT demonstrated inconsistent correlation direction. For example, DTF and HT at CM20 were significantly positively correlated with DTF ($r = 0.26$); however, in CM21, DTF and HT were significantly negatively correlated ($r = -0.31$), while PT21 and UM22 showed no correlation.

4.4.4. Linkage Map Analysis

A high-density linkage map was developed utilizing the 60K *Brassica napus* Infinium array. A

Table 4.4: Correlation coefficient (r) among traits calculated in L/R DH population grown in Carman 2020 (CM20), Carman 2021 (CM21), Portage la Prairie 2021 (PT21), and Winnipeg 2022 (UM21) using Spearman ranking for lodging trait (LODG), and Pearson method for days-to-flower (DTF), height (HT), and days-to-maturity (DTM). Significant levels are indicated using asterisks.

		Spearman	**Pearson**	**Pearson**
	Trait	**LODG**	**DTF**	**HT**
CM20	**DTF**	-0.006		
	HT	-0.07	0.26***	
	DTM	0.32***	0.23***	0.16***
CM21	**DTF**	0.36***		
	HT	0.01	-0.31***	
	DTM	0.53***	0.63***	-0.04
PT21	**DTF**	0.12**		
	HT	-0.10*	-0.02	
	DTM	0.16***	0.31***	0.22***
UM22	**DTF**	-0.16***		
	HT	0.01	0.00	
	DTM	0.04	0.17***	0.25***

****p < 0.001; **p < 0.01; *p < 0.05*

total of 7,086 SNPs were selected for mapping in the L/R population. These SNP markers were distributed across 26 different linkage groups, which covered a length of 1559 cM, with an average marker interval of 0.22 cM (**Table 4.5)**. When these markers were anchored to the physical chromosome, linkage maps were aligned to all the chromosomes of *B. napus* cv. 'Darmor-*bzh*' (**Table 4.5)**. The number of markers in each linkage group varies from 12 to 2014. LG-8 was the longest LG, which mapped to A03 and covered 146.16 cM. The shortest LG was LG-24, which mapped to C04 and covered 1.01 cM. In many cases, multiple linkage groups were anchored to the same physical chromosome (e.g., LG-5 and LG-7 on A02, LG-6 and LG-13 on A05, LG-2 and LG-16 on A07).

4.5. QTL Analysis

4.5.1. Lodging

In the L/R DH population, ICIM mapping methods with an additive effect module from IciMapping were utilized to identify seven additive QTLs associated with LODG **(Table 4.6)**. These QTL were located on A5, C02, C04, C05, and C07 and were named qLODG.L/R-A05, qLODG.L/R-C02, qLODG.L/R-C04, qLODG.L/R-C05, qLODG.L/R-C07.1, qLODG.L/R-C07.2, and qLODG.L/R-C07.3. In all cases, the male of CRB141047-3-9-3 increased lodging resistance at each of these QTL. The male alleles contributing to the increase in lodging resistance were expected as the male was the resistant parent. Only qLODG.L/R.C07.2 was considered stable QTL, as it was detected in multiple environments. The phenotypic variation explained ranged from

Table 4.5: Chromosome location on *Brassica. napus*-Damor reference genome and map coverage in cM of linkage groups constructed in MSTmap using L/R DH Brassica napus population consisting of 195 individuals.

Linkage Group	Number of Markers	Total Map Coverage (cM)	Chromosome (*B. napus*-Damor)
LG-0	552	141.3	A01
LG-5	219	80.93	A02
LG-7	12	7.23	A02
LG-8	495	146.16	A03
LG-3	211	90.16	A04
LG-6	194	65.5	A05
LG-13	67	27.94	A05
LG-15	234	140.57	A06
LG-2	218	45.82	A07
LG-16	73	30.96	A07
LG-14	91	28.21	A08
LG-4	106	41.41	A09
LG-18	56	32.39	A10
LG-1	2014	116.86	C01
LG-22	307	45.63	C02
LG-9	781	127.05	C03

Table 4.5: Continued

Linkage Group	Number of Markers	Total Map Coverage (cM)	Chromosome (*B. napus*-Damor)
LG-12	48	51.62	C04
LG-24	53	1.01	C04
LG-21	135	61.37	C05
LG-25	23	20.02	C05
LG-17	143	17.21	C06
LG-26	83	10.9	C06
LG-10	545	106.46	C07
LG-19	211	61.65	C08
LG-20	170	60.71	C09
Total	7041	1559.07	

Linkage groups with less than ten markers, and markers mapped into the genetic distance of 0.00 cM, are not shown. This results in the exclusion of 45 markers from the table.

Table 4.6: Detection of Additive-Effect QTL for Days-to-Flower, Days-to-Maturity, Height, and Lodging in a Bi-Parental L/R DH population grown in CM20, CM21, PT21, and UM22. ICIM mapping method was utilized.

Trait	Environment	LG	Chr.	Genetic Position	LOD	PVE (%)	Add	CI (cM)	PI (Mb)
Lodging									
qLODG.L/R-A05	CM20	6	A05	1.20	4.89	8.86	0.17	0.00-1.3	35.511-35.796
qLODG.L/R-C02	UM22	22	C02	2.60	3.15	8.44	0.12	2.60-3.10	57.463-57.497
qLODG.L/R-C04	CMB	12	C04	4.70	3.73	6.77	0.11	4.30-4.80	55.877-57.387
qLODG.L/R-C05	CM20	25	C05	16.00	3.15	5.63	0.14	15.90-16.10	1.07-1.136
qLODG.L/R-C07.1	CM20	10	C07	18.30	4.60	8.31	0.17	17.60-18.40	25.55-27.098
qLODG.L/R-C07.2	PT21	10	C07	52.20	3.51	8.03	0.15	42.20-55.10	31.18-36.265
	CMB	10	C07	55.10	6.11	11.35	0.13	46.50-55.10	34.229-36.265
qLODG.L/R-C07.3	CM21	10	C07	74.50	4.99	11.52	0.17	73.90-74.50	41.843-42.261
Days-to-flower									
qDTF.L/R-A01	CMB	0	A01	1.10	5.52	8.27	-0.46	1.10-3.20	1.443-1.883
	PT21	0	A01	9.30	4.73	7.80	-0.35	8.20-11.80	2.014-2.19

Table 4.6: Continued

Trait	Environment	LG	Chr.	Genetic Position	LOD	PVE (%)	Add	CI (cM)	PI (Mb)
qDTF.L/R-A02	CM20	5	A02	39.10	4.11	7.59	0.48	36.10-40.10	5.19-6.87
	CMB	5	A02	40.00	4.00	5.89	0.39	36.10-40.10	5.19-6.87
qDTF.L/R-A07	PT21	2	A07	19.60	4.60	7.60	0.35	19.60-20.90	16.11-16.40
qDTF.L/R-A10	CM20	18	A10	13.10	5.80	10.88	-0.58	13.10-13.20	16.20-16.33
	CMB	18	A10	21.50	3.72	5.45	-0.37	20.20-23.50	15.75-16.12
	PT21	18	A10	26.70	3.09	5.02	-0.28	26.10-27.90	15.19-15.834
qDTF.L/R-C04	CM20	12	C04	0.00	3.45	6.27	-0.45	0.00-0.50	52.94-53.96
	PT21	24	C04	0.30	4.81	7.96	-0.36	0.00-1.00	33.26-36.06
	UM22	24	C04	0.30	4.28	9.53	-0.57	0.00-1.00	33.26-36.06
	CMB	24	C04	0.30	5.93	8.94	-0.49	0.00-1.00	33.26-36.06
qDTF.L/R-C07	CMB	10	C07	81.00	3.54	5.17	0.36	77.80-81.00	42.32-43.27
Height									
qHT.L/R-A09	PT21	4	A09	19.00	3.81	9.45	2.02	18.10-19.10	20.927-33.03

Table 4.6: Continued

Trait	Environment	LG	Chr.	Genetic Position	LOD	PVE (%)	Add	CI (cM)	PI (Mb)
qHT.L/R-A10	CM20	18	A10	13.00	3.94	9.18	-2.11	11.60-13.20	16.201-16.40
	CMB	18	A10	13.10	3.00	5.76	-1.35	11.60-13.20	16.20-16.40
qHT.L/R-C04	CMB	12	C04	9.30	3.08	5.93	-1.39	6.30-9.40	57.39-60.29
Days-to-maturity									
qDTM.L/R-C02	PT21	22	C02	29.20	5.13	11.58	-0.72	27.60-29.30	16.39-24.23
	CMB	22	C02	36.30	3.31	6.57	-0.28	35.10-36.70	11.32-14.58
qDTM.L/R-C04	PT21	24	C04	0.30	4.07	9.05	-0.64	0.00-1.00	33.26-36.06
	CM21	24	C04	0.60	3.24	7.31	-0.53	0.00-1.00	33.26-36.06
	CMB	24	C04	1.00	4.40	8.79	-0.33	0.30-1.00	33.26-36.06
qDTM.L/R-C07	CMB	10	C07	27.00	2.99	5.92	0.27	24.50-32.30	27.90-30.37

QTL, Quantitative Trait Loci; Env., Environment; LG, Linkage Group; Chr., Chromosome; Pos., Genetic position on the linkage group in cM; LOD, Logarithm of odds peak; LOD threshold for ICIM is 3.0 resulted from a 1000 premutation test at the threshold of $\alpha = 0.05$; PVE, phenotypic variation explained (r^2; %); Add, Additive effect of allele substitution. A positive number indicated that the female parent allele increased the respective quantitative trait and vice-versa; CI, Genetic Confident Interval (LOD ±1); PI, Physical Confident Interval; DTF, Days-to-flower, DTM, Days-to-maturity, HT, Plant height (cm); CMB, combined data set; CM20, Carman 2020; CM21, Carman 2021; PT21, Portage la Prairie 2021; UM22, Winnipeg 2022. **Bolded QTL indicate that the region was detected in more than one environment.**

5.63 % to 11.52 %. Two QTL on C07 exhibited the highest phenotypic variation explained among all QTL. The qLODG.L/R-C07.2 and qLODG.L/R-C07.3 explained 11.35 % and 11.52 % of the observed phenotypic variation, respectively.

4.5.2. Days-to-flower

Six additive-QTL for DTF were found in the L/R DH population. The QTL were resided on A01, A02, A07, A10, C04, and C07, and were named qDTF.L/R-A01, qDTF.L/R-A02, qDTF.L/R-A07, qDTF.L/R-A10, qDTF.L/R-C04, and qDTF.L/R-C07 **(Table 4.6).** The female parent (9-1 LLM03/HR202-B-9-1) delayed days-to-flower at qDTF.L/R-A02, qDTF.L/R-A07, and qDTF.L/R-C07. In contrast, the male parent (CRB141047-3-9-3) alleles delayed the flowering date at qDTF.L/R-A01, qDTF.L/R-A10, qDTF.L/R-C04. The phenotypic value explained ranged between 5.02 to 10.88 %. Four QTL (qDTF.L/R-A01, qDTF.L/R-A02, qDTF.L/R-A10, qDTF.L/R-C04) were considered as stable QTL as they were detected in multiple environments. qDTF.L/R-A10 was co-aligned with qHT.L/R-A10 **(Table 4.6)**. Similarly, qDTF.L/R-C04 was co-localized with qDTM.L/R-C04. The overlapping mapping interval between DTF and HT on chromosome A10, and between DTF and DTM chromosome C04 implied that these QTL might be tightly linked or had pleiotropic effects. The significant correlation observed in **(Table 4.4)** may further support such co-localization between QTL for DTF and HT and between QTL for DTF and DTM**.**

4.5.3. Height

Three additive-QTL for HT were detected. The QTL were located on A09, A10, and C04 , and labelled as qHT.L/R-A09, qHT.L/R-A10, and qHT.L/R-C04 **(Table 4.6)**. The female parent (9-1

LLM03/HR202-B-9-1) alleles reduced plant height at qHT.L/R-A10 and qHT.L/R-C04. The male parent (CRB141047-3-9-3) alleles reduced plant height at qHT.L/R-A09. In three QTL found, qHT.L/R-A10 was the only stable QTL found. The phenotypic value explained for these QTL ranged between 5.76 to 9.45 %.

4.5.4. Days-to-maturity

Three additive-QTL for DTM were discovered in the L/R DH population. The QTL on C02, C04, and C07 were named qDTM.L/R-C02, qDTM.L/R-C04, and qDTM.L/R-C07 **(Table 4.6)**. The female parent (9-1 LLM03/HR202-B-9-1) alleles delayed maturity date at qDTM.L/R-C07. The male parent (CRB141047-3-9-3) alleles delayed the maturity date at qDTM.L/R-C02 and qDTM.L/R-C04. In the three detected QTL, qDTM.L/R-C02 and qDTM.L/R-C04 were considered stable QTL as they were found in multiple environments. The phenotypic value explained for these QTL ranged between 5.92 to 11.58%.

4.6. Candidate transcripts exploring for lodging

The physical confidence intervals of QTL with phenotypic values explained over 10 % (i.e. qLODG.L/R-C07.2, and qLODG.L/R-C07.3) were utilized to screen for candidate protein transcripts. Based on GO terms, eight candidate protein transcripts for LODG were identified **(Table 4.7)**. Candidate transcripts were involved in auxin-activated, brassinosteroid-meditated, gibberellic acid-mediated, and plant root development.

4.7. Discussion

Crop lodging, the permanent displacement of the stem from its vertical growth position, commonly occurs in cereal and canola crops (Pinthus, 1974; Wu et al., 2021a). Lodging hinders *B. napus* yield and seed quality and complicates mechanical harvesting. As biotic stresses become more prevalent, lodging has increased in frequency of occurrence (Wu et al., 2020; Wu & Ma, 2018). Hence, identifying lodging-resistant loci is necessary to achieve a high yield in *B. napus* production.

Recognizing the significance of genetic factors in enhancing lodging resistance, this investigation employed a visual lodging evaluation strategy, previously used in soybean, pea, and sorghum (Gawłowska et al., 2021; Wang et al., 2020; Yamaguchi et al., 2015), to discover QTL linked to lodging in *B.napus*. This study identified seven lodging-associated QTL in four environments over three years of field experiments. These QTL were anchored to *Brassica napus* cv. 'Darmor-*bzh*' reference genome (Rousseau-Gueutin et al., 2020) to enable a comparison of QTL between traits and QTL identified in the literature. Among these, two QTL were co-located with previously identified QTL for lodging-related traits. For instance, qLODG.L/R.C07.2 in this study was mapped to the same location as QTL for *Sclerotinia sclerotiorum* resistance (Shao et al. 2022). Similarly, qLODG.L/R.C07.3 in the current study was found approximately 1 Mb from QTL for *S. sclerotiorum* stem rot resistance and lodging (Li et al., 2018; Shao et al., 2022). Several QTL

Table 4.7: Candidate transcripts for lodging in the DH L/R population based on physical interval of QTL that had phenotypic explained above 10 %.

QTL	Protein Accession	Gene Ontology	Description
qLODG.L/R-C07.2			
	C07p29770.1_BnaDAR	brassinosteroid mediated signaling pathway	Kinase family protein
	C07p29920.1_BnaDAR	ethylene-activated signaling pathway	Ethylene-responsive transcription factor RAP2-2
	C07p30680.1_BnaDAR	lateral root development	Lon protease homolog 2, peroxisomal
	C07p30970.1_BnaDAR	ethylene-activated signaling pathway	Ethylene-responsive transcription factor 5
	C07p31400.1_BnaDAR	auxin-activated signaling pathway;	Serine/threonine-protein kinase D6PKL2
	C07p31650.1_BnaDAR	auxin-activated signaling pathway	Auxin-responsive protein
	C07p32180.1_BnaDAR	gibberellic acid mediated signaling pathway regulation of nitrogen utilization;	DELLA protein GAI
qLODG.L/R-C07.3			
	C07p40190.1_BnaDAR	root morphogenesis	Protein WVD2-like 1

Candidate transcripts were screened using the physical interval of QTL for lodging that have a phenotypic value explained above 10 %. Gene ontology terms associated with plant-type cell wall, cellulose, lignin, root development, and ethylene, gibberellin, auxin and brassinosteroid-related activities were utilized to select the transcript.

discovered in this study (e.g., qLODG.L/R-A05, qLODG.L/R-C04, qLODG.L/R-C05, and qLODG.L/R-C07.1) were not previously reported in the literature. Therefore, these QTL are considered as novel QTL and may be useful in improving lodging resistance in *B. napus.* A possible explanation for why these novel QTL were not discovered in previous mapping studies could be the population size, the mapping method used, the heritabilities of the target QTL, and the type and number of markers incorporated (Hwang & Lee, 2019). Nevertheless, the detection of these novel QTL in this study highlights the resistant parent as a valuable resource for enhancing lodging resistance in *B. napus*.

Among the identified QTL, candidate transcripts were screened using GO annotations previously associated with lodging resistance (Li et al., 2022; Liu et al., 2022; Niu et al., 2021; Shah et al., 2019). These candidate transcripts include kinase family proteins, Lon protease homolog 2, WAVE-DAMPENED 2, ethylene-responsive transcription factor 5, and DELLA protein GAI. In *A. thaliana,* protein kinase was shown to regulate stem elongation and vascular development (Matschi et al., 2013). Specifically, mutation of calcium-dependent kinase was reported to modify key regulators of gibberellic acid, such as the expression of NAC transcription factors (Matschi et al., 2013). In sorghum, protein kinase transcribed from the *Dw2* gene was identified to influence stem length (Hilley et al., 2017). Lon protease homolog 2, peroxisomal (LON2) enables the import of matrix protein into peroxisomes in *Arabidopsis* (Lingard & Bartel, 2009). Mutant LON2 exhibited an unresponsive effect to IBA-induced lateral root formation (Lingard & Bartel, 2009). *Arabidopsis WAVE-DAMPENED 2 (WVD2)* was identified as a gene that affects root helical growth handedness (Perrin et al., 2007; Yuen et al., 2003). Elevated expression of *WVD2* or other members of the *WVD2-LIKE (WDL)* genes family, such as *WDLD1-LIKE 1,* exhibited short stems

and roots and suppressed root-waving phenotype (Perrin et al., 2007). ETHYLENE RESPONSE FACTOR5 (ERF5) is a member of the ethylene-responsive factor superfamily and mainly responds to abiotic stress in plants (Dubois et al., 2013; Müller & Munné-Bosch, 2015; Pan et al., 2012). In tomatoes, Pan et al. (2012) showed that *ERF5* expression was induced in the stems and roots under heat stress. Overexpression of *ERF5* during the early development stage was reported to inhibit root and stem growth while allowing the tomato plant to produce more side shoots (Pan et al., 2012). In *Arabidopsis*, Dubois et al. (2013) further illustrated that ERF5 restricted leaf growth while the mutant form of this protein showed an increase of rosette area by 33 % under drought stress. DELLA protein GAI is a growth repressor protein that can be degraded by gibberellin (GA) (Ubeda-Tomás et al., 2009). According to Boccaccini et al. (2014), gain-of-function mutant plants had reduced plant height and became insensitive to GA. Interestingly, the DELLA protein was demonstrated to be a downstream regulator of *ERF5* (Dubois et al., 2013). The identified candidate transcripts suggest that complex regulatory signaling govern lodging resistance.

The correlation table provides valuable insight into the interrelationships among LODG, DTF, HT, and DTM. The correlation between LODG and DTF varied across locations, mirroring the inconsistency found in the literature. For example, soybean studies reported both non-significant and significant correlations between LODG and DTF across different site-years (Gawłowska et al., 2022). On the other hand, the negative correlation between LODG and DTF was also found in soybeans and wheat (Kebede et al., 2001; Tripathi et al., 2003). For the correlation between LODG and HT, the weak correlation observed in PT21 (r= -0.10) suggests that taller plants exhibit a reduced susceptibility to lodging. This inverse correlation between LODG and HT contradicts evidence found in other crops where taller plants are more prone to lodging (Feng et al., 2019; Hu

et al., 2022). As in the previous chapter described, taller genotypes observed on the field often matured late, with poor pod sets. Less pod sets mean less force is applied to the stem, leading to less lodging. Transitioning to the correlation between LODG and DTM, a positive correlation was found, indicating that delayed maturity may contribute to increased lodging severity. The positive correlation between LODG and DTM is aligned with soybeans (Hwang & Lee, 2019). Furthermore, the positive correlation between DTM and HT suggests that plants with delayed maturity exhibit increased height. This finding is consistent with previous Tartary buckwheat and soybean studies, which showed a significant positive correlation between DTM and HT (Krishna Joshi, 2010; Liu et al., 2011). In summary, the analysis of trait correlation reveals a complex relationship of these traits to one another as one trait can significantly influence the outcome of other traits.

4.8. Future direction

It is challenging to compare the identified QTL with the literature due to the diverse genetic maps and various segregating populations used in other studies. However, it is possible to map the QTL from different studies on the physical chromosomes using *B. napus* reference genome to further validate the QTL for lodging and other traits detected in this study. Moreover, QTL for lodging found in this study may lay a foundation for the potential identification of makers for marker-assisted selection to accelerate breeding progress in developing high-yield and high lodging-resistant cultivars.

5. General Discussion and Conclusions

Increased yield in canola production is driving cultivar development. As yield increases, the weight and force imposed on the plant's center of gravity will continue to increase, thereby intensifying the lodging risk. This phenomenon underscores the need for high-yielding cultivars to have stronger stems and roots, enabling canola plants to withstand these additional forces. Hence, it is crucial to identify genomic regions associated with strong stem and root lodging resistance.

In recent years, QTL for plant height (Miller et al., 2018; Shen et al., 2018), stem strength (Lemloh et al., 2014; Miller et al., 2018), stem wall thickness (Tripathi et al., 2003), and stem diameter (Wu & Ma, 2022) were used to screen for lodging resistance in *B. napus*. However, QTL for lodging has not been directly utilized in this crop. Visual field ratings in soybeans have been employed to discover QTL for lodging (Hwang & Lee, 2019). As a result, it was hypothesized that QTL for lodging could also be detected in *B. napus*. This hypothesis was tested using Z/B and L/R DH populations corresponding to chapters three and four of this thesis. In addition, plant height is known to correlate with lodging resistance in many crops (Piñera-Chavez et al., 2016; Wu & Ma, 2022; Xue et al., 2017), and it was predicted that QTL for height would also be found along with QTL for lodging. As expected, QTL for lodging was identified along with QTL for other key traits, including flowering data and maturity. Furthermore, the co-localization of QTL for lodging with QTL for flowering time, plant height and maturity were also found (**Figure 3.1**). This finding is useful as it provides additional information regarding the complex interaction/relativeness between QTL for lodging, flowering time, plant height and maturity at the genetic level.

In the comparative analysis of QTL for lodging between the two populations (i.e Z/B and L/R populations), it was observed that QTL for lodging identified in Z/B resided on chromosomes distinct from those the QTL detected in the L/R population, except for QTL on the chromosome of A05. Despite that, it is not likely that qLODG/Z/B-A05 and qLODG.L/R-A05 are overlapping as these QTL are approximately 9 Mb apart on the physical map (**Table 3.7 & Table *4.6***). The non-overlapping of QTL for lodging identified in both populations is likely because they were developed from parents with diverse genetic backgrounds. In addition, the loci diversity for lodging QTL underscores the genetic complexity of genes controlling lodging in *B. napus*. Nevertheless, QTL for lodging detected in both populations were reported as QTL for stem rot resistance caused by *Sclerotinia sclerotiorum* (Li et al., 2018; Shao et al., 2022). The co-alignment of QTL for lodging found in this study and QTL for stem rot resistance identified in the literature suggest that these QTL may play a duo role or reside in close proximity to one another. Further research into the genetics and physiological connections between these traits may be essential.

A linkage map is a road map that pinpoints the position and relative genetic distance between markers (Nadeem et al., 2018). Among the two linkage maps constructed in the two DH populations (Z/B and L/R), several chromosomes (i.e. A02, A04, A09, and C09) were excluded from the linkage map in the Z/B population, indicating that there are no polymorphic markers found on these chromosomes or the filtering process removed polymorphic SNP with marker allele frequencies less than 0.4 . The lack of polymorphic markers in these regions is likely due to the lack of genetic variation between the two parents in the Z/B population. Maximizing genotypic diversity in these chromosomes is crucial to ensure good coverage in future studies.

In conclusion, QTL for lodging identified in two different DH populations provide evidence that multiple genetic loci exist in governing lodging in *B. napus.* The alignment of QTL for lodging with QTL for lodging-related traits further validates the result of this study. It is possible to utilize the QTL for lodging to develop markers and employ marker-assisted selection to screen for individuals carrying alleles for lodging resistance at these loci, thereby improving lodging resistance in elite *B. napus* genotypes.

6. Future Research

Phenotyping for lodging by visual rating has effectively identified QTL for lodging in the current study. However, one of the downsides of lodging rating is that it could have some level of bias. In other words, different raters could provide different scores to a given row of plants. In addition, it is difficult to distinguish the slight differences between rows. The utilization of unmanned aerial vehicles (UAVs) has been successful in evaluating lodging in other crops. This system is less biased as it is based on spectral images of the field and can detect subtle changes between lodging levels (Sun et al., 2019; Wu & Ma, 2022). As a result, utilizing such a high-throughput system could further enhance the accuracy of lodging scores compared to the human visual-based method. Combining this with the lodging scale rating from 1 to 9 (Li et al., 2023) instead of 1 to 5 would allow for better separation of different degrees of lodging.

Multiple loci often control lodging. Phenotyping efficiency could be further improved by phenotyping a secondary trait with a strong genetic correlation with lodging (Moose & Mumm, 2008). For instance, traits such as stem diameter, stem strength, and stem lignin content could be utilized within this population. Including these traits could reveal a mechanism for lodging resistance and provide additional selection criteria for use in a breeding program.

Environmental conditions strongly influence lodging (Baker et al., 2014; Chauhan et al., 2019; Niu et al., 2022; Stubbs et al., 2020). The absence of weather conditions that induce lodging hinders the ability to detect lodging-resistant genotypes as they are indistinguishable from lodging-susceptible genotypes. Thus, conducting QTL mapping using four environments within a 200 km radius is fairly limited. More diverse environments are likely needed to account for such

environmental variations. Having diverse environmental growing conditions would also be more beneficial because some minor QTL can sometimes only be detected in a specific environment. Diversity in environments allows one to identify these minor QTL for lodging and strengthen the stability of major QTL as they are likely detected in multiple environments.

Regarding the QTL identified in both populations within this thesis, it is critical to validate these QTL by testing them in other populations with different genetic backgrounds. In the next step approach, it is possible to fine-mapping the QTL regions to identify specific genes responsible for lodging resistance. Subsequently, cloning genes within these loci can further elucidate the function and mechanism of these genes in lodging resistance. It could also be appropriate to conduct transcriptomic analysis to compare gene expression levels between lodging-resistant and susceptible genotypes. Functional validation, via gene knockout or gene silencing, could also be conducted on the candidate transcript identified to confirm the role of these genes in controlling lodging resistance. Molecular markers associated with lodging resistance identified in the two populations can then be used in marker-assisted selection. The implementation of this knowledge can significantly accelerate the development of high-yielding *B. napus* cultivars while maintaining high lodging resistance.

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