LODGING-RELATED TRAITS IN WHEAT IN RESPONSE TO NITROGEN FERTILIZATION AND ALTERATION OF GIBBERELLIN LEVEL

By

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ABSTRACT

Kaur, Gurnoor. M.Sc., The University of Manitoba, November 2024. Lodging-related traits in wheat in response to nitrogen fertilization and alteration of gibberellin level. M.Sc. Supervisor: Dr. Belay T. Ayele.

Wheat production is limited by numerous factors, including lodging, which refers to permanent displacement of a plant from its upright position due to falling over of the plant or breakage of its stem. The incidence of lodging is determined by genetic factors such as those that regulate gibberellin (GA) metabolism and thereby plant morphological traits, environmental factors such as heavy wind and rain, and crop management practices including nitrogen (N) fertilization. The present study investigated lodging-related morphological traits, which includes plant height, bending moment, internode breaking strength and lodging index, in response to varying N fertilization rates as well as treatment of plants with a plant growth regulator (PGR) that inhibits GA biosynthesis using two spring wheat cultivars with contrasting plant height. The findings of this thesis project indicated that high N fertilization rates increase plant height, bending moment and lodging index in both cultivars. Moreover, the taller cultivar exhibited higher bending moment and lodging index in response to nitrogen than the shorter cultivar. To gain insights into the molecular basis regulating GA metabolism and lodging-related morphological traits, the expression patterns of GA biosynthetic and catabolism genes were examined in the second basal internodes, which revealed differential expression patterns of these genes in response to varying N rates in both cultivars. Consistently, treatment of the tall cultivar plants with the PGR led to the reduction of plant height, which was associated with reductions in bending moment and lodging index.

FOREWORD

This thesis is written in manuscript style. A general introduction about the research project and a literature review precedes the manuscript. An abstract, introduction, materials and methods, results and discussion form a complete manuscript. The manuscript is followed by a general discussion and conclusions, a list of references and appendices.

1.0 GENERAL INTRODUCTION

Wheat is one of the most important cereal crops used for numerous purposes worldwide such as food, livestock feed, and industrial applications due to its agronomic adaptability, convenience of grain storage and ease of converting grain into flour for making many different food products (Giraldo et al., 2019; Khan, 2016). It is a staple food crop being consumed by an estimated 40% of the global population (Igrejas et al., 2020), and its demand is rising due to the rapidly growing world population. However, wheat production is negatively affected by numerous factors that cause significant yield and quality losses. One of these factors is lodging, which is the state of permanent displacement of shoots from their vertical stature that may ultimately lead to falling over of the plant or even breakage of the stems (Pinthus, 1974). It usually occurs as a combined effect of inadequate anchorage strength of the crop and impact of abiotic stresses such as rain, wind, and hailstorms (Khobra et al., 2019). Lodging can cause up to 50% yield reduction and compromises the end-use quality. It also creates challenges during harvesting, and thereby contributes to higher overall production costs (Berry et al., 2004; Islam et al., 2007; Stapper and Fischer, 1990).

Lodging is a complex phenomenon caused by the interaction of genetic factors that control morphological and anatomical traits as well as environmental and agronomic factors. Among the plant morphological factors, greater plant height is a main contributor in the occurrence of lodging throughout all developmental stages (Berry and Berry, 2015; Navabi et al., 2006). Therefore, producing semi-dwarf cultivars has been a major target of crop breeding for improving lodging resistance as they have the potential to withstand lodging pressure due to a reduced centre of gravity and above ground weight (Berry et al., 2000; Griffiths et al., 2012). The growth promoting plant hormone, gibberellin (GA), is one of the crucial endogenous factors that regulate

plant height. During the Green Revolution, significant yield increase was accomplished through the cultivation of wheat varieties that consisted of "Reduced height (*Rht*)" dwarfing genes. The *Rht* genes encode GA-insensitive non-functional DELLA proteins that limit plant response to GA, and thereby result in semi-dwarf plants (Hedden, 2003; Würschum et al., 2017).

Stem strength which is determined by thickness and overall robustness of the stem, is another important factor that contributes to lodging resistance by enhancing the stability of the plant (Neenan and Spencer, 1975). Specifically, the basal internodes of the stem provide a lever to hold the plant upright, thus, improving stem's mechanical strength is critical in increasing lodging resistance (Peng et al., 2014). It has been reported that culm breakage in wheat plants primarily occurs at the lower or basal internodes and the incidence of culm breakage increases with increased rate of nitrogen (N) application (Kong et al., 2014). Thus, N fertilization at an elevated rate, which is a prerequisite to ensure high yield, is a prime concern for the occurrence of lodging since it causes an increase in plant height and decreases in culm diameter and thickness due to reduced lignification in the culms, making plants susceptible to lodging (Tripathi et al., 2003; Zhang et al., 2016; Zhang et al., 2017b).

It has been observed previously that lowering the rate of N fertilization in spring wheat decreases height but increases stem diameter and wall width, both of which ultimately improve stem strength (Berry et al., 2000). In addition to the use of semi-dwarf cultivars, plant growth regulators (PGRs) that can reduce plant height and improve stem mechanical strength can be considered as an alternative solution to combat the problem of lodging. PGRs are defined as organic compounds that promote, inhibit, or modify any physiological process in plants at low dosages (Basra, 2000; Rademacher, 2015). Plant growth retardants, which are also known as plant growth inhibitors, form a significant group of PGRs, with GA biosynthesis inhibitors being the

most prominent due to their commercial value and the acreage of crops on which they are applied (Rademacher, 2015). The level of bioactive GAs in plants is regulated by a balance between its biosynthesis and catabolism (Yamaguchi, 2008). PGRs that inhibit GA biosynthesis are used to prevent lodging in cereals and other crops worldwide by reducing the level of GA in plants, which leads to repression of cell division and elongation and therefore plant height (Rademacher and Brahm, 2010; Rademacher, 2015). To further our understanding of genotypic difference in lodging resistance in response to N fertilization and/or PGRs in spring wheat, this study was designed to address the following objectives.

1. To investigate genotypic difference in lodging-related morphological traits in response to N fertilization and PGR treatment.
2. To examine changes in the expression patterns of GA metabolism genes under different N rates.

2.0 LITERATURE REVIEW

2.1 Wheat: a major cereal crop

Wheat (*Triticum aestivum* L.) was first cultivated in the Middle East around 10,000 years ago, making it one of the earliest domesticated food crops (Dvorak et al., 1998). It has been the staple food for millions of people across Europe, West Asia and North Africa for 8000 years due to its agronomic adaptability, and ease of grain storage and ability to convert its grain into flour for making different kind of foods (Giraldo et al., 2019). It belongs to the genus *Triticum*, tribe *Triticeae* and family *Poaceae* also known as *Gramineae*. Although there are 18 allopolyploid and 13 diploid wheat species of genera *Aegilops* and *Triticum* (Feldman and Levy, 2012); two wheat species *Triticum aestivum* (common wheat) and *Triticum turgidum* var. *durum* (durum wheat) account for over 95% of total wheat consumption (Randhawa et al., 2013). Wheat is widely cultivated on approximately 17% of the total cropped areas, spanning subtropical, mediterranean-type and temperate regions of both hemispheres (Levy and Feldman, 2022; Peng et al., 2011). Due to its substantial genetic diversity and adaptability to a wide range of climatic conditions, wheat is the most extensively grown commercial crop, enabling plant breeders to develop more than 25,000 varieties (Shewry, 2009). It serves as the staple food for over 40% of the global population, primarily in North America, Europe, and regions of western and northern Asia (Peng et al., 2011).

2.2 Evolution of wheat

The *Poaceae* family, commonly known as the grass family, originated around 50 to 70 million years ago (Mya) (Kellogg, 2000), with the divergence of sub-family *Pooideae* (including wheat, oats, and barley) approximately 20 Mya (Inda et al., 2008). The diploid ancestors of allopolyploid wheat evolved from a common progenitor approximately 2.5 to 4.5 Mya (Feldman & Levy, 2015).

Wheat has undergone evolution through crossing between species of the genus *Aegilops* and *Triticum*, which was proceeded by genome doubling. The allotetraploid form of wheat emerged approximately 300,000 to 500,000 years ago (Huang et al., 2002).

The modern bread wheat is an allohexaploid species comprised of 21 chromosome pairs (2n = 6x = 42) organized in three subgenomes A, B, and D, which originated from events of hybridization involving three diploid progenitors, namely *T. urartu* (AA; 2n=2x=14, wild einkorn), a yet-undiscovered extant *Aegilops* species closely related to *Ae. speltoides* (BB; 2n=14), and *Ae. Tauschii* (DD; 2n=14) (Kihara, 1944; McFadden and Sears, 1946; Zaharieva and Monneveux, 2006). The first polyploidization event occurred between *Triticum urartu* and B genome ancestor that is closely related to *Ae. speltoides* (goat grass), around 300,000 to 500,000 years before present (BP), which resulted in the formation of an allotetraploid wheat, *T. turgidum* ssp. *dicoccoides* (2n=4x=28; AABB, wild emmer) (Eversole et al., 2014). The earliest proof of wild emmer being used by man for milling and baking flour was discovered at Ohallo in Israel, a settlement established by hunter-gatherers approximately 19,000 years before present (BP) (Feldman and Kislev, 2007).

About 10,000 years ago, hunter-gatherers began to cultivate wild emmer, and its cultivation spread eastwards from the Fertile Crescent, followed by the development of cultivated emmer (*T. turgidum* ssp. *dicoccum*, 2n = 4x = 28, genome AABB) that hybridized spontaneously with another goat grass (*Ae. tauschii*, 2n = 2x = 14, genome DD) about 9,000 BP and produced an early spelt (*T. aestivum* ssp. *spelta*, 2n = 6x = 42, genome AABBDD) (Feldman and Levy, 2012). Around 8,500 BP, a natural mutation altered the structure of the ears in both emmer and spelt, transitioning them into a more easily threshed form, which eventually gave rise to the free-threshing ears characteristic feature of durum wheat (*T. durum*) and modern bread wheat (*T. aestivum*) (Peng et

al., 2011). These allopolyploids, which exhibit broader morphological variation and greater ecological diversity, are dispersed over larger area geographically than their diploid ancestors (Kimber and Feldman, 1987; Zohary and Feldman, 1962).

2.3 Domestication of wheat

Domestication refers to the process of selective breeding, leading to enhanced adaptability of plants or animals to human cultivation or husbandry (Brown, 2010). It renders all cultivated crops, including wheat, for their cultivation in agricultural environments in order to meet human needs (Gustafson et al., 2009). During the Natufian period, which spans from approximately 13,000 to 10,300 BP, the hunter-gatherers inhabiting the Levant (the western part of the Fertile Crescent) gathered the grains of wild cereals such as wheat, oat, rye, barley, and *Aegilops*. However, there is no evidence of cultivation during this timeframe (Feldman and Levy, 2023; Harris, 1998). The domestication of wheat occurred about 10,000 years ago in the Fertile Crescent which includes parts of the modern Middle East (Peng et al., 2011). This period is referred to as the "Neolithic revolution" or the "agricultural revolution," when a shift from the lifestyle of hunting and collecting food to sedentary agriculture took place (Dubcovsky and Dvorak, 2007). Historical records indicate that only wild einkorn and wild emmer wheats underwent the process of domestication selection.

The wild einkorn wheat (*T. monococcum*) was initially found as a weed growing in the Middle East and was subsequently domesticated in the Karacadag mountain range located in southeast Turkey. It is a type of diploid wheat which was domesticated from its wild progenitor *T. boeoticum*, about 10,400 years ago. The domestication of einkorn wheat was aided by a genetic mutation leading to non-brittle rachis (Faris, 2014), which transformed the harvesting process and

allowed for a more effective grain collection (Heun et al., 1997). Over the past 5,000 years, einkorn wheat has predominantly been replaced by tetraploid and hexaploid varieties. Currently, einkorn remains as a relic crop that is cultivated in selected Mediterranean countries primarily for animal feed (Peng et al., 2011).

The domestication of emmer wheat (*T. dicoccum*) marked a significant advancement in agricultural history, particularly in understanding of the domestication process of hexaploid wheat. Emmer wheat stands out as the sole true wild polyploid wheat within its lineage and serves as the ancestor of present-day durum and modern wheat cultivars. It was naturally growing in Fertile Crescent where it was discovered in 1906 by Aaron Aaronsohn in Israel. Archaeological evidence dating back to the period of 10,300 to 9,500 BP indicates that wild emmer was first cultivated in the southern Levant. Domesticated emmer emerged several hundred years later in southeast Turkey, around 9,500 to 9,000 BP, and it was cultivated alongside wild emmer in numerous Levantine sites in southeast Turkey for over a millennium (Peng et al., 2011). This coexistence led to spontaneous hybridizations, resulting in the gene transfer including the transfer of non-brittleness gene between the wild and domesticated populations (Feldman and Kislev, 2007; Rahman et al., 2020).

Furthermore, a tetraploid wheat species, *T. turgidum* sssp. *dicoccum*, originated in southeastern Turkey and underwent domestication as durum wheat approximately 9,000 years ago (Gill and Friebe, 2002; Heun et al., 1997). Earlier evidence suggests that hexaploid bread wheat species were domesticated and cultivated in the Fertile Crescent around 8,000 years ago (Rahman et al., 2020). Today, the wheat species like einkorn, emmer, and spelt, also known as hulled wheat, is grown in some parts of Spain, Turkey, the Balkans, and the Indian subcontinent (Peng et al.,

2011) while the modern wheat varieties represent 95% of wheat grown worldwide and are consumed as primary food and calorie source.

2.4 History of Wheat in Canada

After its origin in the Fertile Crescent, the common bread wheat first dispersed to Asia, Africa, and Europe, and then North America. The records of wheat cultivation in North America, particularly in Canada, date to the late 15th and 16th centuries. In Canada, wheat was first grown in 1605 by French settlers at Port Royal, Nova Scotia (Hopper, 1923) while the exports were initiated in 1654. The cultivation of wheat was initiated by settlers in Selkirk, Manitoba in 1812. These settlers were sent by Lord Selkirk from Scotland and eventually established 'Red River settlement'. In early 1813, they cultivated winter wheat seeds brought from their home countries along with some spring wheat, however, the problem of late maturity made the crop vulnerable to frost damage, lodging, and stem rust ultimately marking it as an unsuccessful attempt (Buller, 1919; Newman, 1928). After the initial setbacks of wheat farming in Western Canada between due to harsh winter conditions, late maturity of the available wheat varieties, and lack of farming expertise and equipment, some progress was achieved by 1815 as farmers adapted to these challenges (Buller, 1919). The soil along the Red River's bank was very fertile as reflected by high yields of other crops such as potatoes and turnips. However, it was reported that the wheat crop on the settlement was affected by grasshoppers in 1818 and 1819, resulting in huge crop losses. As a result, no seed remained from the first variety grown by the settlement (Buller, 1919). In 1842, David Fife, an Ontario farmer received wheat seeds from Glasgow, Scotland and developed a hard red spring wheat variety called "Red Fife". After many trials and failures, cultivar Red Fife gained widespread recognition for its excellent yield and milling quality by 1870. Nonetheless, it

struggled to withstand frost during extreme low temperature. Furthermore, Red Fife was crossed with Hard Red Calcutta in 1903 by Sir Charles Saunders to develop the cultivar Marquis, which became the foundation of Western Canadian wheat development (Buller, 1919; DePauw and Hunt, 2001). By 1909, this cultivar was widely adopted all over Canada, resulting in a notable rise in wheat production from 2 million tonnes in 1904 to 7.7 million in 1913, popularizing the hard red spring wheat in Canada (Campbell, 2015; Charmet, 2011).

Presently, the Canadian wheat varieties are divided into two categories by region i.e. Eastern Canadian wheat classes and Western Canadian wheat classes, which are further grouped according to their functional characteristics. Eastern Canadian wheat classes include classes like Canada Eastern Red Spring (CERS), Canada Eastern Soft Red Winter (CESRW), Canada Eastern Amber Durum (CEAD), Canada Eastern Hard Red Winter (CEWHRW), Canada Eastern White Winter (CEWW) and Canada Eastern Feed (CE Feed). In Western Canada, wheat is classified into nine milling classes including Canada Northern Hard Red (CNHR), Canada Prairie Spring Red (CPSR), Canada Prairie Spring White (CPSW), Canada Western Extra Strong (CWES), Canada Western Hard White Spring (CWHWS), Canada Western Red Spring (CWRS), Canada Western Amber Durum (CWAD), Canada Western Red Winter (CWRW) and Canada Western Soft White Spring (CWSWS) (Government of Canada, 2019).

2.5 Wheat production and its uses

Wheat grain is an important source of human nutrition since it consists of 75-80% carbohydrates, 9-18% proteins, dietary fibres, vitamins (particularly B group), iron, calcium and many more macro and micro-nutrients, making it a staple food for about 40% of the world population (Igrejas et al., 2020). Species within the genus *Triticum* are used for different purposes such as pasta, bread, and

noodles worldwide, of these, *T. aestivum* (bread wheat) and *T. turgidum* (durum wheat) are most prominent and commonly used for making bread and pasta, respectively. Processed wheat grain is used to produce a wide range of functional food products due to its unique composition of gluten and protein (Shewry and Hey, 2015). The gluten in wheat flour is mainly composed of endosperm proteins, gliadins and glutenins (Shewry, 2019). These proteins play a crucial role in enhancing the baking quality of the flour as they determine the elasticity and extensibility of the dough to be processed into various baked goods (Delcour et al., 2012). In addition to human consumption, wheat is used in brewing beer, distilling vodka, and producing biofuel. By-products from processing procedures such as wheat bran and secondary flours are commonly sold as feed for animals and poultry (Shewry, 2009). Statistically, more than two-thirds of the world's wheat production is allocated for human consumption, with approximately 20% utilized as livestock feed, and around 3 to 5% designated for seed, industrial use, and other purposes (Melini and Carcea, 2016). Owing to its potential to make distinctive food products and changes in eating habits due to urbanization, the demand for wheat is increasing in many parts of the world even in regions that do not have suitable climate for its cultivation.

In the 1960s, the Green Revolution significantly boosted wheat productivity, especially in developing nations, by introducing high-yield varieties and the use of fertilizers, pesticides, and herbicides. As a result, wheat production doubled between 1965 and 1970, making wheat the most widely cultivated crop globally (Curtis and Halford, 2014). On a global scale, over 219 million hectares of land was under wheat cultivation in 2022 , which produced approximately 784 million metric tonnes during the 2023/2024 marketing year (Shahbande, 2024). China and India are reported to be the major producers of wheat with the production of 136.6 and 110.5 million metric tonnes in 2023/2024 marketing year, respectively (USDA, 2024).

Wheat is Canada's most extensively grown field crop, and the country ranks among the top global exporters of durum wheat (Cereals Canada, 2022). In Canada, both winter wheat, which is planted in the fall and harvested in the summer, and spring wheat, planted in the spring and harvested in late summer, are widely cultivated (DePauw et al., 2011). Due to efforts in development of high yielding and disease resistant cultivars, wheat yields in Canada have shown significant increase, from 1512 kg/ha during 1961 to 1970 to 2478 kg/ha during 2000 to 2010 (Randhawa et al, 2013; Statistics Canada, 2021). In the 2021 growing season, Canadian farmers produced about 22 million tonnes of wheat, of which Manitoba accounted for 17% of total production despite the occurrence of extreme dry heat conditions (Government of Manitoba, 2021). In 2022, Canada produced 33.8 million tonnes of wheat, which represented a 51.7% increase as compared to that produced in 2021; Saskatchewan and Alberta produced 14.8 and 11.3 million tonnes, respectively, while Manitoba produced 4.8 million tonnes (Statistics Canada, 2022). In 2023, Canada was the 6th largest wheat producer in the world with 32 million tonnes of production. The western Canadian provinces produced over 90% of the total wheat produced in Canada (Cereals Canada, 2023).

Wheat demand is expected to increase from 700 million tonnes in 2021 to over 900 million tonnes in 2050 so as to feed the predicted world population of 9 billion by 2050 (Grote et al., 2021; Nelson et al., 2010). The need to increase wheat yield with the existing cultivated land requires a "Second Green Revolution" aimed at boosting its yield and productivity (Baulcombe et al., 2009). Moreover, research efforts should be focused on enhancing the crop's resistance against biotic and abiotic stresses. This can be achieved through the development of genomic tools for introducing varieties with disease and pest resistance along with sustainable agricultural practices to conserve water, maintain soil fertility, and promote genetic diversity in wheat.

2.6 Constraints of wheat production

Wheat production is negatively affected by several biotic and abiotic factors that results in tremendous yield and quality losses. Biotic stress is caused by several living organisms including pathogens such as fungi, bacteria and viruses, and insect pests and weeds, that interact with crops to hinder their growth and productivity. The most economically important diseases that limit wheat production in Canada are fusarium head blight, powdery mildew, leaf and stem rust, and loose smut, all of which are caused by fungal pathogens (Aboukhaddour et al., 2020). Abiotic stresses such as flooding, drought, salinity, and high temperature also have significant negative effects on wheat production (Mariani & Ferrante, 2017). For example, owing to the extreme drought condition that occurred in 2021, the total wheat yield in Canada was only 22 million tonnes, which represents a yield reduction of 38.5% as compared to that of 2020 (Statistics Canada, 2021). Lodging is another major constraint which usually occurs as a combined effect of inadequate anchorage strength of the crop and impact of abiotic stresses such as rain, wind, and hailstorms (Khobra et al., 2019). Lodging can cause yield losses of up to 40% if it occurs within the 10 days after heading (Manitoba Crop Alliance, 2023). Moreover, lodging risk in spring wheat has increased with the introduction of high-yielding cultivars that require high nitrogen (N) fertilization to enhance grain yield and protein content (Mangin et al., 2022). Thus, effective genetic tools and crop management practices are required to reduce the risk of lodging in wheat.

2.7 Lodging

Lodging is defined as the state of permanent displacement of shoots of small grain cereals from their upright position due to combined effect of plant genetic factors that influence plant height,

anchorage and stem strength; environmental conditions such as rain, wind and hailstorms; and agronomic practices such as fertilizer management (Khobra et al., 2019; Pinthus, 1974). Lodging ultimately results in falling over of the plant or even breakage of the stems (Pinthus, 1974), which usually occurs after emergence of the spike or the panicle (Berry et al., 2004). Lodging is one of the major constraints that limits cereal productivity worldwide (Fischer & Stapper, 1987) as it can cause significant reduction in grain yield, by up to 50%, and in end use quality (Berry et al. 2004; Stapper and Fischer, 1990). The distribution and degree of lodging determine the severity of lodging (Pinthus, 1974). Lodging disrupts the typical arrangement of the crop's canopy, leading to a decline in the plant's ability to perform photosynthesis and produce dry matter (Hitaka, 1969). Severe lodging negatively affects the movement of water, nutrients, and assimilates through the xylem and phloem, resulting in a decrease in the amount of assimilates available for grain filling. Elevated moisture levels in lodged plants provide favourable conditions for fungal growth and onset of diseases, which have adverse effect on grain quality and appearance (Islam et al., 2007). Additionally, grains in lodged plants may sprout on the spike, especially in cultivars with insufficient level of seed dormancy, leading to significant losses in yield and quality of grains. Moreover, lodging poses challenges in the harvesting process and consequently contributes to an overall increase in production cost (Islam et al., 2007). Lodging can be grouped either as root lodging or stem lodging.

2.7.1 Root lodging

Root lodging refers to the leaning of straight and intact plant stems from the crown when the root fails to maintain strong soil contact or has a poor anchorage in soil (Berry et al., 2003; Pinthus, 1974). It occurs when the roots are unable handle the stress caused by wind and rain, especially

when the above-ground parts of the plant exert pressure on the root (Udagawa and Oda, 1967). Irrigation or rainfall saturates the top layer of the soil, and this causes weakening of the plant's anchorage, making the plant susceptible to tilting/lodging even by a gentle wind (Udagawa and Oda, 1967). Root lodging may uproot or displace the root system within the soil due to forces from the above-ground parts, and this often occurs earlier in the growing season such as during flowering in cereals (Sterling et al., 2003). In general, root lodging typically occurs when heavy rainfall weakens the strength of cohesive binding of soil particles surrounding the root system (Shah et al., 2019). Thus, breeding programs focused on developing cultivars with improved root anchorage under varying soil moisture conditions can provide a sustainable solution to minimize the impact of root lodging (Kashiwagi et al., 2005).

2.7.2 Stem lodging

Stem lodging refers to the bending or breaking of the lower internodes of the culm due to adverse weather events such as heavy rainfall or strong winds. It is observed specifically in plants firmly anchored by a dry and compact upper soil layer (Pinthus, 1974). It can be classified as bending or breaking type based on how the stem responds. In bending type of stem lodging, the stem bends at the middle of the internode; however, this is less detrimental since the bent culm can still transport photosynthates to the grain to support grain filling. In contrast, breaking type of stem lodging, where the stem fractures below the third internode, is more severe and permanently damages the plant's ability to transport nutrients (Khobra et al., 2019). In wheat, barley and oats, stem lodging is usually caused by the buckling of one of the two basal internodes that causes the upper stem and ear to lie parallel to the ground (Berry et al., 2004). Stem lodging is related to plant height, culm diameter and thickness, stem strength, lignin and cellulose accumulation in the stem

wall, and spike weight (Shah et al., 2019). It can be induced by external environmental forces such as hail and storm, and it is enhanced by the insect damage to the culms or foot rot as well as the weight of the crop itself (Pinthus, 1974). Moreover, high plant density and nutrient imbalances can lead to taller and thinner stems that are more prone to lodging (Berry et al., 2004). Overall, stem lodging is a complex phenomenon influenced by various morphological factors including stem strength and plant height as well as environmental factors and crop management practices. Developing lodging-resistant cultivars and the use of plant growth regulators (PGRs) are among the strategies that have been explored to mitigate stem lodging (Berry et al., 2004; Ennos, 1993).

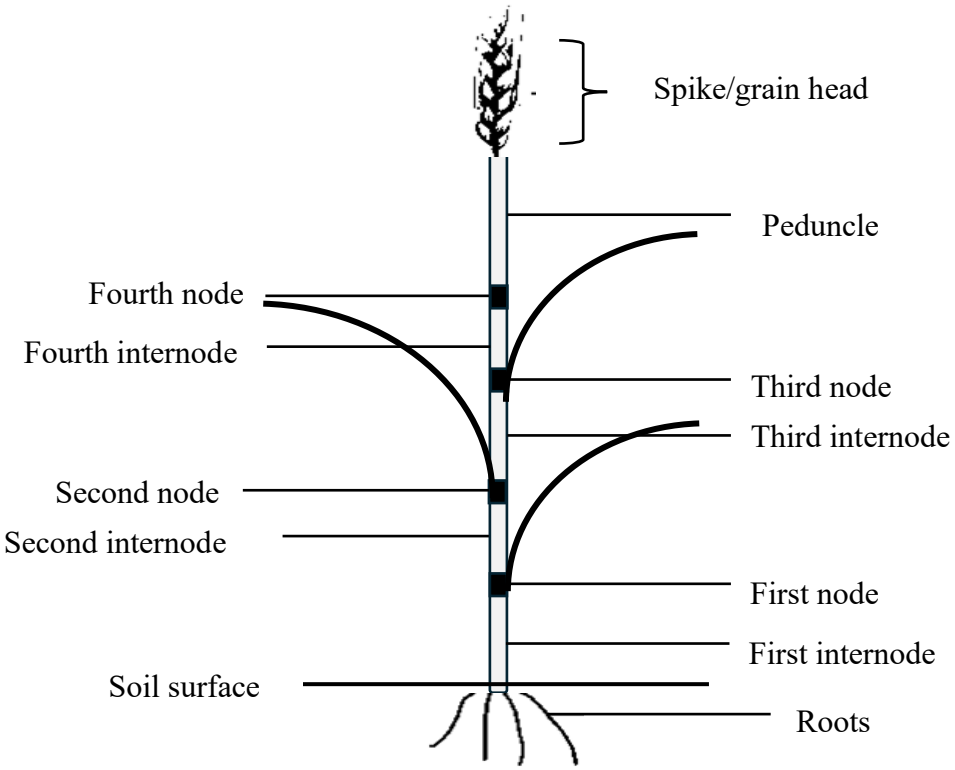

Figure 2.1. Schematic diagram of a wheat plant.

2.8 Crop and other factors associated with lodging

Lodging is a complex phenomenon caused by a combination of morphological, anatomical, metabolic, environmental and agronomic/crop management factors.

2.8.1 Morphological factors

Plant height, internode length and stem diameter are the key plant morphological traits that significantly influence lodging (Shah et al., 2019). The overall height of a wheat plant encompasses the spike, peduncle, and the combined length of all above-ground internodes, which together determine its resistance against lodging. In cereals, excessive plant height is a significant contributor for the occurrence of lodging throughout all developmental stages (Berry and Berry, 2015). In general, genotypes that produce tall plants are highly susceptible to lodging while those producing shorter plants have the potential to withstand lodging pressure due to lower centre of gravity as well as above ground weight (Berry et al., 2003; Wu et al., 2022). Therefore, producing semi-dwarf cultivars has been a major target of wheat breeding for improving lodging resistance (Griffiths et al., 2012). A key factor in regulating plant height is gibberellin (GA), a growth-promoting plant hormone. Several genetic studies have established that most semidwarf wheat cultivars are developed by mutations in GA metabolism and signaling genes (Sakamato et al., 2003, 2004).

During the Green Revolution, breeders mitigated the risk of lodging by incorporating semi-dwarfing genes like *Rht-B1b* and *Rht-D1b* into wheat to develop cultivars with reduced plant height (Wilhelm et al., 2013). Furthermore, short primary (basal) internodes provide a lever to hold the plant upright to support an ideal culm structure for lodging resistance, indicating a negative correlation of the length of the basal internodes with the lodging resistance (Peng et al.,

2014; Tripathi et al., 2003). Several studies reported that lodging index (LI) is positively correlated with plant height (Kashiwagi et al., 2005), number of internodes and internodal length (Huang et al., 2006; Kelbert et al., 2004), indicating that these plant characteristics significantly influence resistance to lodging.

Stem strength is the other important factor that affects lodging resistance (Wang et al., 2006). The basal part of the culm internode has a significant role in lodging resistance as it provides a lever to hold the plant upright. Hence, increasing the physical strength of the basal part of the culm internodes can improve lodging resistance (Peng et al., 2014). Mechanical strength of the stem is positively regulated by culm diameter and thickness. According to a study by Kelbert et al. (2004), lodging-resistant wheat genotypes exhibit higher culm wall thickness than genotypes that are susceptible to lodging. It has been reported in wheat (Berry et al., 2003) and rice (Islam et al., 2007; Okuno et al.,2014; Zhang et al., 2017b) that greater culm diameter is strongly associated with greater culm wall thickness, which is an important contributor of lodging resistance.

Cell wall components such as lignin and cellulose play a crucial role in enhancing the mechanical strength of plant culms through reinforcing the rigidity of the basal stem and thereby improving lodging resistance in cereal crops (Kong et al., 2013). During secondary cell wall formation, lignin accumulates within the carbohydrate matrix, making the plant body more robust and enabling it to grow upright (Del et al., 2012). Additionally, culm wall thickness shows a positive correlation with the levels of lignin, cellulose, and water-soluble and structural carbohydrates (Khobra et al., 2019). Studies have demonstrated a significant association between the total lignin content in the basal second internode and the structural integrity and elasticity of stems in wheat (Zheng et al., 2017) and rice (Okuno et al., 2014), indicating lignin's pivotal role in improving breaking strength (BS) of the stem. Several studies have reported an association of

lodging resistance with deposition of lignin, pectin, cellulose, and protein concentrations in plant stems (Shah et al., 2019). For example, in wheat, the lignin accumulation in higher amounts have been found to enhance the physical stability of culm internodes and thereby the lodging resistance (Peng et al., 2014). Moreover, lower lignin accumulation has been shown to correspond to increased vulnerability to lodging as wheat varieties that are susceptible to lodging are found to exhibit lower levels of hemicellulose and/or lignin in their culms compared to lodging-resistant varieties (Chen et al., 2011; Peng et al., 2014). The activity of lignin biosynthetic enzymes, which include tyrosine ammonia-lyase (TAL), peroxidase (POD), and phenylalanine ammonia lyase (PAL), is significantly linked with accumulation and build-up of lignin in wheat culms (Khan et al., 2003). Consistently, strong associations between lodging resistance and the expression levels of key lignin biosynthesis genes including, *4-coumarate:CoA ligase1* (*4CL1*), *cinnamoyl-CoA reductase2* (*CCR2*), *p-coumarate 3-hydroxylase1* (*C3H1*), *ferulate 5-hydroxylase2* (*F5H2*), and *caffeic acid O-methyltransferase2* (*COMT2*) have been reported in the internodes of wheat culm (Nguyen et al., 2016). Lodging resistance in wheat, thus, might be improved by increasing lignin accumulation in with the stem/internodes via enhancing the expression of genes and activities of the enzymes involved for lignin formation.

2.8.2 Anatomical factors

Wheat culms mainly consist of ground tissue (parenchyma), mechanical tissue (sclerenchyma) and two rows of vascular bundles, with smaller vascular bundles embedded in outer sclerenchyma tissues and the larger vascular bundles embedded in inner parenchyma tissues (Khobra et al., 2019). There is a higher proportion of sclerenchymatous tissues in completely or partially filled stems, and this contributes to better resistance to lodging than wheat lines with hollow stem; this

is because enhanced sclerification strengthens soft tissues and prevent collapsing and bulking of stems (Kong et al., 2013). Furthermore, enhanced pith parenchyma density renders good shock absorbing capacity and thereby increases culm's stability and ability to withstand environmental forces such as wind and rain (Kokubo et al., 1989). Since solid stemmed wheat genotypes have the greatest amount of pith, these genotypes are likely to be more resistant to lodging than those with hollow stems. Studies by Khanna (1991) and Hamilton (1951) found that stem lodging decreased with increased number of vascular bundles in wheat, triticale, rye, and oat. Moreover, a previous study indicated a significant positive correlation between vascular bundles per mm^2 and bending resistance (Wang et al., 2006).

2.8.3 Agronomic factors

Agronomic practices such as early sowing, high plant density, enhanced soil fertility and high rate of nitrogen (N) fertilization are some of the agronomic practices applied by farmers to increase crop yield. However, these practices may also contribute to lodging. N fertilization is a prerequisite to ensure high yield, however, it is a prime concern for the occurrence of lodging. Several studies have highlighted the intricate relationship between nitrogen fertilization and lodging in cereal crops (Ma et al., 2017; Mangin et al., 2022; Zhang et al., 2017a). Culm strength which is determined by thickness and overall robustness of the stem, is critical in providing resistance to lodging as it imparts stability to hold the plant upright. Higher rates of N application have been reported to elongate the lower culm internodes and reduce lignification and mechanical strength of internodes, leading to the development of thin and weak basal internodes with reduced BS (Zhang et al., 2016; Zhang et al., 2017b). In addition, an increase in the photosynthetic potential of plants due to high N fertilization results in dense crop canopy, which in turn causes shading to

the lower parts of the plant due to low red to far red ratios (Khobra et al., 2019). As a result of shading, plants exhibit shade avoidance response, which is characterized by an increase in basal internode length and overall plant height along with a decrease in stem diameter, wall thickness, and lignin accumulation, as observed in both Arabidopsis (Pantazopoulou et al., 2021) and wheat (Luo et al., 2022). The prevalence of excessive stem elongation and thinner stem leads to weakening of the structural integrity of wheat stem and reduction of its breaking strength, which ultimately increases the risk of lodging (Luo et al., 2022). The enhanced vegetative growth due to high N fertilization also reduces root penetration into the underground soil, increasing root lodging risk. It has been reported that lowering the rate of N fertilization in spring wheat decreases height, but increases stem diameter and wall width, improving stem strength (Barry et al., 2000). In contrast, wheat genotypes grown under higher levels of N show higher LI due to decreased lignin accumulation, thinner stems, and increased angle of lodging (Tripathi et al., 2003).

2.9 Gibberellins

Gibberellins (GAs) are one of the plant hormones that are produced endogenously by plants at very low concentration and regulate a wide range of plant growth and developmental processes and mediate plant response to several environmental factors (Davies, 2010). They belong to a class of tetracyclic diterpenoid carboxylic acids produced through complex biosynthetic pathways (Hedden, 2020). Research on GA began in the late 19th-century in Japan, when scientists discovered that a fungal infection caused a rice disease, which was later named Bakanae or "foolish seedling" disease. This disease, which led to excessive stem elongation, pale green leaves, infertility and reduced yields, was eventually linked to the fungus *Gibberella fujikuroi* that produces the GA hormone responsible for the observed abnormal shoot growth (Hori, 1898).

Further studies confirmed that the culture filtrates of fungus *Gibberella fujikuroi* caused these symptoms, leading to the isolation of the water-soluble growth-promoting compound that was named 'gibberellin' by Yabuta (Hedden and Sponsel, 2015). However, this discovery remained unnoticed due to language barriers and times of war. It was only after the Second World War that the primary work in Japan was recognized by plant scientists worldwide (Takahashi, 1998). The presence of GA in plants as an endogenous growth regulator was reported almost 70 years ago due to its ability to restore the height of dwarf mutants of pea and maize (Hedden and Phillips, 2001). To date, about 140 GAs have been identified from higher plants, bacteria and fungi, however, only a small fraction are biologically active, which includes GA_1, GA_3, GA_4 and GA_7 (Camara et al., 2018; Hedden & Thomas, 2012; Rademacher, 2015). Biologically active GAs play key roles in diverse aspects of plant growth and development such as stem elongation, seed germination, leaf expansion, and flower and seed development (Vishal & Kumar, 2018). The non-bioactive forms either act as precursors for bioactive forms or inactivated metabolites (Yamaguchi, 2008).

2.10 Gibberellin metabolism

Gibberellin metabolism is defined as the biosynthesis of bioactive forms of GAs from their precursors and the catabolism/inactivation of the bioactive GAs to their respective inactive forms (Sponsel & Hedden, 2010). The levels of bioactive GAs in a plant tissue are regulated by the rate of their biosynthesis and catabolism (Yamaguchi, 2008).

2.10.1 Gibberellin biosynthesis

The GA biosynthesis pathway can be categorized into three stages based on the type of enzymes involved and their sites of action: the first stage is catalysed by terpene synthases (TPSs) and takes

place in the proplastids while the second stage is catalyzed by cytochrome P450 monooxygenases (P450s) and occurs in the endoplasmic reticulum. The third stage of GA biosynthesis takes place in the cytosol by the actions of 2-oxoglutarate-dependent dioxygenases (2ODDs) (Hedden and Phillips, 2000). GA biosynthesis pathway begins with *trans*-geranylgeranyl diphosphate (GGDP), which is a common C_{20} precursor for diterpenoids (Yamaguchi, 2008). Thus, first stage involves the conversion of GGDP to *ent*-kaurene by two terpene synthases; GGDP is converted to *ent*-copalyl diphosphate via *ent*-copalyl diphosphate synthase (CPS) and *ent*-kaurene synthase (KS) catalyses the formation of a tetracyclic hydrocarbon intermediate, *ent*-kaurene from *ent*-copalyl diphosphate. The synthesis of *ent*-kaurene from GGPP takes place in the stroma of proplastids or developing chloroplasts, where there is presumably less competition for GGPP from the primary biosynthesis pathways of chlorophyll and carotenoid compared to mature chloroplasts (Hedden, 2020). *ent*-kaurene is then transported to the outer membrane of endoplasmic reticulum, where it is first converted to *ent*-kaurenol and then to *ent*-kaurenal via sequential oxidation on C-19 by the action of a P450 monooxygenase, *ent*-kaurene oxidase (KO), to produces *ent*-kaurenoic acid (Yamaguchi, 2008). The conversion of *ent*-kaurenoic acid to GA_{12} is mediated by another P450-dependent enzyme, kaurenoic acid oxidase (KAO). It involves the sequential conversion of *ent*-kaurenoic acid to *ent*-7a-hydroxykaurenoic and then to GA_{12}-aldehyde and GA_{12} (Hedden and Phillips, 2000). GA_{12} is then pooled in the cytosol where the 2ODD enzymes, GA 20-oxidase (GA20ox) and GA 3-oxidase (GA3ox), catalyse oxidation reactions at C-20 and C-3, respectively, to produce one of the bioactive forms of GA, which is GA_4. Alternatively, GA 13-oxidase (GA13ox) mediates an oxidative reaction to convert GA_{12} into GA_{53}, which serves as a precursor to GA_1 in the 13-hydroxylated pathway. Accordingly, GA_{53} undergoes a series of oxidation reactions catalysed by GA20ox and GA3ox to produce the other form of bioactive GA, GA_1

(Yamaguchi, 2008). The enzyme GA20ox utilizes C20-GAs as substrates to produce C19-GAs (GA_9 and GA_{20}) via removal of the C-20 atom and formation of a lactone. Finally, bioactive GAs, GA_4 and GA_1, are then synthesised from GA_9 and GA_{20}, respectively, by the activity of GA3ox (Hedden and Phillips, 2000).

2.10.2 Gibberellin catabolism

GAs can be metabolically inactivated in a number of ways; however, they are catabolized primarily through 2β-hydroxylation by the actions of GA 2-oxidases (GA2oxs) on either the bioactive GAs or their precursors (Hedden, 2020). The ability to inactivate GAs through 2β-hydroxylation exists exclusively in higher plants, including gymnosperms and angiosperms (Hedden, 2020). GA2oxs can be categorized into three classes based on their phylogenetic relationships. The class I or II GA2oxs use C19-GAs as their substrates, which includes some bioactive GAs and their immediate precursors such as GA_9 and GA_{20}. The class III GA2oxs accepts only C20-GAs, therefore, are known to deplete the levels of precursor GAs earlier in the pathway such as GA_{12} and GA_{53}, which could otherwise be changed to biologically active GAs (Yamaguchi, 2008).

Furthermore, a study in rice by Zhu et al. (2006) revealed another GA inactivation mechanism, which is catalysed by a P450 monooxygenase enzyme designated as elongated uppermost internode (EUI). EUI, also known GA 16α,17-epoxidase, catalyses epoxidation of the 16,17- double bond of the non-13-hydroxylated GAs (GA_4, GA_9, GA_{12}) to reduce GA level and restrict elongation of the upper internodes (Zhu et al., 2006). In addition to these mechanisms, GAMT1 and GAMT2 (gibberellin methyltransferases) were discovered to methylate the C-6 carboxy group of active GAs and their precursors using S-adenosyl-L-methionine as a substrate, leading to the deactivation of bioactive GAs in Arabidopsis (Varbanova et al., 2007). Another

mechanism of GA inactivation is conjugation, typically to glucose, which involves reversible esterification with the formation of either GA-O-glucosyl ethers (GA-O-Glc) or GA-glucosyl esters (GA-Glc ester), depending on the specific hydroxyl or carboxyl groups involved. The GA-Glc esters sequester bioactive GAs and release them when needed. In contrast, GA-O-Glcs outcomes: GA-2-O-glcs produce inactive forms after hydrolysis, whereas GA-3-O-glcs produces bioactive GAs (Hedden, 2020; Sponsel and Hedden, 2010). This specificity in synthesis and hydrolysis plays a critical role in regulating the pool of bioactive GAs in plants (Hedden, 2020; Sponsel and Hedden, 2010).

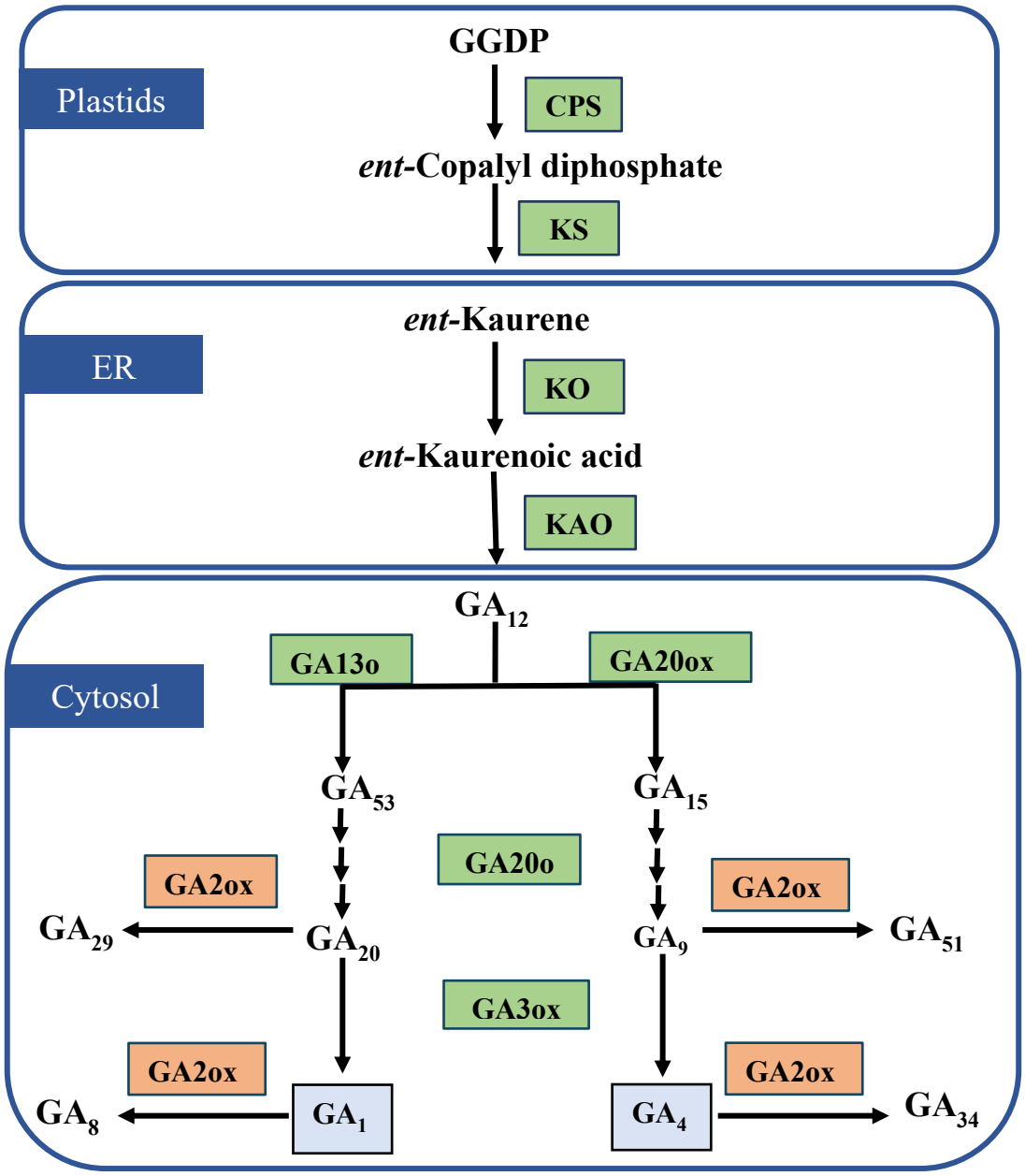

Figure 2.2. A simplified GA metabolism pathway in plants (adapted from Yamaguchi, 2008). GA1 and GA4 are bioactive GAs. GGDP, geranylgeranyl diphosphate; CPS, ent-copalyl diphosphate synthase; KS, ent-kaurene synthase; KO, ent-kaurene oxidase; KAO, ent-kaurenoic acid oxidase; GA13ox, GA 13-oxidase; GA20ox, GA 20-oxidase; GA3ox, GA 3-oxidase; GA2ox, GA 2-oxidase. ER, endoplasmic reticulum.

2.11 Gibberellin metabolism genes

Enzymes that catalyse the early steps of the GA biosynthesis, TPSs and cytochrome P450s, (CPS, KS, KO, and KAO) are encoded by one or two genes, whereas enzymes that catalyse the later stages of GA biosynthesis (2ODDs) are encoded by multigene families (Yamaguchi, 2008). In Arabidopsis, CPS, KS, KO are each encoded by a single gene, and the non-functional alleles of these genes (*ga1*, *ga2*, and *ga3*) result in severe GA-deficient dwarfism (Koornneef and van der Veen, 1980). The P450 monooxygenase of Arabidopsis, KAO is encoded by two genes, *CYP88A3* and *CYP88A4*. *CYP88A4* was observed to have greater expression levels in the stem and inflorescence compared to *CYP88A3* in Arabidopsis (Helliwell et al., 2001). Although there are two genes that encode a functional CPS in rice, only one of them is likely to participate in GA biosynthesis (Sakamoto et al., 2004). On chromosome 6 of rice, there are five *KO*-like genes positioned sequentially, of these, *KO1* (*CYP701A7*), *KO2* (*CYP701A6*) and *KO5* (*CYP701A9*) are integral to GA biosynthesis (Hedden, 2020; Itoh et al., 2004; Sakamoto et al., 2004). Although the wheat KO family has not been functionally studied yet, it's worth noting that treating wheat seedlings with the KO inhibitor paclobutrazol led to the accumulation of certain compounds, including 3α-hydroxy-*ent*-kaurene. This compound is likely produced by a gene related to KO; a KO paralog (Croker et al., 1995; Hedden, 2020).

Members of the gene families encoding the 2 ODDs have distinct functions and expression patterns at developmental stage and at the tissue level and in response to environment factors (Han and Zhu, 2011). For instance, a study in Arabidopsis showed that all five *GA20ox* genes produce functional enzymes except *GA20ox5*, which encodes functionally different enzyme that is responsible for aldehyde synthesis without subsequent conversion to the C-19 GAs. It has been shown that functional disruption of *GA20ox1*, *GA20ox2* and *GA20ox3* leads to a decrease in seed

germination, infertility and dwarfism. In contrast, *GA20ox4* and *GA20ox5* genes were found to have minimal effects on these traits (Plackett et al., 2012). In the rice genome, four *GA20ox* genes (*GA20ox1, -2, -3, -4*) have been identified (Sakamato et al., 2004). Among the four genes, plant height is regulated mainly by both *GA20ox1*and *GA20ox2* (Oikawa et al., 2004; Spielmeyer et al., 2002). Expression study of *GA20ox* genes revealed that *GA20ox2/SD* is a dominant *GA20ox* gene in rice stems (Sakamato et al., 2004). In agreement with this report, loss-of function mutation in *GA20ox2* (*sd1*)of rice played a role during the Green Revolution as it resulted in the semi-dwarf rice with greater lodging resistance and improved harvest index, facilitating the increased use of nitrogen based fertilizers (Gao and Chu, 2020; Han and Zhu, 201; Jennings, 1964)

Among the four *GA3ox* genes of Arabidopsis, *GA3ox1* exhibits predominant expression during vegetative growth while a null allele of this gene, designated as *ga3ox1-2*, results in semi-dwarfism (Chiang et al., 1995; Phillips et al., 1995). The expression patterns of *GA3ox* genes suggest that *GA3ox1* and *GA3ox2* genes play major roles in germination and vegetative growth, while *GA3ox1*, *GA3ox3* and *GA3ox4* are prominent genes for the reproductive organ development in Arabidopsis (Mitchum et al., 2006). In rice, two *GA3ox* genes (*GA3ox1, -2*) have been identified (Itoh et al., 2001).*OsGA3ox2* of rice is expressed in various organs, such as shoots and roots, and its null mutant alleles exhibit a severe dwarf phenotype, suggesting reduction of GA production in the shoots (Kawai et al., 2022). *OsGA3ox1* is expressed specifically at the late developmental stages in reproductive organs such as pollen, anther filaments, and lodicules but not in vegetative organs (Itoh et al., 2002). These reports indicate that *OsGA3ox2* contributes to the GA biosynthesis for shoot elongation, whereas *OsGA3ox1* plays a role in reproductive organ development (Kawai et al., 2022).

Nine *GA2ox* genes (*GA2ox1, -2, -3, -4, -6, -7, -8, -9* and *-10*) have been identified in Arabidopsis, and their enzyme functions were confirmed through expression in *E. coli* (Rieu et al., 2008; Thomas et al., 1999; Yamaguchi, 2008). GA2ox acts on C19- GAs such as the active forms GA_4 and GA_1, as well as their precursors. In vivo evidence for their role in GA deactivation comes from studies where overexpression of these genes in Arabidopsis leads to dwarfism and reduced levels of bioactive GAs (Wang et al., 2004). Ten *GA2ox* genes (*GA2ox1- GA2ox10*) have been reported in rice (Sakamoto et al., 2001). Of these, overexpression of *GA2ox1* was shown to result in significant plant height reduction with no formation of seeds in rice (Sakamoto et al., 2001, 2003), while overexpression of *GA2ox6* or *GA2ox9* genes leads to moderate reduction of plant height with the formation of normal seeds (Lo et al., 2008). Collectively, these studies demonstrate the potential of overexpressing *GA2ox* genes to develop semidwarf lines with different levels of height reduction (Hedden, 2020).

In wheat, four homoeologous sets of *GA20ox* genes (*GA20ox1, -2, -3* and *-4*), two sets of *GA3ox* genes (*GA3ox2* and *GA3ox3*) and eight sets of GA2ox genes (GA2ox1, *-3, -4, -6, -7, -8, -9* and *-10*) have been identified (Pearce et al., 2015). In the leaf and stem tissues, the *TaGA20ox1* gene exhibits high level of expression during rapid growth phases of development whereas *TaGA20ox2* is highly expressed following the period of peak elongation in mature tissues. This implies that *TaGA20ox1* likely plays a more significant role in regulating growth rate of wheat shoots and therefore final organ size. Of the two *GA3ox* genes (*GA3ox2* and *GA3ox3*) reported in wheat, *TaGA3ox2* was found to have predominant expression in stems/internodes (Appleford et al., 2006). Among the GA catabolism genes, *TaGA2ox3, TaGA2ox4* and T*aGA2ox9* are found to be expressed in most of the vegetative tissues such as roots, shoots and spikes, respectively (Pearce et al., 2015). It has been reported that wheat reduced height alleles, *Rht18* and *Rht14*, show

increased expression level of *TaGA2oxA9* in stems and therefore reduced GA level, and loss-of-function *taga2ox9* mutants generated from Rht18 exhibit overgrowth phenotypes as compared to the tall parent of Rht18 due to an increase in GA level, indicating that this gene plays a key role in controlling stem height (Ford et al., 2018).

2.12 Gibberellin signaling

GA controls vital aspects of plant growth and development via interaction of its metabolism and signaling pathway. The majority of the components of the GA signaling pathway have been identified through genetic studies on rice and Arabidopsis. The GA-insensitive mutants exhibit a dwarf phenotype similar to the GA-deficient mutants, however, they are unable to respond to exogenous GA. Molecular characterization of GA response mutants led to the discovery of three major components of GA signaling, which includes the GA receptor (GID1), the F-box protein component of an E3 ubiquitin ligase that specifically targets DELLAs, another component of GA signaling that acts as a growth repressor (Davière and Achard, 2013; Harberd et al., 2009).

The GA signal is perceived by a soluble plasma membrane receptor protein, GA-INSENSITIVE DWARF1 (GID1), which was first discovered in rice (Ueguchi-Tanaka et al., 2005) Later, the Arabidopsis *GID1a*, *GID1b* and *GID1c* were identified as genes encoding GA-binding proteins that can rescue the GA-insensitive dwarf phenotype of *gid1* mutants of rice.

The DELLA proteins belong to the plant-specific GRAS family of putative transcription regulators that repress almost all known GA dependent processes, while bioactive GAs relieve their repressive activity. DELLA genes are categorized as negative regulators of GA signaling since gain-of function mutants of the DELLA genes exhibit dwarfism due to a decrease in GA signaling whereas the loss-of function mutations lead to increased GA signaling and thereby,

resulting in a tall or slender phenotype (Hauvermale et al., 2012). There are five DELLA proteins in Arabidopsis, which includes RGA, GAI, RGA-LIKE1 (RGL1), RGL2 and RGL3, whereas rice, barley and wheat each have only one DELLA protein, namely SLENDER RICE1 (SLR1), SLENDER1 (SLN1) and Reduced height-1 (Rht-1), respectively (Gao and Chu, 2020).

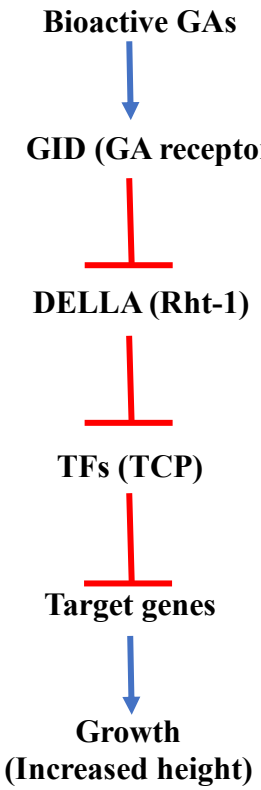

Figure 2.3. A simplified diagram of GA signalling pathway. GA, Gibberellin; DELLA, growth repressor DELLA proteins such as Rht-1 in wheat; TFs, Transcription factors such as TCP, TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTOR, that regulate plant height (Davière et al., 2014; Silverstone et al., 2001; Ikeda et al., 2001).

2.13 Use of plant growth regulators for lodging resistance

Plant growth regulators (PGRs) are organic compounds that promote, inhibit, or modify plant physiological process at low concentrations, and the term includes both naturally occurring and synthetic compounds or their chemical analogues (Basra, 2000). Plant growth retardants, also

known as plant growth inhibitors, constitute a significant group among these compounds. They represent a distinguished category of synthetic compounds that reduce stem elongation and generally enhance the green colour of the leaves (Gianfagna, 1995). They hinder the cell division in the subapical meristem of the shoot, but generally, have nominal impact on the leaf production or root growth (Gianfagna, 1995). Among the growth retardant PGRs, GA biosynthesis inhibitors form the most prominent group in terms of their commercial value and the acreage of crops on which they are applied (Rademacher, 2015).

2.14 Inhibitors of GA biosynthesis

GA biosynthesis inhibitors reduce the formation of bioactive GA and therefore affect many GA-regulated plant developmental processes such as inhibiting stem elongation and thereby resulting in reduced plant height (Rademacher and Brahm, 2010). These PGRs are therefore used to prevent lodging in cereal and other crops around the world (Rademacher, 2015).

The GA biosynthesis inhibitors can be categorized into four groups depending on the specific steps of GA biosynthesis they target, including quaternary ammonium compounds, compounds with an N-containing heterocycle, structural mimics of 2-oxoglutaric acid, and the 16, 17 dihydro-GAs.

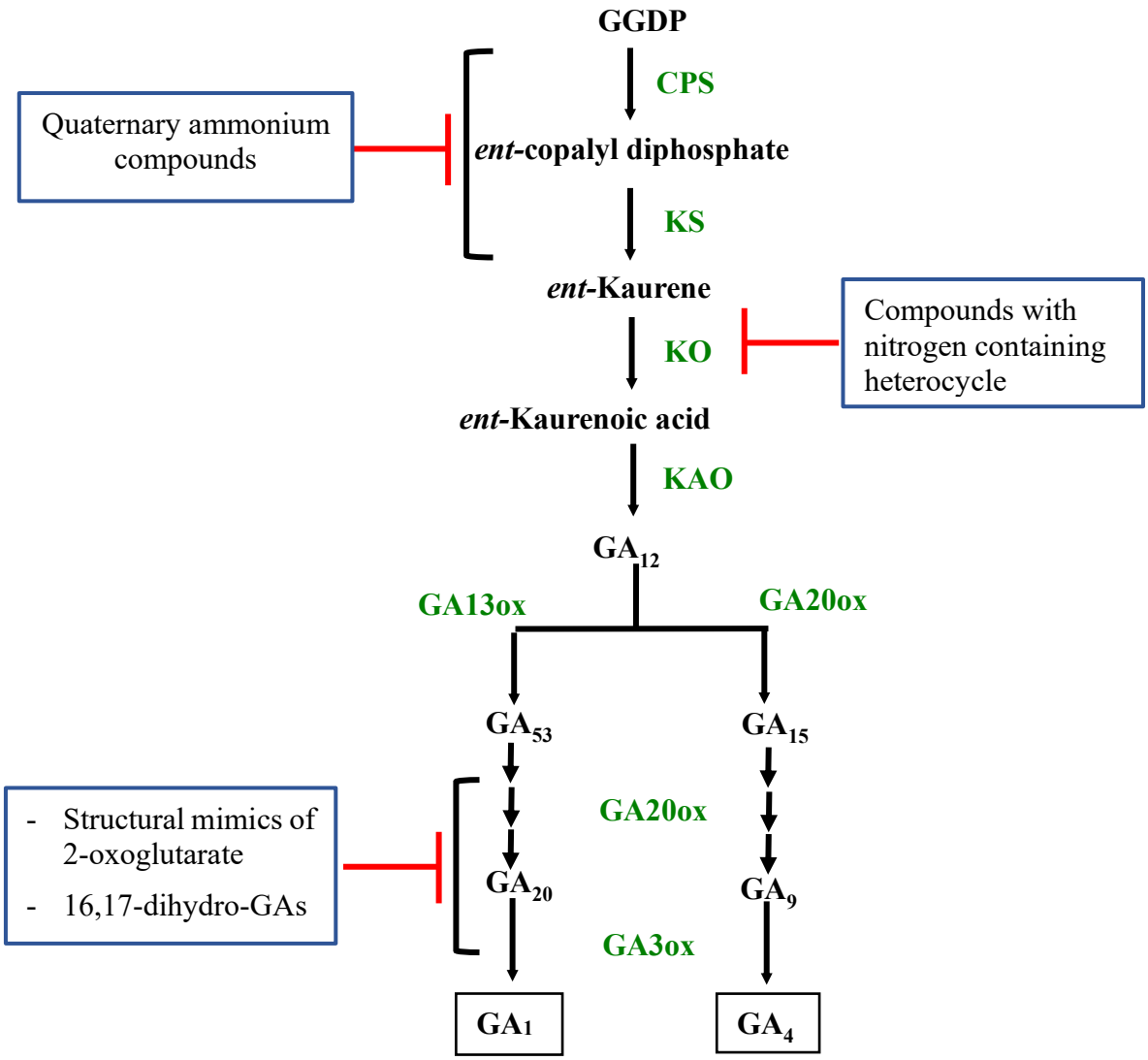

Figure 2.4. Inhibitors of GA biosynthesis and target enzymes (Rademacher, 2016). GA_1 and GA_4 are bioactive GAs. GGDP, geranylgeranyl diphosphate; CPS, *ent*-copalyl diphosphate synthase; KS, *ent*-kaurene synthase; KO, *ent*-kaurene oxidase; KAO, *ent*-kaurenoic acid oxidase; GA13ox, GA 13-oxidase; GA20ox, GA 20-oxidase; GA2ox, GA 2-oxidase and GA3ox, GA 3-oxidase.

2.14.1 Quaternary ammonium compounds

The first category of GA biosynthesis inhibitors are quaternary ammonium compounds that target the early stages of GA biosynthesis and thereby inhibit the activity of TPSs that catalyse the

formation of *ent*-kaurene. The quaternary ammonium compounds such as chlormequat chloride and mepiquat chloride are a representative group of onium-type compounds. Chlormequat chloride was first discovered in 1960 by a chemistry professor at Michigan State University, N.E. Tolbert, to produce plants with reduced height and thicker stems. The onium-type compounds consist of either a positively charged ammonium, phosphonium or sulphonium group and they block the biosynthesis of GAs directly before *ent*-kaurene (Rademacher, 2000). Chlormequat chloride along with related compounds inhibit the activity of copalyl-diphosphate synthase in both GA-producing fungus *G. fujikuroi* and higher plants. It also inhibits the activity of *ent*-kaurene synthase, although to a lesser extent (Shechter and West, 1969). It has been reported previously that chlormequat chloride decreases the levels of GA_1 in shoots as well as grains of wheat (Lenton et al., 1987). Similarly, it reduces the levels of all GAs in the later stages of GA biosynthesis including GA_{12}, GA_{53}, GA_{44}, GA_{19}, GA_{20}, GA_1, GA_8, in sorghum (Lee et al., 1998). The potential of this PGR as an anti-lodging agent in wheat was first recognized in Germany and Austria (Rademacher, 2015), and it is still the most commonly used PGR in cereals including wheat, rye, triticale, and oats. It is sold under the commercial name of Cycocel by BASF, which is its major producer and supplier (Rademacher, 2016). Mepiquat chloride is another widely used PGR following its introduction in 1979. It inhibits the biosynthesis of GA by blocking the conversion of GGDP to copalyl diphosphate and the formation of *ent*-kaurene (Rademacher, 2000). As a result, it decreases the level of GA produced and reduces the internode length and plant height and thereby lessens the risk of lodging and stabilises the stem, as reported in cotton (Wang et al., 2014).

2.14.2 Compounds with N-containing heterocycle

The second class of GA biosynthesis inhibitors represent compounds comprising a nitrogen-containing heterocycle, which block the enzymatic action of P450 monooxygenases that catalyse the oxidation reactions converting *ent*-kaurene to *ent*-kaurenoic acid (Rademacher, 2000). The biosynthetic steps after the formation of *ent*-kaurenoic acid, do not seem to be affected, despite the involvement of monooxygenase enzymes in catalysing those reactions. This group of chemicals includes pyrimidines, ancymidol and flurprimidol, which have major commercial application in ornamental plants; members of the triazole family such as tebuconazole and metconazole, which are used as fungicides as well as PGRs in crops; and paclobutrazol, unicazole and uniconazole-P that are known to control lodging in crops such as rice (Rademacher, 2015). The shared characteristic feature of all these inhibitors is the presence of a lone electron pair on the sp^2-hybridized nitrogen, which is situated at the periphery of the molecule in their heterocyclic ring. It is suggested that this electron pair likely displaces oxygen from its binding site to target monooxygenases with cytochrome P450 at the protoheme iron and inhibit their enzymatic action (Rademacher et al., 1987).

Ancymidol and flurprimidol are pyrimidine compounds that share a similar chemical structure, and they were brought to the market by Elanco Products, which later become a part of Dow AgroSciences in 1971 and 1989, respectively. They are used for inhibiting the growth rate of a wide range of mono- and dicotyledonous species, including turf grasses, ornamental cover plants, herbaceous and woody ornamentals, and a wide range of trees for gardens and parks (Rademacher, 2016). Paclobutrazol, uniconazole and uniconazole-P, which represent closely related members of triazole family are used to control the vegetative growth of avocadoes, mangoes, and litchis in countries with warmer climates (Rademacher, 2015). Paclobutrazol consists mainly of the (2RS,

3RS) diastereoisomer with (2S, 3S) and (2R, 3R)-enantiomers. Among these, (2S, 3S)-enantiomer is the more specific inhibitor of GA biosynthesis due to its structural similarity to *ent*-kaurene and thereby possess growth-regulatory activity (Sugavanam, 1984). On the other hand, the (2R, 3R)-enantiomer, which relates closely to lanosterol, is more active in inhibiting ergosterol biosynthesis (Burden et al., 1987; Rademacher, 2020). Thus, it is likely that these two enantiomers of paclobutrazol compete with the substrates of GA or sterol biosynthesis. The influences of plant growth retardants both on GA and sterol formation will affect the longitudinal shoot growth (Rademacher, 2020). Like paclobutrazol, the growth-retarding activity of uniconazole is widely linked to one enantiomer, however, the fungicidal or sterol-reducing effects have not been investigated (Sugavanam, 1984). Some triazole-type compounds such as tebuconazole and metconazole control shoot growth in oilseed rape by blocking GA biosynthesis, although they were first introduced as fungicides. In addition, metconazole is also applied as a PGR in ornamentals in Germany (Rademacher, 2016).

2.14.3 Structural mimics of 2-oxoglutaric acid

The third category of chemical inhibitors of GA biosynthesis are structural mimics of 2-oxoglutarate that acts as a co-substrate for oxidation of GA_{12} aldehyde by 2ODDs in the later stage of GA biosynthesis. This group of chemicals consist of particularly of acylcyclohexanediones, prohexadione-calcium and trinexapac-ethyl. A succinic acid derivative, daminozide, is also grouped under this category. These chemical inhibitors compete with the 2-oxoglutarate to block the subsequent oxidation of GA_{12} to biologically active GAs or their precursors (Sponsel, 1995). Acylcyclohexanediones seem to primarily target GA3ox, which is responsible for the conversion

of GA_9 or GA_{20} to biologically active GAs, as well as GA2ox, which converts biologically active GAs to inactive forms (Griggs et al., 1991).

Prohexadione-calcium was first marketed in 1994 as an anti-lodging agent of rice in Japan. BASF initiated commercialization of this compound in conjunction with mepiquat chloride as a stem stabilizer in cereals in 1998 (Rademacher, 2015). On the other hand, elevated doses of acylcyclohexanediones, prohexadione-calcium and trinexapac-ethyl also results in notable side-effects such as the inhibition of anthocyanin synthesis in various plant parts, including flowers by targeting 2-ODDs, particularly flavanone 3-hydroxylase, a key enzyme in anthocyanidin biosynthesis (Rademacher et al., 1992). Previously, daminozide was in use to reduce vegetative growth and improve colours and firmness of fruits in several fruit tree species such as apple. Additionally, it has been used to increase harvest efficiency in peanut by making them compact. Currently, the use of daminozide is limited to ornamental plants since its use on food crops was banned in 1989 due to toxicological concerns (Rademacher, 2015).

2.14.4 16, 17-dihydro-gibberellins

The fourth category of GA biosynthesis chemical inhibitors are 16, 17-dihydro-GAs, which represents the most recent group of GA-related growth retardants. They inhibit the activity of dioxygenases, which catalyse the late stages of GA metabolism, particularly GA3oxs (Rademacher, 2016). Applying 16,17-dihydro-GA5 to *Lolium temulentum* and sorghum plants induces changes in GA levels comparable to those triggered by acylcyclohexanediones (Foster et al., 1997; Junttila et al., 1997). In these studies, the levels of GA_1 were found to decline while that of GA_{20} increased (Rademacher, 2016). After systematic testing of several 16,17-dihydro-GA5 derivatives, exo-16,17-dihydro-GA5-13-acetate was identified as the most potent growth retardant

for graminaceous plants because of its highly effective competition with natural GA substrates in grasses. Despite this outcome, its synthesis in large quantities from GA_3 was deemed too costly for commercial purposes and it showed minimal effectiveness in reducing shoot growth in other plant species (Rademacher et al., 1999).

A systematic application of PGRs commenced in the 1930s, with the use of ethylene and acetylene in pineapple to induce flowering and fruit formation. Since then, various PGRs have been developed and used worldwide for different purposes in crop management (Radmacher, 2015). About one-fourth of the global sales of PGRs are represented by anti-lodging products. These products are commonly used in various countries including France, Germany, and the UK, for the cultivation of small grains cereals and oilseed rape (Radmacher, 2015). Application of PGRs to reduce lodging is highly prevalent in cereal crops. For example, in the year 2016, 90% of spring wheat (*Triticum aestivum*), 71% of winter rye (*Secale cereale*), and 83% of the winter barley (*Hordeum vulgare*) acreage in the UK was treated with anti-lodging products (Garthwaite et al., 2017). PGRs are also used on 84% of the winter wheat acres in UK, averaging 1.7 applications per year. This high usage of PGRs can be attributed to the wetter climate and longer growing season in the region that leads to severe lodging every three to four years with average yield losses of 25% (Strydhorst et al., 2018). In contrast, the usage of anti-lodging products for small grain cereals is minor in countries with relatively low crop production intensity. In the US, Canada, and Australia, in particular, the major restrictions for the use of PGRs include climatic factors, unfavorable temperatures as well as insufficient moisture (Radmacher, 2016).

While PGRs have been used in Canadian agriculture for several decades, their adoption has been influenced by various factors including environmental conditions, regulatory issues, and market access concerns (Strydhorst et al., 2018). Moddus (active ingredient: Trinexapac-ethyl),

Manipulator (active ingredient: chlormequat chloride) and Ethrel (active ingredient: ethephon) are the three PGRs currently registered in Canada for use to manage lodging in cereal crops (Alberta Grains, 2022). The ethylene-releasing agent, Ethrel is registered for use on wheat as it decreases plant height and increases stem wall thickness, but it may also increase tillering. The other two PGRs are GA biosynthesis inhibitors that reduce stem elongation, shorten the crop, and reduce lodging, and they are registered for use on spring wheat, winter wheat, barley and oats (Strydhorst et al., 2018). Ongoing research and development efforts aim to expand the use of PGRs in Canadian crop production, potentially leading to more widespread adoption in the future.

2.15 Plant growth regulators, nitrogen fertilization and lodging

The extent of lodging is strongly associated with culm morphological and anatomical traits, which can be influenced by application of growth inhibitors as well as N fertilization, among other factors. PGRs are often used to restrain elongation of internodes, improve lodging traits, and protect yield potential. Although N fertilization is required to ensure high yield, application of nitrogen at higher rates can increase plant height and decrease culm diameter and thickness, which results in weakening the basal internodes. A previous study investigated lodging behaviour and morphological traits in 12 spring wheat cultivars in response to different N rates and ethephon as a PGR (Tripathi et al., 2003). This study showed that ethephon application resulted in the reduction of plant height, peduncle length as well as length of the third internode while the number of tillers per m^2 and stem wall thickness were found to increase significantly. Moreover, the lodging angle and percentage increased with an increase in the level of N to up to 240 kg/ha; however, further increase in N level did not have effect on lodging severity (Tripathi et al., 2003). A study in winter wheat also demonstrated that exogenous application of a GA growth inhibitor, paclobutrazol,

significantly decreased lodging by altering plant height and physical strength of the basal part of the culm internode. This was primarily accomplished by altering the activity of lignin biosynthesis enzymes and lignin accumulation in the basal internode (Peng et al., 2014). A study on the effect of N on lodging resistance in winter wheat showed that basal internode characteristics including stem wall thickness, filling degree, lignin and cellulose content exhibit a positive correlation with culm lodging resistance index. Furthermore, as the level of N fertilization increases, for example to 270 kg/ha, basal internodes became slender and weak due to a decrease in the chemical composition and stem strength, which ultimately increases the risk of lodging (Zhang et al., 2017a). However, a recent study revealed that applying an optimal rate of N fertilization (~200 kg per ha) leads to significant increases in lignin accumulation, BS and culm lodging resistance index as compared to N rate of 270 kg/ha (Chen et al., 2018). Furthermore, Sun et al. (2021) found that N metabolism and N transporters are induced by treatment with bioactive GA but inhibited by treatment with prohexadione calcium, a GA biosynthesis inhibitor, suggesting that GA is required for N absorption and it promotes N metabolism. Consistent with this observation, recent studies reported that a decrease in the level of bioactive GAs and accumulation of DELLA proteins cause inhibition of N uptake (Hawkesford et al., 2014; Li et al., 2020). In addition, accumulation of DELLA proteins results in semi-dwarfism, which reduces the plant response to N and thus reducing the uptake of nitrogen. Despite these studies, there is still scarce information about the combined effects of N fertilization level and inhibition of GA biosynthesis on lodging in wheat.

2.16 Use of genetic approaches for lodging resistance

A major factor contributing to the occurrence of lodging in wheat plants is excessive plant height. As a result, reducing plant height has historically been the focus of research efforts to improve

lodging resistance (Peng et al, 2014). Several genetic studies have demonstrated that most of the semi-dwarf cultivars in wheat are due to mutations in GA metabolism and signaling genes (Sakamato et al., 2003, 2004). During Green Revolution, plant breeders enhanced lodging resistance and yield potential by integrating *Reduced height* (*Rht*) dwarfing genes from the Norin 10, a Japanese wheat variety, into spring wheat (Hedden, 2003). The introduction of gene not only reduced plant height, increased stem diameter and wall width that contributed to addressing the problem of lodging but also improved harvest index (Hedden, 2003; Wilhelm et al., 2013). To date, 23 *Rht* genes have been reported in wheat; however, few of these genes that reduce height without adverse effects on yield, are used by wheat breeders as most of them also have negative effect on grain yield (Tian et al., 2017). These are classified into GA-responsive and GA-insensitive categories reflecting the relative magnitude of their responses to exogenous GAs (Chen et al., 2015; Tian et al., 2017). The most prominent *Rht* genes are the two GA insensitive *Rht-1* homoeoloci, *Rht-B1* and *Rht-D1*, which have been employed in wheat breeding worldwide since the Green Revolution. These genes encode truncated DELLA proteins that are insensitive to GA, resulting in constitutive stem growth repression (Würschum et al., 2017). However, the GA insensitivity and reductions in cell size due to the *Rht-1* loci not only affect stem elongation/plant height, but also other GA-dependent developmental processes such as root elongation (Bai et al., 2013), early seedling vigor essential for seedling establishment and growth (Rebetzke et al., 2001), and coleoptile length that reduces the rate of seedling emergence, all of which can lead to reduction in yield (Rebetzke et al., 2014; Würschum et al., 2017). These limitations have led to the search for alternative dwarfing alleles that can reduce plant height without negatively impacting coleoptile length and seedling growth. Researchers have successfully identified several such alleles and made significant progress in understanding their mechanisms of action, aiming to

optimize plant height while maintaining early-stage growth (Pearce, 2021). For instance, *Rht12*, a dominant GA-responsive gene has a greater effect on reducing plant height than either of the Green Revolution genes (*Rht-B1b* and *Rht-D1b*), reducing wheat plant height by ~46% with fewer negative effects on yield (Sun et al., 2019). Furthermore, another GA-responsive dwarfing gene, *Rht18* was observed to decrease GA_1 level by increasing the expression of *TaGA2oxA9* gene which encodes GA2ox, a GA metabolic enzyme which decreases GA levels (Ford et al., 2018). Both *Rht12* and *Rht18* are unrelated to DELLAs and have potential for use in breeding programmes (Sun et al., 2019). Additional GA-responsive genes, *Rht24* and *Rht25*, were also identified in common wheat and should be evaluated for their potential as major height-reducing genes to improve wheat yield (Mo et al., 2018; Würschum et al., 2017). Cloning and functional characterization of additional *Rht* genes will enhance our understanding of the genetic mechanisms influencing plant height and discovery of novel genetic variations that help breeders make informed choices when selecting and combining alleles for crop improvement.

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3.0 LODGING-RELATED TRAITS IN WHEAT IN RESPONSE TO NITROGEN FERTILIZATION AND ALTERATION OF GIBBERELLIN LEVEL

Abstract

Lodging, defined as the permanent displacement of a plant from its upright position, is a major factor that affects wheat yield and quality worldwide. Lodging results in either the falling over of the plant or breakage of the stem due to coupling of several plant/genetic, agronomical and environmental factors. This research investigates the effect of nitrogen (N) fertilization rates as well as plant growth regulator (PGR) treatment on various lodging related morphological traits by using two spring wheat cultivars namely, Invader, a taller cultivar and Wildcat, a shorter cultivar. Our results indicate that elevated N rates resulted in 21% and 24% increase in plant height, 2.6- and 3.0-fold increase in bending moment and 1.8- and 2.7-fold increase in lodging index, in the taller and shorter cultivar, respectively. Furthermore, treatment of the taller cultivar plants with PGR led to the reduction of plant height by 10% to 22% irrespective of N fertilization rates. This decrease in plant height was associated with a reduction in bending moment by 12% to 37%, which led to a decrease in lodging index by ~23%. Furthermore, expression analysis of GA metabolic genes (*GA20ox, GA3ox, GA2ox*) in the second basal internodes of both cultivars under varying N rates (control, 180 kg/ha, 280 kg/ha) revealed differences in their expression patterns, explaining their roles in mediating variations in lodging-related traits. The expression level of GA biosynthesis genes (*GA20ox1, GA20ox3, GA20ox4, GA3ox3*) were upregulated at the 280 kg/ha N rate in the Wildcat while the expression of GA catabolic gene, *GA2ox3*, was downregulated in both cultivars at 180 kg/ha and 280 kg/ha N rates. These results provided valuable insights into the impact of genotypic variations, N fertilization and PGR treatment on lodging in wheat.

3.1 Introduction

Wheat (*Triticum aestivum* L.) is among the most important cereal crops worldwide, owing to its genetic variability and potential to adapt to a wide range of climatic conditions (Levy and Feldman, 2022; Shewry, 2009). Being the staple food crop for over two-third of the global population, its demand is increasing due to the surge in world population and impact of urbanization, necessitating an increase in its production (Igrejas et al., 2020). However, wheat yield and quality are limited by several biotic and abiotic factors as well as other factors such as lodging, which is defined as the state of permanent displacement of plant stems from their upright position as a result of either stem breakage or bending or leaning of straight and intact culms from the crown (Berry et al., 2003; Pinthus, 1974). Lodging in crops significantly impairs nutrient translocation and grain filling, while promoting plant susceptibility to disease due to elevated humidity levels. Consequently, it reduces grain yield and quality, potentially leading to considerable economic losses in crop production (Islam et al., 2007).

Lodging is complex phenomenon usually influenced by a range of morphological, anatomical and environmental factors along with the crop management practices. Plant height is one of the key morphological traits that plays a prominent role in determining the likelihood of lodging (Shah et al., 2017). The height of a plant is closely associated with lodging resistance throughout all developmental stages (Berry et al. 2003; Berry and Berry, 2015). While cultivars with tall stature are prone to lodging, shorter cultivars tend to have lower centre of gravity and self-weight movement of shoot that enables them to withstand lodging pressure (Berry et al. 2003; Okuno et al., 2014). Several studies in wheat have demonstrated the positive correlation of plant height with lodging index (LI) (Navabi et al., 2006; Verma et al., 2005). In addition to plant height, other morphological parameters such as stem strength affect lodging resistance (Wang et al.,

2006). Particularly, the basal part of the culm provides support as a lever to hold the plant upright, hence, its enhanced mechanical strength contributes to lodging resistance (Peng et al., 2014). Stem lodging in several cereal crops including wheat, barley (*Hordeum vulgare*) and oats (*Avena sativa*), has been reported to be caused by the breakage of one of the bottom two internodes, resulting in the fall over of the plant (Berry et al., 2004). Moreover, other studies have shown a negative correlation between length of the basal internodes and LI/lodging score (Huang et al., 2006; Kelbert et al., 2004), indicating that shorter basal internodes represent an ideal culm structure for lodging resistance (Peng et al., 2014; Tripathi et al., 2003). Lodging resistance is also influenced by mechanical strength of the culms which is determined by culm diameter and culm wall thickness (Berry et al., 2003). Accordingly, stem strength and lodging resistance in wheat have been shown to be closely associated with culm diameter, which in turn exhibits a positive correlation with culm wall thickness (Kelbert et al., 2004). Cell wall components such as lignin and cellulose also play significant role in providing mechanical strength to plant tissues and therefore lodging resistance. For example, a previous study in wheat showed a positive correlation between stem strength and lignin levels in the stem; lower lignin accumulation in the stem leads to increased vulnerability to lodging (Peng et al., 2014).

Crop management practices such as nitrogen (N) fertilization plays a critical role in lodging. While N is an essential nutrient that promotes plant growth and higher yields, its excessive application can increase the risk of lodging in plants. Elevated rate of N fertilization leads to the formation of dense crop canopy because of enhanced photosynthetic capacity, and this causes shading and therefore inhibition of light penetration at the base of the plant. This lack of exposure of the basal portion of the plant to sunlight results in reduced stem diameter, culm wall thickness, and lignin accumulation and increased elongation of the basal internodes, which increases the risk

of lodging, for example, in wheat (Luo et al., 2022) and Arabidopsis (Pantazopoulou et al., 2021). It has also been shown that wheat and rice (*Oryza sativa*) plants grown under high levels of N produce thinner stems and exhibit a higher LI (Tripathi et al., 2003; Yang et al., 2009). In addition, culm breakage at the lower basal internodes of wheat has been shown to be induced by high rates of N fertilization. On the other hand, N fertilization at low rates have been observed to decrease plant height, increase stem diameter and culm wall width, leading to improved stem strength in spring wheat (Berry et al., 2000).

The plant hormone gibberellin (GA) is a key factor in regulating plant height. Previous genetic studies have shown that loss of function of key GA biosynthesis and signaling genes as well as overexpression of GA catabolism genes lead to decrease in plant height (Cheng et al., 2019). The level of bioactive GAs in plants is determined by its biosynthesis, which is regulated by GA 20-oxidase (GA20ox) and GA 3-oxidase (GA3ox), and its catabolism, which is regulated by GA 2-oxidase (GA2ox) (Yamaguchi, 2008). In addition to its level, GA signaling also plays a role in the regulation of plant height. One of the major components of GA signaling is DELLA protein that acts as a negative regulator of plant GA response. During the Green Revolution, wheat breeders were able to produce semi-dwarf wheat cultivars with improved lodging resistance as well as an increased harvest index by introducing *Rht-B1b* and *Rht-D1b* genes, which encode GA-insensitive non-functional DELLA proteins thereby inhibiting the response of wheat plants to GA (Hedden, 2003; Wilhelm et al., 2013). While semi-dwarf genes have significantly improved lodging resistance in wheat, they have certain limitations as they impair seedling emergence in dry conditions, reduce crop adaptability in certain environments and pose potential yield constraints due to excessive plant shortening (Mangin et al., 2022; Zhang et al., 2017a). For example, the use of semi-dwarfing genes has been rendered insufficient to control lodging in the Canadian Prairies

due to rising occurrence of extreme weather events, coupled with the management for higher yield potential (Cogato et al., 2019; Mangin et al., 2022). Therefore, additional strategies such as the use of plant growth regulators (PGRs) along with optimized N fertilization can allow farmers to minimize the negative effects of lodging while maintaining high crop yield.

PGRs are natural or synthetic compounds that regulate various plant developmental events such as shoot elongation (Rademacher, 2015), and can be used as alternative approaches to enhance lodging resistance in cereals. Among the PGRs, plant growth retardants such as those that inhibit GA biosynthesis represent a diverse group of synthetic compounds that hinder plant growth without disrupting their developmental pattern (Rademacher, 2000; Rademacher, 2015). Application of GA biosynthesis inhibitors to plants reduces the synthesis of GA, and this negatively affects cell division and elongation, leading to reduction in plant height and increased lodging resistance (Rademacher and Brahm, 2010; Rademacher, 2015). Several studies have demonstrated the effect of GA biosynthesis inhibiting PGRs on lodging. For example, application of paclobutrazol, a GA biosynthesis inhibitor, to winter wheat plants significantly decreased the risk of lodging by altering plant height as well as physical strength of the basal internodes (Peng et al., 2014). Similarly, reduced plant height and LI were observed in common buckwheat treated with uniconazole, another GA biosynthesis inhibitor (Wang et al., 2015).

To better understand of the relationship between N fertilization and lodging in spring wheat, this study investigated various lodging-related morphological traits and expression patterns of GA metabolism genes in two cultivars that exhibit contrasting plant height under different N fertilization rates. The study also examined the effect of a PGR that inhibits GA biosynthesis on different morphological parameters that are associated with lodging in plants grown under different rates of N.

3.2 Materials and methods

3.2.1 Plant materials and experimental design

Two red spring wheat cultivars with contrasting plant height, namely Invader (tall) and Wildcat (dwarf), were used in this study. Field trials were conducted at the Point, University of Manitoba over two growing seasons (2022 and 2023). The experiments were laid out in a split-plot design with nine randomized blocks. A seeding rate of 100 seeds per meter was applied, resulting in 300 seeds being planted along each 3-meter row. Soil samples were tested prior to planting to determine residual N level, and additional N was applied in the form of granular urea at two rates. The main plots were total N levels, including control (residual N - 20 kg/ha), 180 kg/h and 280 kg/ha while the subplots were the two genotypes. During the 2023 field trial, PGR was applied as additional treatment in which plants were treated with a GA biosynthesis inhibitor, Chlormequat chloride (Manipulator 620) at the rate of 1.8 L/ha when the second node was visible, which represents growth stage 7 based on Feekes wheat growth scale.

3.2.2 Morphological parameters

Several morphological traits associated with lodging were recorded as described previously (Zhang et al., 2017a). In short, at the milk developmental stage, fifteen representative plants per treatment were collected to measure plant height, second internode length and diameter, culm breaking strength (BS), bending moment (BM), and lodging index (LI). Plant height was measured from the base of the plant to the tip of the spike excluding the awns. In addition, the length, diameter and fresh weight of the second basal internodes were recorded. The diameter of the

internodes was measured after removing the leaf sheath at the middle section of the internodes. BS of the second internode was measured with a plant stem strength tester (Model YYD-1; Graigar). BM was determined as a product of plant height from the base of second internode (cm) multiplied by fresh weight of the plant from the base of second internode to the tip. LI was calculated as a product of BM divided by BS (Ookawa & Ishihara, 1992). For gene expression analysis, second internode tissues were also harvested at milk developmental stage of the plant in liquid nitrogen and then immediately stored at -80°C until further use.

3.2.3 RNA extraction

Total RNA was isolated from the second basal internodes of main stems of both cultivars using TRIzol method as described previously (Nguyen et al., 2016). Approximately 100-150 mg of the tissues was ground into fine powder in a mortar and pestle using liquid nitrogen, which was then transferred to fresh sterile centrifuge tube, and then mixed with 1 ml of TRIzol reagent. After vortexing, the mixture was incubated at room temperature for 10 minutes followed by centrifugation at 12,000 g at 4°C for 5 minutes. The clear supernatant was then transferred to fresh tubes and incubated at room temperature for ~ 5 minutes. Afterwards, 0.2 ml of chloroform per 1 ml of TRIzol was added followed by another incubation at room temperature for 3 minutes and then centrifugation at 12,000 g at 4°C for 15 minutes. The supernatant containing the RNA was transferred to fresh sterile tubes. Subsequently, 0.5 ml isopropanol per 1 ml TRIzol was added to the aqueous phase, followed by incubation for 10 minutes at 4°C and centrifugation at 16,200 g for 10 minutes at 4°C. After discarding the supernatant, the RNA-containing pellet was washed with 1 ml of 75% ethanol, vortexed and centrifuged at 7,500g for 5 minutes at 4°C. After removing the supernatant, the pellet was air-dried at room temperature for approximately 5 minutes.

Subsequently, the RNA pellet was dissolved in 50 µl of diethyl pyrocarbonate (DEPC) water, followed by incubation at 55°C in a water bath for 10 minutes and then stored at -80°C until further use.

3.2.4 DNase treatment

The total RNA sample was digested with DNase (DNA-free kit; Ambion, Austin, TX) to eliminate genomic DNA contamination. To this end, 5 µL of 10X DNase I buffer (equivalent to 0.1 of the total RNA volume) and 1 µL of rDNase I enzyme were gently mixed with 50 µL of total RNA (10 µg) to achieve a final reaction volume of 56 µL. The mixture was then incubated at 37°C for 30 minutes with a gentle tapping every 10 minutes. Subsequently, 5 µL of DNase inactivation reagent was added and samples were incubated for 5 minutes at room temperature. After centrifugation at 10,000 g for 2 minutes, the supernatant containing RNA was transferred to a fresh sterile tube for further use. The integrity of the resulting RNA was verified by gel electrophoresis while the purity and concentration were determined using an epoch multi-sample spectrophotometer (Biotek Instruments, Winooski, VT, USA).

3.2.5 cDNA synthesis

The first strand cDNA was synthesised, using the iScript reverse transcription supermix (Bio-Rad, Hercules, CA, USA). The reaction mix consisted of 4 µL of 5X iScript reverse transcription supermix, 1 µg of total RNA, and nuclease-free water that brought the total reaction volume to 20 µl. The entire reaction mixture underwent incubation in a thermal cycler, which included priming for 5 minutes at 25°C, reverse transcription for 30 minutes at 42°C and inactivation of the reaction at 85°C for 5 min. Subsequently, the resulting cDNA was diluted 20X and stored at -20°C until employed for qPCR analysis.

3.2.6 Real-time qPCR assay

The quantitative PCR assays were conducted using the CFX96 real-time PCR system (Bio-Rad, Hercules, CA, USA). Each qPCR reaction mixture consisted of 5 µl of diluted cDNA, 1.25 µl forward primer (5 µM; final concentration 300 nM) and 1.25 µl reverse primer (5 µM; final concentration 300 nM) of each targeted gene, 2.5 µl nuclease-free water and 10 µl of Eva Green Supermix (BioRad, Hercules, CA, USA), making a total volume of 20 µl. The reaction mixtures were subjected to the following thermocycling conditions: initial denaturation and enzyme activation at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing at 60˚C for 30 seconds. The last step involved extension at 72˚C for 30 seconds. The relative transcript levels of each gene were calculated using the $2^{-\Delta\Delta}$Ct method as reported in Livak and Schmittgen (2001). The wheat *β*-Actin gene (*Taβactin*) was used as a reference gene for normalization. The primer sequences used for qPCR analysis of the target genes, including *TaGA20oxs, TaGA3oxs* and *TaGA2oxs* are shown in Appendix 1. These primer sequences have been previously reported by Izydorczyk et al. (2011) and Pearce et al. (2015).

3.2.7 Statistical analysis

Two-way analysis of variance (ANOVA) was performed to determine statistical significance among samples for the different parameters examined using least significant difference (LSD) test at $P<0.05$.

3.3 Results

3.3.1 Nitrogen treatment and plant morphological traits

This study examined the effect of different rates of N fertilization on lodging related morphological traits which includes plant height, fresh weight, BM, LI as well as length, diameter and BS of the second internodes.

3.3.1.1 Plant height, fresh weight and bending moment

An increase in the rate of N resulted in an increase in plant height in both cultivars (Figure 3.1A). The increase in the rate of N from the control (residual) rate to 280 kg/ha resulted in significant increase of plant height by about 21% and 24% in the taller cultivar, Invader, and shorter cultivar, Wildcat, respectively (Figure 3.1A). Plant height of Invader and Wildcat plants also increased as N rate increased from control to 180 kg/ha N rate by 8% and 17%, respectively, but the increases were found to be not significant. Overall, at each N rate considered in this study, Invader plants were, ~17% taller than Wildcat plants (Figure 3.1A).

Data pooled from the two growing seasons showed an increase in plant height by 19% in Invader and 14% in Wildcat at 280 kg/ha N rate compared to that observed at the control N rate (Appendix 2A). In addition, plant height in Invader increased by 11% at 180 kg/ha while that of Wildcat showed a non-significant increase of 8% at the same N rate. The pooled data also revealed that Invader plants are on average 15% taller than Wildcat plants at each N rate, and this result is consistent with that obtained from the first growing season (Appendix 2A).

A significant increase in plant fresh weight in response to the increased N rate was observed in Invader. Compared to that observed under control rate of N, plant fresh weight increased by about 29% in response to 180 kg/ha N rate and further increase of the N rate to 280

kg/ha resulted in ~51% increase in plant fresh weight (Figure 3.1B). Correspondingly, BM in Invader plants increased with an increase in N rate, exhibiting a 33% increase in response to 180 kg/ha N rate and 61% increase in response to 280 kg/ha N rate compared to the control N rate (Figure 3.1C). However, the increment in weight and BM at 180 kg/ha N rate were not statistically significant. For Wildcat, 28% and 35% increase in plant fresh weight was observed in response to 180 kg/ha and 280 kg/ha N rates, respectively, relative to that observed in response to the control rate of N (Figure 3.1B). However, the changes in plant fresh weight between the 180 kg/ha and 280 kg/ha N rates were found not to be statistically significant. Relative to that observed under the control N rate, the bending moment of Wildcat plants increased by 61% at 180 kg/ha N rate although not significant, while the 280 kg/ha N rate led to ~67% increase in BM (Figure 3.1C).

The fresh weight of Wildcat plants was higher (~11%) than that of Invader at control and 180 kg/ha. In contrast, the fresh weight of Invader plants was higher (14%) than that of Wildcat at 280 kg/ha N rate. However, there was no significant difference in fresh weight between the two cultivars at both 180 kg/ha and 280 kg/ha N rates. Invader plants had significantly higher (~31%) BM than Wildcat at 280 kg/ha N rate. No significant difference was observed between the two cultivars at the control and 180 kg/ha N rates.

The data pooled from both growing seasons also showed that the fresh weight of Invader and Wildcat plants increased by 34% and 21%, respectively, as the N rate increases from control to 180 kg/ha (Appendix 2B). Consistently, Invader and Wildcat plants exhibited 23% (non-significant) and 36% increases in BM at 180 kg/ha N rate, respectively (Appendix 2C). Furthermore, an increase in the N rate from control to 280 kg/ha resulted in 49% increase in plant fresh weight of Invader and a 16% (non-significant) increase in Wildcat, and this pattern of increase is in accordance with that observed in the first growing season (Appendix 2B). As a result,

the BM also increased by 46% in Invader and 36% in Wildcat in response to the 280 kg/ha N rate (Appendix 2C). Similar to the data obtained from the first growing season, data pooled from both growing seasons revealed that Wildcat plants have ~26% and 12% higher fresh weight than that of Invader at the control and 180 kg/ha N rates, whereas the fresh weight of Invader was 18% higher than that of Wildcat at 280 kg/ha N rate. However, there was no significant difference in fresh weight between the two cultivars at any of the three N rates (Appendix 2B). Additionally, Invader plants showed significantly higher BM than Wildcat, with a ~33% difference at 280 kg/ha N rate, while no difference was found between the two cultivars at the control and 180 kg/ha N rates, which is consistent with the data from the first growing season (Appendix 2C).

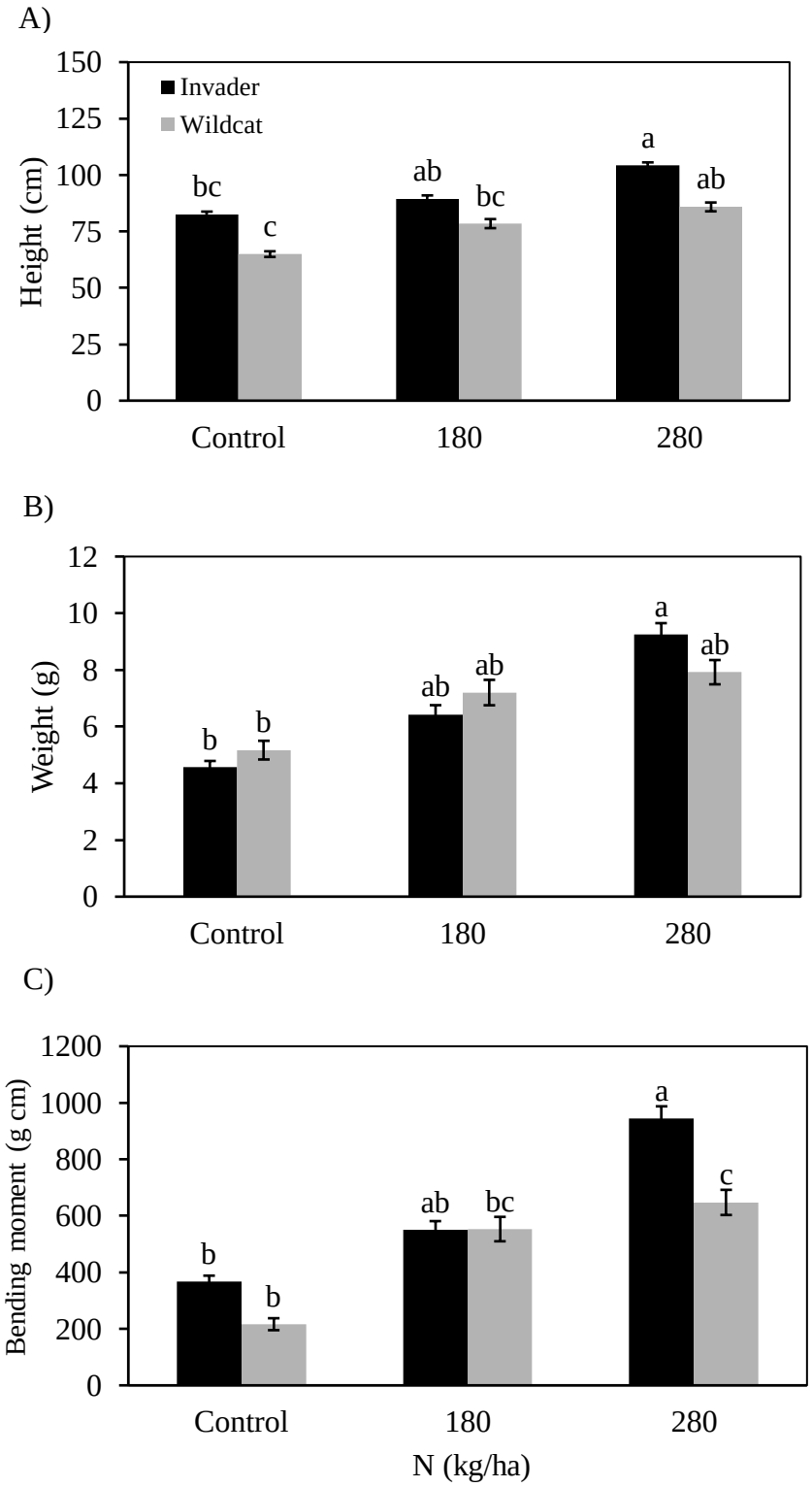

Figure 3.1. Plant height (A), fresh weight (B) and bending moment (C) of Invader and Wildcat plants at three nitrogen (N) rates (control, 180 kg/ha and 280 kg/ha). Data are means of 15 plants ± SE. Different letters indicate statistically significant differences among treatments at $P<0.05$ (LSD test).

3.3.1.2 Basal internode length, diameter and breaking strength

The second basal internode of Wildcat plants exhibited 19% and 24% increases as the rate of N increased from control to 180 kg/ha and 280 kg/ha, respectively (Figure 3.2A). Compared to those grown under control N rate, Invader plants grown under 180 kg/ha and 280 kg/ha N rates showed ~14% and 8% increases in length of second basal internode, although the increases were not statistically significant (Figure 3.2A). The second basal internode of Invader plants was longer than that of Wildcat plants at the control and 180 kg/ha N rates; Invader plants showed ~20% and 15% longer second internodes at the control and 180 kg/ha N rate, respectively (Figure 3.2A). There was only small difference(~2%) in second internode length between the two cultivars at 280 kg/ha N rate. The two cultivars showed no significant difference in the diameter and BS of the second basal internode regardless of the N rates (Figure 3.2B, -C).

Contrary to the first year data described above, length of the second basal internode pooled from the two growing seasons did not show any significant difference between the N rates in both cultivars (Appendix 3A). However, like that observed in the first growing season, the second internodes of Invader plants were significantly longer than that of Wildcat plants at control (by 17%) as well as 180 kg/ha N rate (by 22%). At the highest N rate of 280 kg/ha, the difference persisted but it was not statistically significant, with Invader plants having 15% longer second internodes than Wildcat plants (Appendix 3A). Although no significant changes in diameter of second basal internode were evident at higher N rates in Invader, a 13.5% increase was found in that of Wildcat at 280 kg/ha N rate (Appendix 3B). Similar to the first growing season data, there was no significant difference in BS of the second basal internodes pooled from the two growing seasons between the two cultivars regardless of the N rates (Appendix 3C).

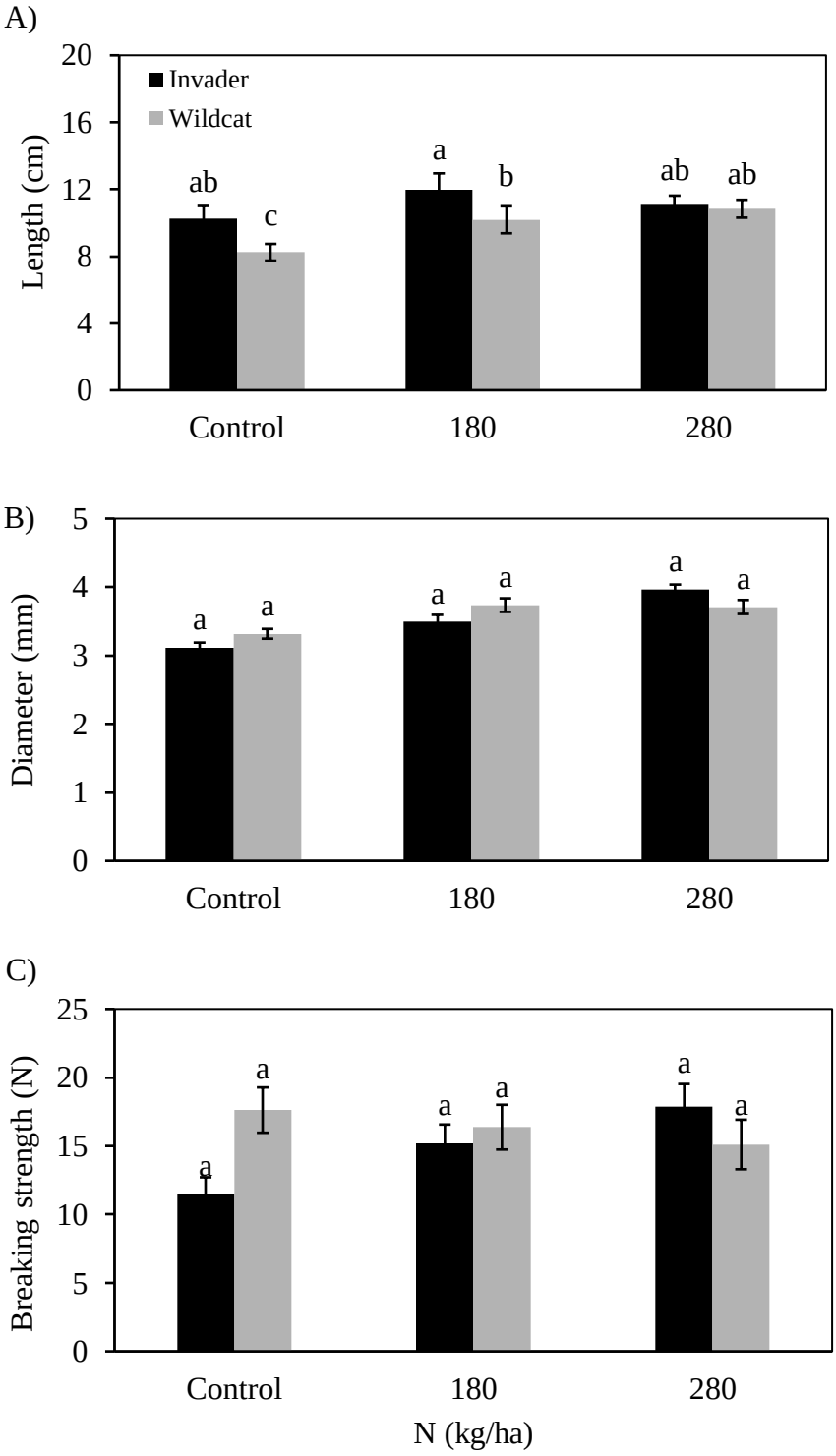

Figure 3.2. Length (A), diameter (B) and breaking strength (C) of the second basal internode of Invader and Wildcat plants at three nitrogen (N) rates (control, 180 kg/ha and 280 kg/ha). Data are means of 15 plants ± SE. Different letters indicate statistically significant differences among treatments at $P<0.05$ (LSD test).

3.3.1.3 Lodging index

Lodging index (LI) showed an increasing trend in response to an increase in N rates. Invader plants grown under the control N rate exhibited a lodging index of 3047.6 g cm/N. As the rate of N increased from control to 180 kg/ha, the LI of Invader plants increased to 4016 g cm/N. Compared to that observed under the control N rate, plants grown under 280 kg/ha N rate showed a LI of 5551.9 g cm/N, which represents a 45% increase in LI. In addition, ~28% increase in the lodging index occurred at 280 kg/ha N rate in comparison to the 180 kg/ha N rate, but this increase was not found to be statistically significant (Figure 3.3). In Wildcat, LI exhibited 64% increase, from 1924.7 g cm/N at the control N rate to 5271.5 g cm/N at 280 kg/ha N rate (Figure 3.3). Similarly, there was a 44% increase in the LI as N rate increased from control to 180 kg/h N rate, but this increase was found not to be significant. LI was further increased by ~34% (non-significant) when N rate was increased from the 180 kg/ha N rate to 280 kg/ha N rate. The LI of Invader plants was higher than that of Wildcat plants at the control (37%), 180 kg/ha (14%) and 280 kg/ha (5%) N rates, although the differences were not significant statistically.

Data pooled from the two growing seasons showed a significant increase in LI of both cultivars at 280 kg/ha N rate, which is consistent with LI data of the first growing season. LI increased by 32% in Invader and 34% in Wildcat due to an increase of N rate from the control to 280 kg/ha (Appendix 4). In addition, ~15% and ~17% increase in the LI occurred as N rate increased from the control to 180 kg/ha, but this increase was not statistically significant. Our data showed that the LI of Invader plants was, on average, 17% higher than that of Wildcat plants, irrespective of the N rate, however, this difference was also not statistically significant (Appendix 4).

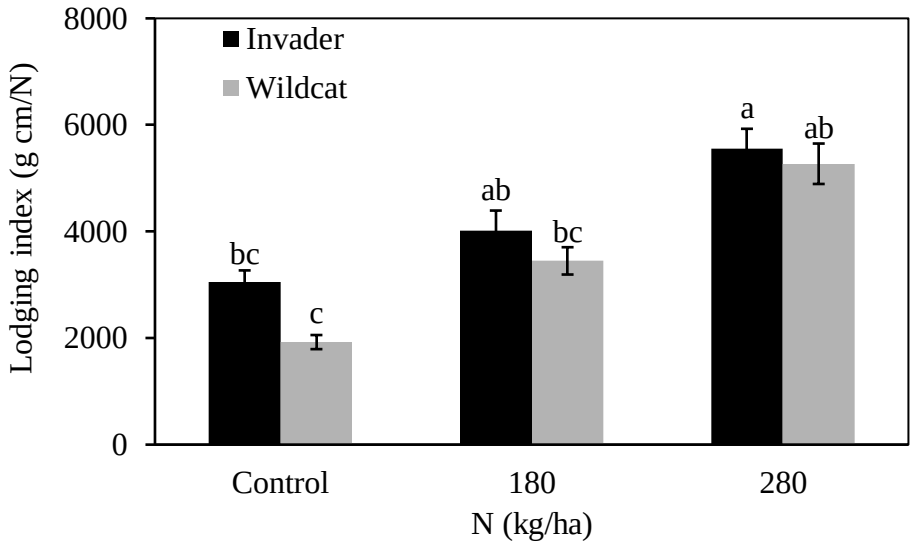

Figure 3.3. Lodging index of Invader and Wildcat plants grown at three nitrogen (N) rates (control, 180 kg/ha and 280 kg/ha). Data are means of 15 plants ± SE. Different letters indicate statistically significant differences among treatments at P<0.05 (LSD test).

3.3.2 Expression patterns of GA metabolism genes in the second basal internodes

In order to assess differences in GA biosynthesis and catabolism in the second basal internodes, the expression levels of GA biosynthetic (*TaGA20ox* and *TaGA3ox*) and catabolic (*TaGA2ox*) genes were examined at the milk stage of their development in the second internodes of Invader and Wildcat plants grown under three different N rates.

3.3.2.1 Expression patterns of *TaGA20ox* genes

This study examined the expression patterns of four *TaGA20ox* genes. The expression level of *TaGA20ox1* in the second internode of Invader exhibited a 4-fold decrease as the N rate increased from control to 180 kg/ha N rate (Figure 3.4A). Further increase of N rate from 180 kg/ha to 280 kg/ha resulted in an increase of its expression level (3-fold), which was still slightly lower (1.4-fold) than that observed in plants grown under control N rate. On the other hand, the second internode of the Wildcat plants showed no difference in expression level of *TaGA20ox1* between

the control and 180 kg/ha N rates. However, while not significant, a further increase in N rate to 280 kg/ha resulted in over 2-fold higher expression level of this gene than that observed in the second internode of plants grown under the control and 180 kg/ha N rates (Figure 3.4A). The expression level of *TaGA20ox1* in Invader was slightly higher than that of Wildcat at the control N rate. In contrast, there was over 2-fold higher expression level of *TaGA20ox1* in Wildcat plants at 180 kg/ha and 280 kg/ha N rates, although the difference was not statistically significant.

The expression level of *TaGA20ox2* in the second internodes of Invader plants was 3.7-fold higher at the 180 kg/ha level than the control N rate (Figure 3.4B). As the N rate increased from 180 kg/h to 280 kg/ha, its expression level decreased significantly (~2-fold) to a similar level observed in the second internode of plants grown under the control N rate. The second internode of Wildcat plants exhibited a similar level of *TaGA20ox2* expression regardless of the N rates. The expression level of *TaGA20ox2* was similar between the two cultivars at control and 280 kg/ha N rates, however, a ~4-fold higher expression of the same gene was observed in Invader plants at 180 kg/ha N rate (Figure 3.4B).

TaGA20ox3 showed the lowest level of expression as compared to the other *TaGA20ox* genes. The second internode of Invader plants grown under 180 kg/ha N rate exhibited a 4.6-fold decrease in *TaGA20ox3* expression level compared to those grown under the control N rate (Figure 3.4C). An increase of the N rate from 180 kg/ha to 280 kg/ha resulted in an increase (3.2-fold) of its expression to a similar level observed in plants grown under the control N rate. In Wildcat plants, no change in the expression level of *TaGA20ox3* in the second internode was observed due to an increase in N rate from control to 180 kg/ha. However, further increase in N rate from 180 kg/ha to 280 kg/ha caused a 2.6-fold increase in its expression compared to those grown under control N rate. An increase in N rate from 180 kg/ha to 280 kg/ha led to ~3-fold increase in

TaGA20ox3 expression. Invader plants exhibited higher (3-fold) expression level of the same gene than Wildcat plants at the control N rate, but both cultivars showed similar expression level at the other N rates.

Compared to the other *GA20ox* genes, *TaGA20ox4* showed the highest expression in the second internode. Compared to Invader plants grown under control N rate, it exhibited a lower expression level (2.6-fold) in the second internode of plants grown at 180 kg/ha N rate, and its expression level almost remained at similar level as the N rate increased from 180 kg/ha to 280 kg/ha (Figure 3.4D). The expression level of *TaGA20ox4* in the second internode of Wildcat increased (over 2-fold) as the rate of N increased from control to 180 kg/ha, and almost no change in its expression was observed due to increase of N rate from 180 kg/ha to 280 kg/ha. This gene had higher expression level in Wildcat at both 180 kg/ha (3.2-fold) and 280 kg/ha (2-fold) than Invader. On the contrary, Invader exhibited higher expression level (1.7-fold) than Wildcat at control N rate. The differences in expression level of *TaGA20ox4* were not significant with respect to N rate as well as cultivars.

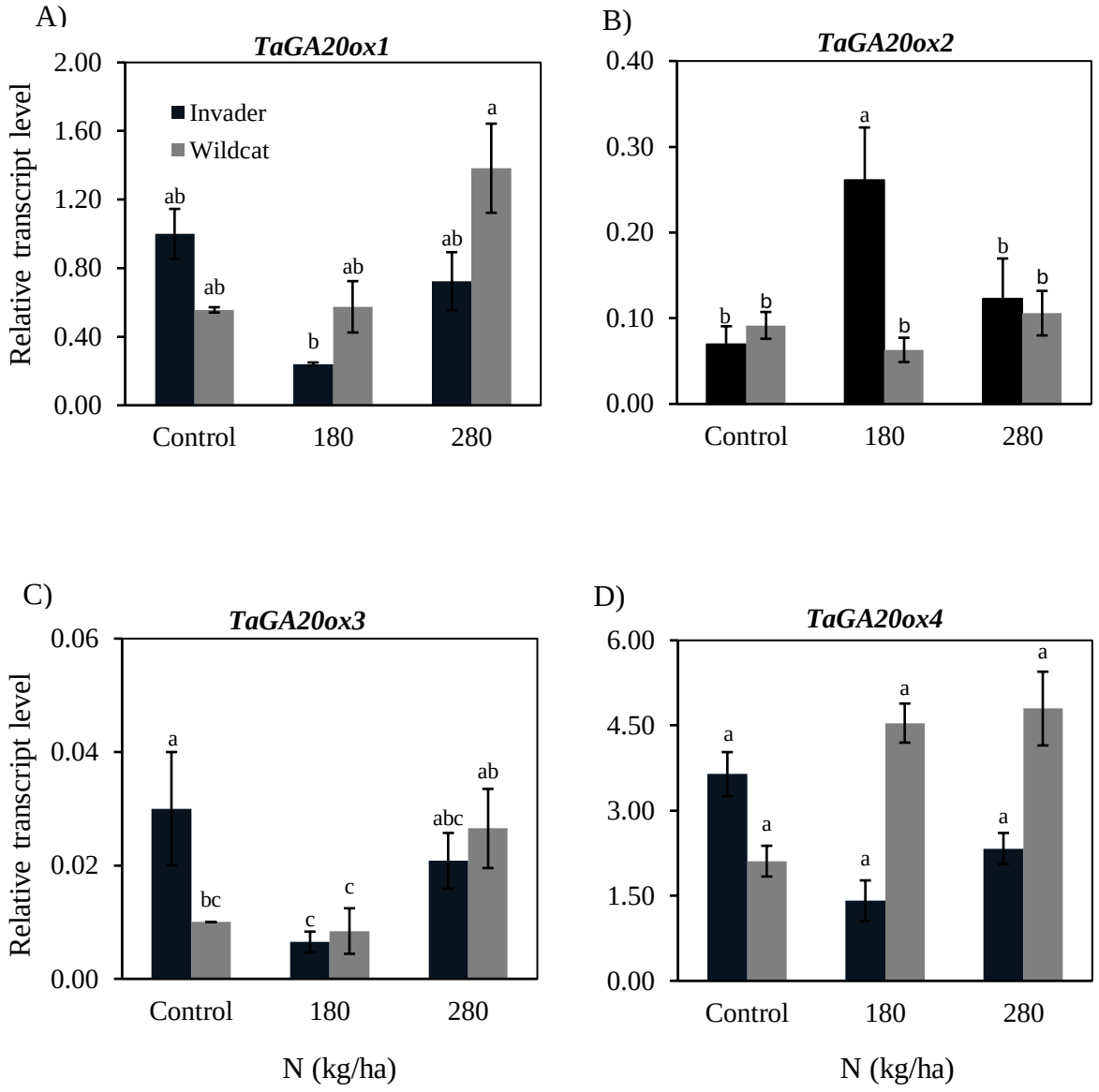

Figure 3.4. Expression levels of *TaGA20ox1* (A), *TaGA20ox2* (B), *TaGA20ox3* (C) and *TaGA20ox4* (D) in the second internodes of Invader and Wildcat plants grown under three nitrogen (N) rates (control, 180 kg/ha and 280 kg/ha). Transcript levels were expressed relative to that observed for *TaGA20ox1* in the second internode of Invader, which was set to a value of 1. Data are means of 2-3 biological replicates ± SE. Different letters indicate statistically significant differences among treatments at $P<0.05$ (LSD test).

3.3.2.2 Expression patterns of *TaGA3ox* genes

The expression level of *TaGA3ox2* in the second internode of Invader plants grown under 180 kg/ha N rate was higher (~4.2-fold) in comparison to those grown under the control N rate, but this was not significant statistically (Figure 3.5A). An increase of N rate from 180 kg/ha to 280 kg/ha resulted in a non-significant decrease (3-fold) of its expression. In Wildcat plants, no change in expression level of *TaGA3ox2* was observed between the second internodes of plants grown under control and 180 kg/ha N rates. However, an increase of N rate from 180 kg/ha to 280 kg/ha resulted in ~2.2-fold increase in its expression level although the increase was not statistically different. The highest difference in the expression level of *TaGA3ox2* between the two cultivars was detected at the 180 kg/ha N rate; Invader exhibited over 5-fold higher expression level than Wildcat. *TaGA3ox3* showed similar expression level in the second internodes of both cultivars at control and 180 kg/ha N rates. Its expression level, however, showed ~5-fold increase in Wildcat as the N rate increased from 180 kg/ha to 280 kg/ha N rate. *TaGA3ox3* exhibited higher expression in Wildcat than that of the Invader at 280 kg/ha N rate, while its expression level between the two cultivars was similar at the other N rates. (Figure 3.5B).

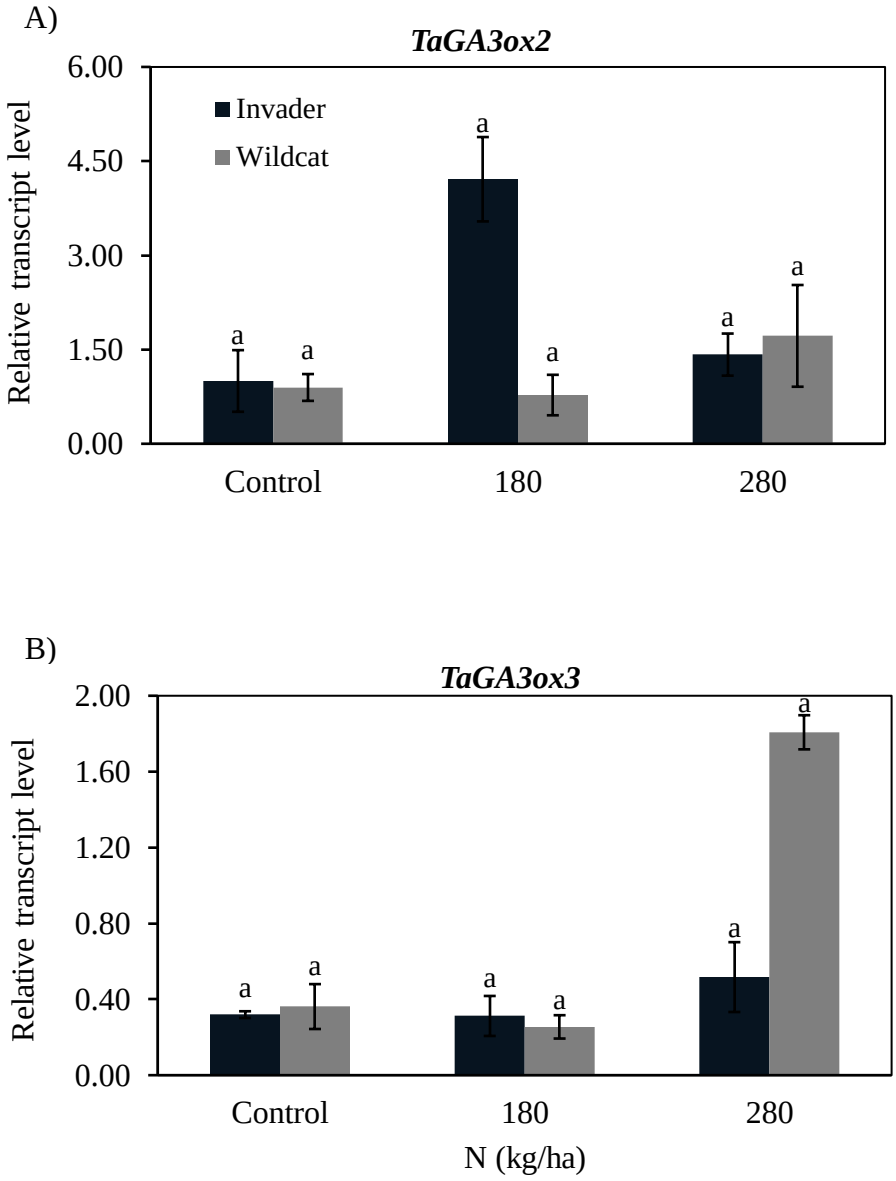

Figure 3.5. Expression levels of *TaGA3ox2* (A) and *TaGA3ox3* (B) in the internodes of Invader and Wildcat plants grown under three nitrogen (N) rates (control, 180 kg/ha and 280 kg/ha). Transcript levels were expressed relative to that observed for *TaGA3ox2* in the second internode of Invader, which was set to a value of 1. Data are means of 2-3 biological replicates ± SE. Different letters indicate statistically significant differences among treatments at $P<0.05$ (LSD test).

3.3.2.3 Expression patterns of *TaGA2ox* genes

The expression level of *TaGA2ox3* in the second internodes of both Invader and Wildcat showed over 2-fold decrease as the rate of N increased from control to 180 kg/ha. The expression level of this gene in both cultivars was further decreased (over 3-fold) at the 280 kg/ha N rate compared to the control N rate. No significant change in their expression level was observed in response to an increase of N rate from 180 kg/ha to 280 kg/ha. Furthermore, no difference in the expression level of *TaGA2ox3* in the second internodes was observed between the two cultivars at any of the N rates considered in the study (Figure 3.6A).

The expression level of *TaGA2ox6* in the second internodes of the two cultivars was similar regardless of the N rate (Figure 3.6B). Invader plants grown under 180 kg/h N rate exhibited over 7-fold increase in expression of *TaGA2ox9* in their second internode compared to those grown at control rate of N (Figure 3.6D). However, as the N rate increased from 180 kg/ha to 280 kg/ha, its expression showed a marked decrease (5.3-fold), resulting in a level similar to that observed at control N rate. Whereas in Wildcat plants, the expression level of this gene was nearly constant regardless of the N rate. The two cultivars showed significant difference in expression level of *TaGA2ox9* at 180 kg/ha N rate, with Invader exhibiting over 3-fold higher expression level than Wildcat. No significant difference was observed between the two cultivars at control and 280 kg/ha N rate (Figure 3.6D).

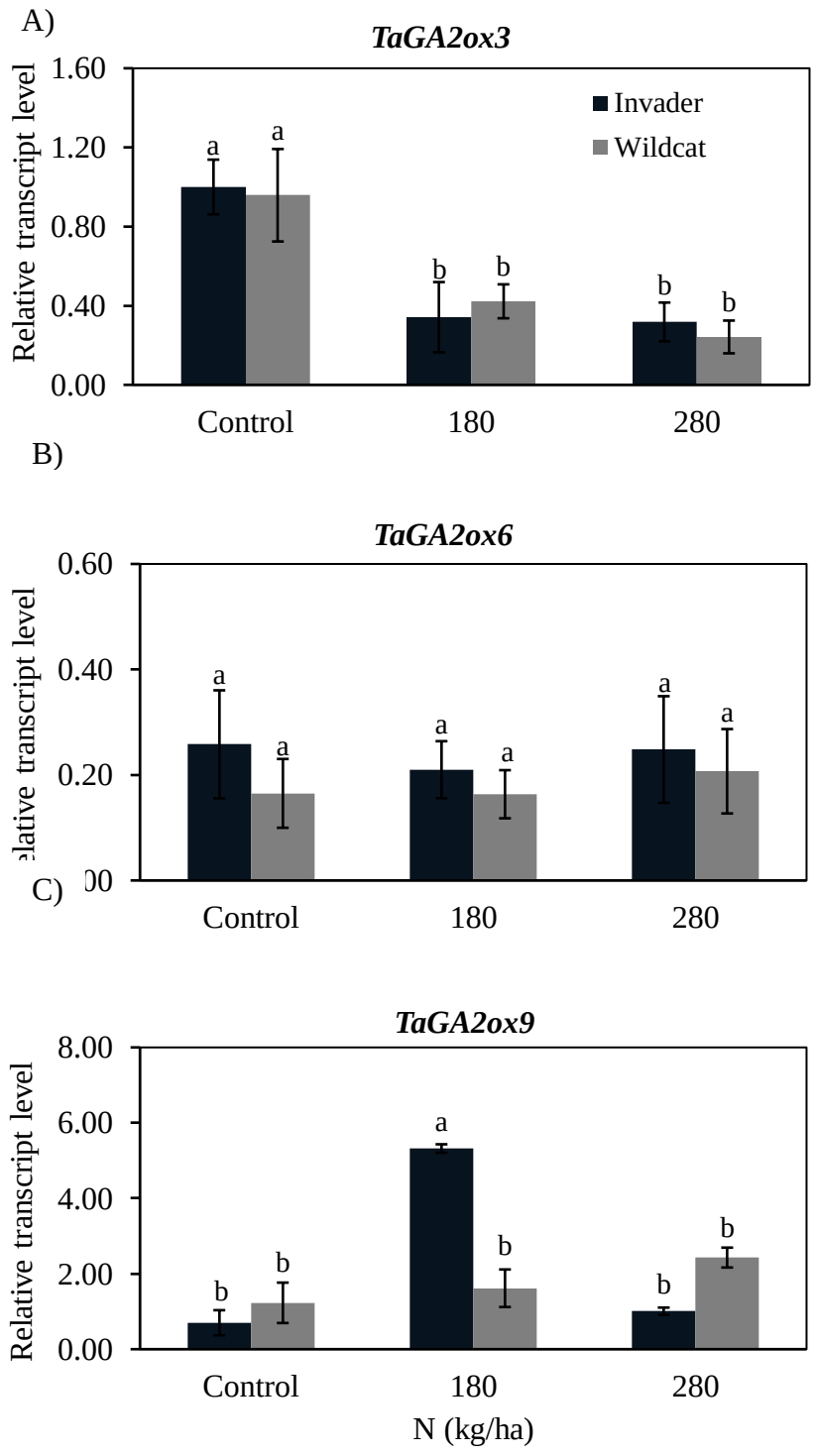

Figure 3.6. Expression levels of *TaGA2ox3* (A), *TaGA2ox6* (B) and *TaGA2ox9* (C) in the internodes of Invader and Wildcat plants grown at three nitrogen (N) rates (control, 180 kg/ha and 280 kg/ha). Data are means of 2-3 biological replicates ± SE. Different letters indicate statistically significant differences among treatments at $P<0.05$ (LSD test).

3.3.3 Interactive effects of plant growth regulator and nitrogen on morphological traits

This study also examined the effect of a GA biosynthesis inhibitor chemical (PGR) on lodging related morphological traits of plants of the two cultivars grown under different N rates.

3.3.3.1 Effect of PGR on plant height under different nitrogen rates

PGR untreated Invader plants exhibited 13.3% increase in plant height at 180 kg/ha N rate and an increase of N rate from 180 kg/ha to 280 kg/ha led to an additional ~4% increase in plant height (Figure 3.7A). Plants at both 180 kg/ha and 280 kg/ha N rates were significantly taller than those grown at control N rate. PGR treated Invader plants also showed significant increase of 23.4% and ~27% in plant height in response to 180 kg/ha and 280 kg/ha N rates, respectively, as compared to the control N rate. On the other hand, Wildcat plants did not show any significant difference in plant height irrespective of N rate and PGR treatment (Figure 3.7B).

Treatment of Invader plants with PGR significantly reduced plant height irrespective of N rate (Figure 3.7A). The PGR treatment resulted in 22% decrease in plant height of Invader plants grown under the control N rate, and 11% and over ~10% plant height reduction at 180 kg/ha and 280 kg/ha N rates (Figure 3.7A). The height of PGR-treated Wildcat plants was 8.4% shorter than that of untreated plants under control N rate. No plant height reduction due to PGR treatment was observed in Wildcat plants grown at 180 kg/ha and 280 kg/ha (Figure 3.7B).

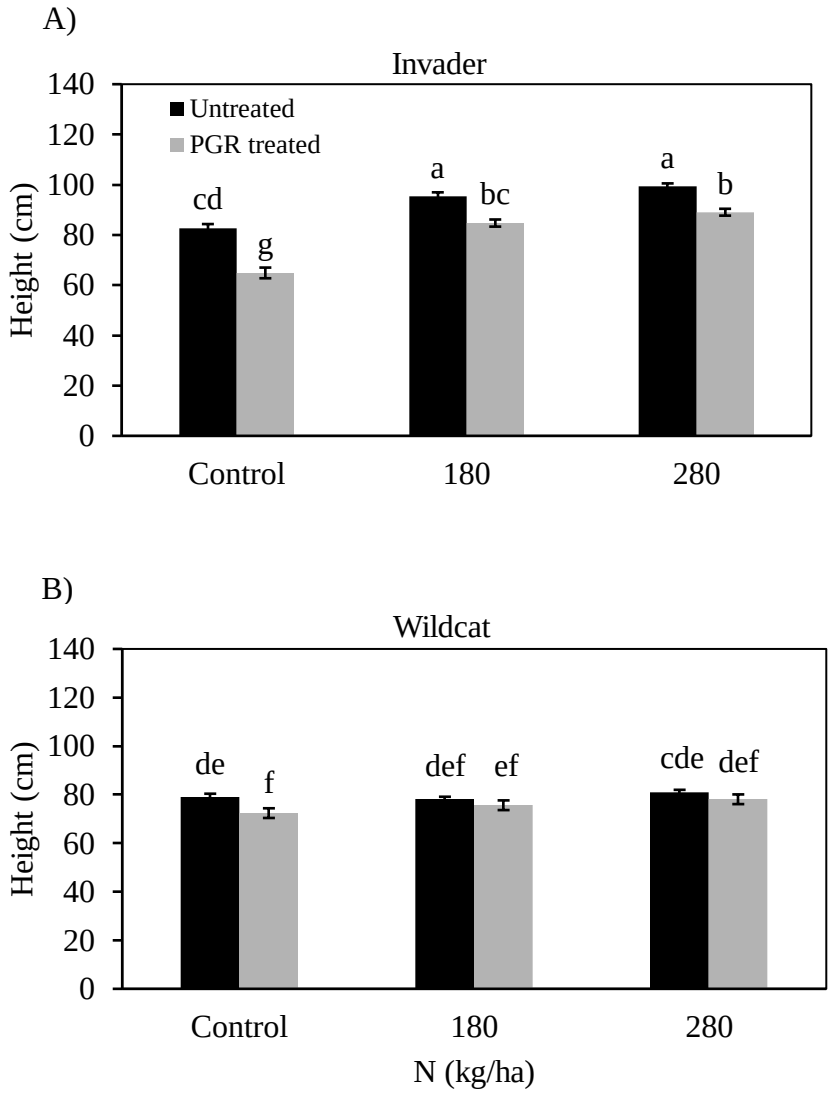

Figure 3.7. Plant height of PGR treated and untreated Invader (A) and Wildcat (B) plants grown under three nitrogen (N) rates (control, 180 kg/ha and 280 kg/ha). Data are means of 15 plants ± SE. Different letters indicate statistically significant differences among treatments at $P<0.05$ (LSD test).

3.3.3.2 Effect of PGR on plant fresh weight under different nitrogen rates

PGR untreated plants of both cultivars did not show any significant difference in fresh weight in response to elevated N rates except the 14% increase observed in Wildcat plants grown at 180 kg/ha (Figure 3.8B). Fresh weight of the PGR treated Invader plants increased by 28% at 180 kg/ha and ~18% at 280 kg/ha N rate compared to the control N rate, and an increase of N rate from 180

kg/ha to 280 kg/ha resulted in slight decrease in fresh weight (Figure 3.8A). Relative to that observed in plants grown under control N rate, fresh weight of PGR treated Wildcat plants also increased by ~14% at 180 kg/ha. However, no significant difference in fresh weight was evident as the N rate increased from 180 kg/ha and 280 kg/ha (Figure 3.8B).

Application of PGR significantly reduced fresh weight of Invader at control (20.7%) and 280 kg/ha (~16%) N rates while no difference was observed at 180 kg/ha N rate (Figure 3.8A). For Wildcat plants, no significant difference in plant fresh weight was found between PGR treated and untreated plants except the ~18% increase observed in PGR treated plants at the 280 kg/ha N rate (Figure 3.8B).

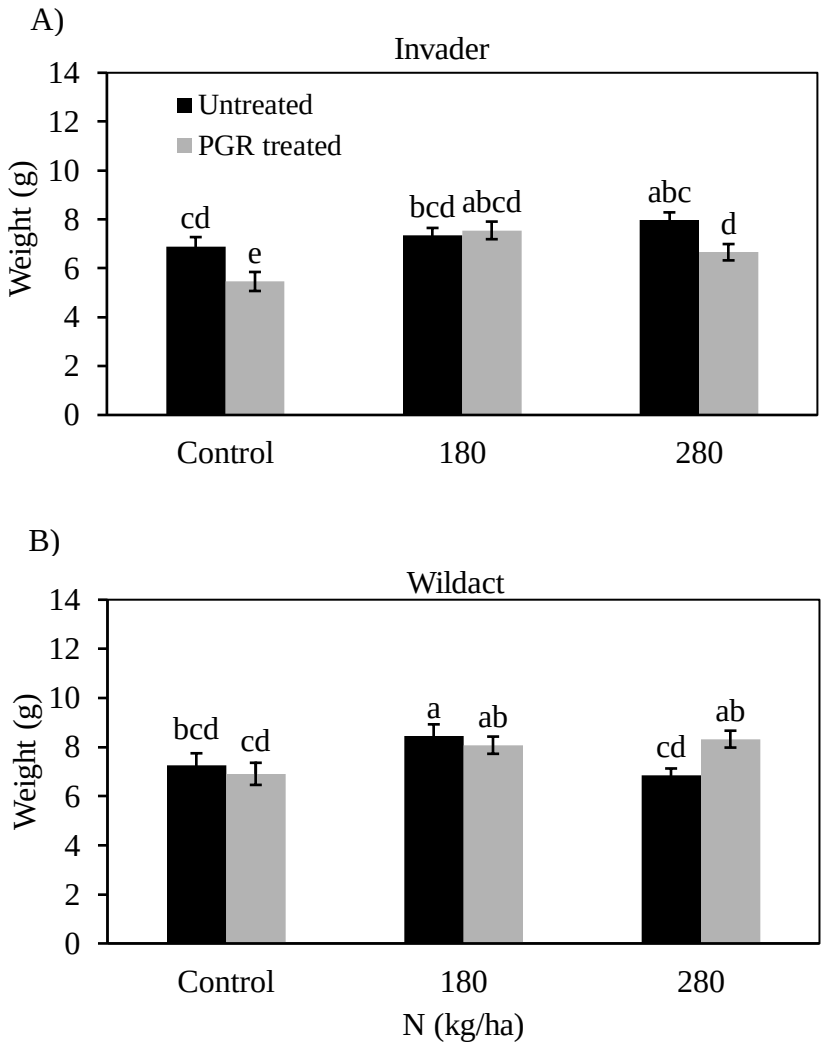

Figure 3.8. Plant fresh weight of PGR treated and untreated Invader (A) and Wildcat (B) plants grown under three nitrogen (N) rates (control, 180 kg/ha and 280 kg/ha). Data are means of 15 plants ± SE. Different letters indicate statistically significant differences among treatments at $P<0.05$ (LSD test).

3.3.3.3 Effect of PGR on bending moment under different nitrogen rates

The bending moment of the untreated Invader plants increased by 21% at 180 kg/ha and by ~28% at 280 kg/ha N rate compared to the control N rate (Figure 3.9A). The BM of Untreated Invader plants grown at both 180 kg/ha and 280 kg/ha N rates were significantly higher than those grown

at control N rate. The bend BM of PGR treated plants of the same cultivar significantly increased by ~43% and 39% in response to 180 kg/ha and 280 kg/ha N rate, respectively, as compared to the control N rate (Figure 3.9A). No significant difference in bending moment was observed between the 180 kg/ha and 280 kg/ha N rates. While the untreated plants of Wildcat showed no variation in BM in response to N rate, PGR treated plants of the same cultivar showed an increase in bending moment at both 180 kg/ha (17.2%) and 280 kg/ha (22.3%) N rates compared to the control N rate (Figure 3.9B).

Application of PGR significantly reduced bending moment of Invader at control (37%) and 280 kg/ha (26%) N rates. Only ~12% reduction in BM was observed at 180 kg/ha N rate, which was not statistically significant (Figure 3.9A). BM of Wildcat plants, did not show any change in response to PGR treatment at all N rates (Figure 3.9B).

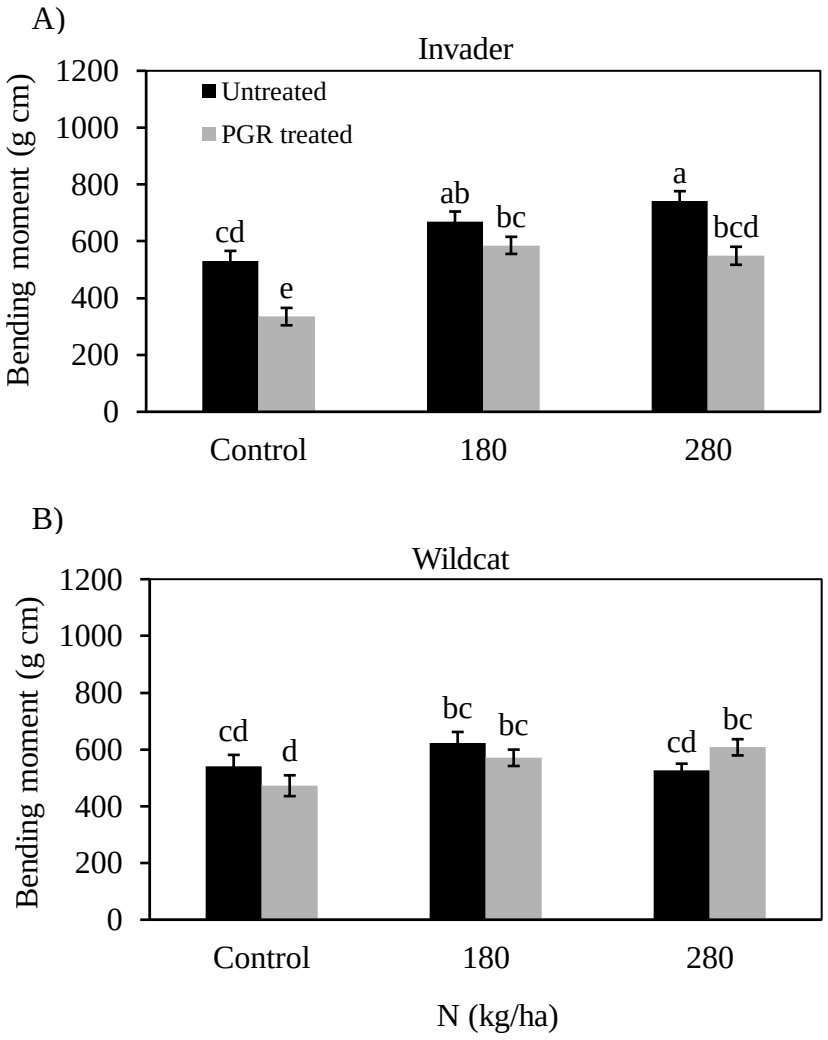

Figure 3.9. Bending moment of PGR treated and untreated Invader (A) and Wildcat (B) plants grown under three nitrogen (N) rates (control, 180 kg/ha and 280 kg/ha). Data are means of 15 plants ± SE. Different letters indicate statistically significant differences among treatments at $P<0.05$ (LSD test).

3.3.3.4 Effect of PGR on second internodes' length under different nitrogen rates

Length of the second internode of the untreated Invader plants did not show significant difference in response to the different N rates (Figure 3.10). Second internodes of PGR treated Invader plants were ~18% and ~34% longer at 180 kg/ha and 280 kg/ha N rate, respectively, compared to the control N rates (Figure 3.10A). No significant change in the length of second basal internodes of

Invader plants was observed in response to PGR treatment. Wildcat plants showed no change in the length of the second internode regardless of N rates and PGR treatment (Figure 3.10A).

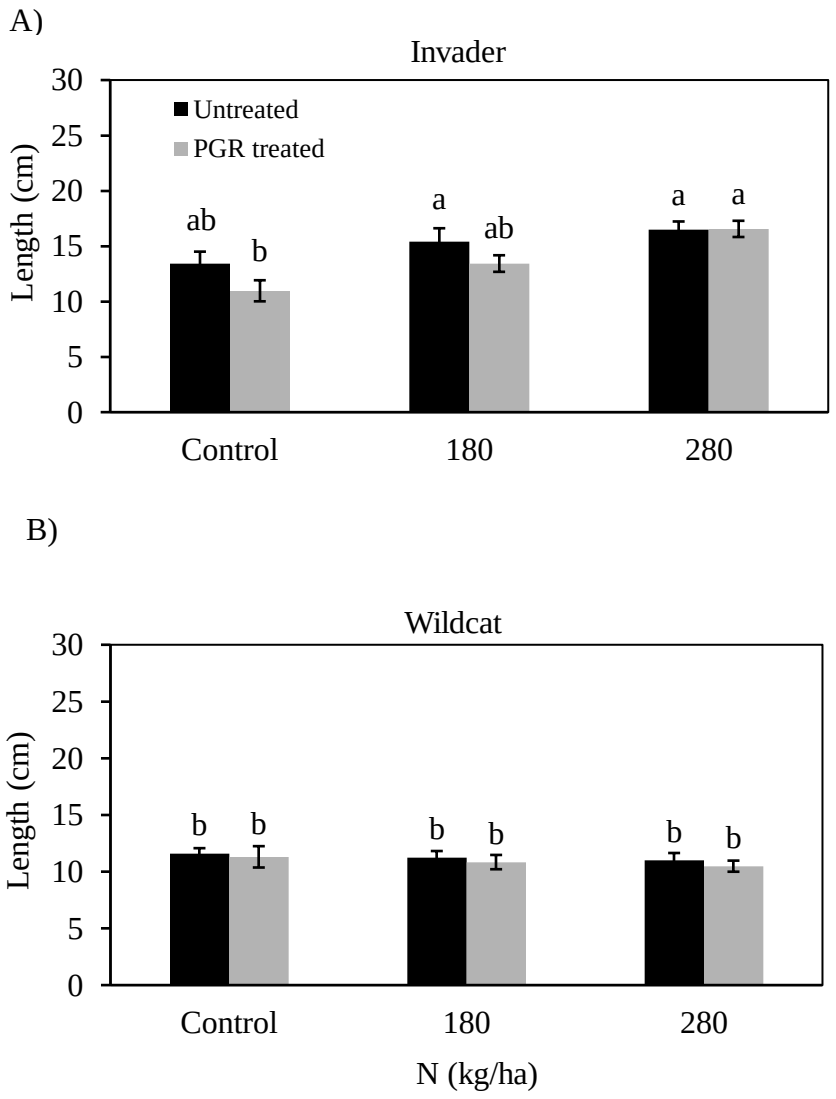

Figure 3.10. Second internode length of PGR treated and untreated Invader (A) and Wildcat (B) plants grown under three nitrogen (N) rates (control, 180 kg/ha and 280 kg/ha). Data are means of 15 plants ± SE. Different letters indicate statistically significant differences among treatments at $P<0.05$ (LSD test).

3.3.3.5 Effect of PGR on second internodes' diameter under different nitrogen rates

The diameter of the second basal internode of PGR untreated Invader plants did not show any significant difference in response to N rates (Figure 3.11A). However, PGR treated plants showed ~19% increase in diameter of the second internode at both 180 kg/ha and 280 kg/ha N rates compared to plants grown at the control N rate. PGR treatment did not affect the diameter of the second internode of Invader plants at 180 kg/ha and 280 kg/ha N rates although it caused a significant decrease (~13%) at the control N rate.

PGR untreated and treated Wildcats plants showed no significant difference in second basal internode diameter at all N rates. However, a slight (~9%) increase of internode diameter was observed at 280 kg/ha N rate due to PGR treatment (Figure 3.11B).

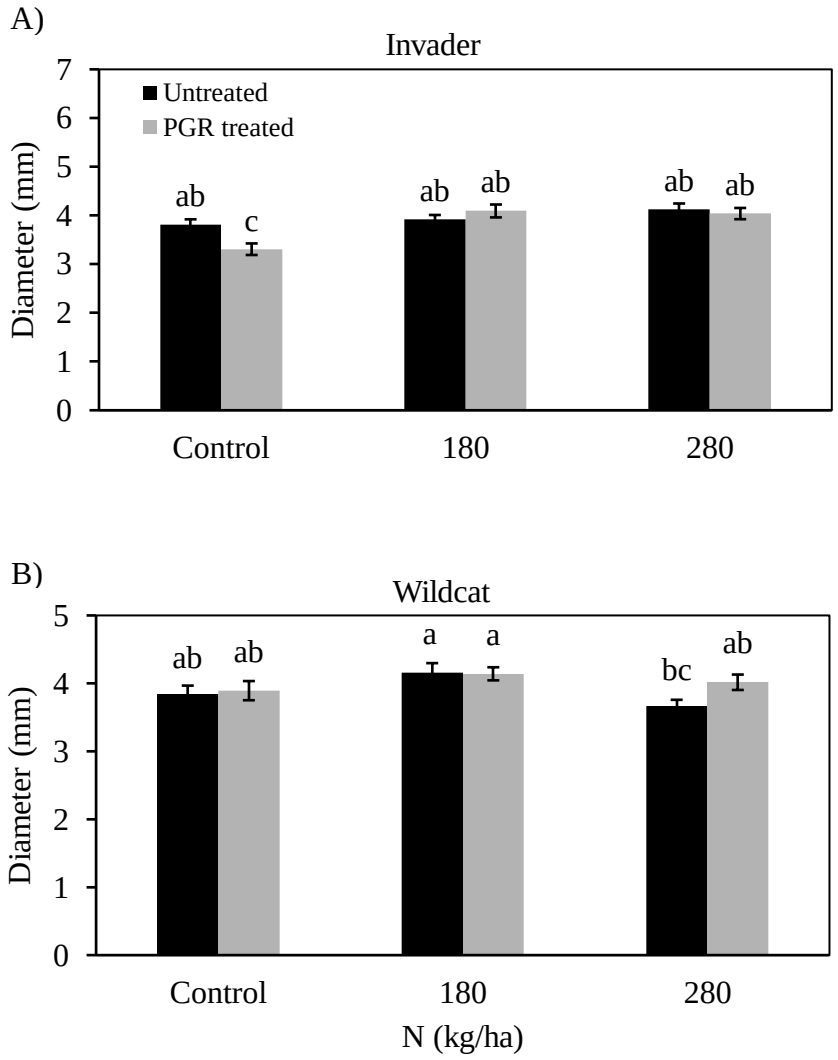

Figure 3.11. Second internode diameter of PGR treated and untreated Invader (A) and Wildcat (B) plants grown under three nitrogen (N) rates (control, 180 kg/ha and 280 kg/ha). Data are means of 15 plants ± SE. Different letters indicate statistically significant differences among treatments at $P<0.05$ (LSD test).

3.3.3.6 Effect of PGR on second internodes' breaking strength under different nitrogen rates

Internode BS of PGR treated Invader plants increased due to increase of N rate from control N rate to 180 kg/ha (23%) and 280 kg/ha (11%) N rates (Figure 3.12A). BS of Invader internodes derived from plants grown under the different N rates was not affected by PGR treatment .

The BS of PGR untreated Wildcat plants increased in response to 180 kg/ha (27%) and 280 kg/ha (~24%) N rates (Figure 3.12B). Similarly, the BS of PGR treated plants of the same cultivar showed an increase as N rate increased from control to 180 kg/ha (8%) and 280 kg/ha (22. Although not statistically significant, PGR treatment increased internode breaking strength of Wildcat plants at control (~18%) and at 280 kg/ha (16%) N rate while no PGR effect on BS was observed at 180 kg/ha N rate.

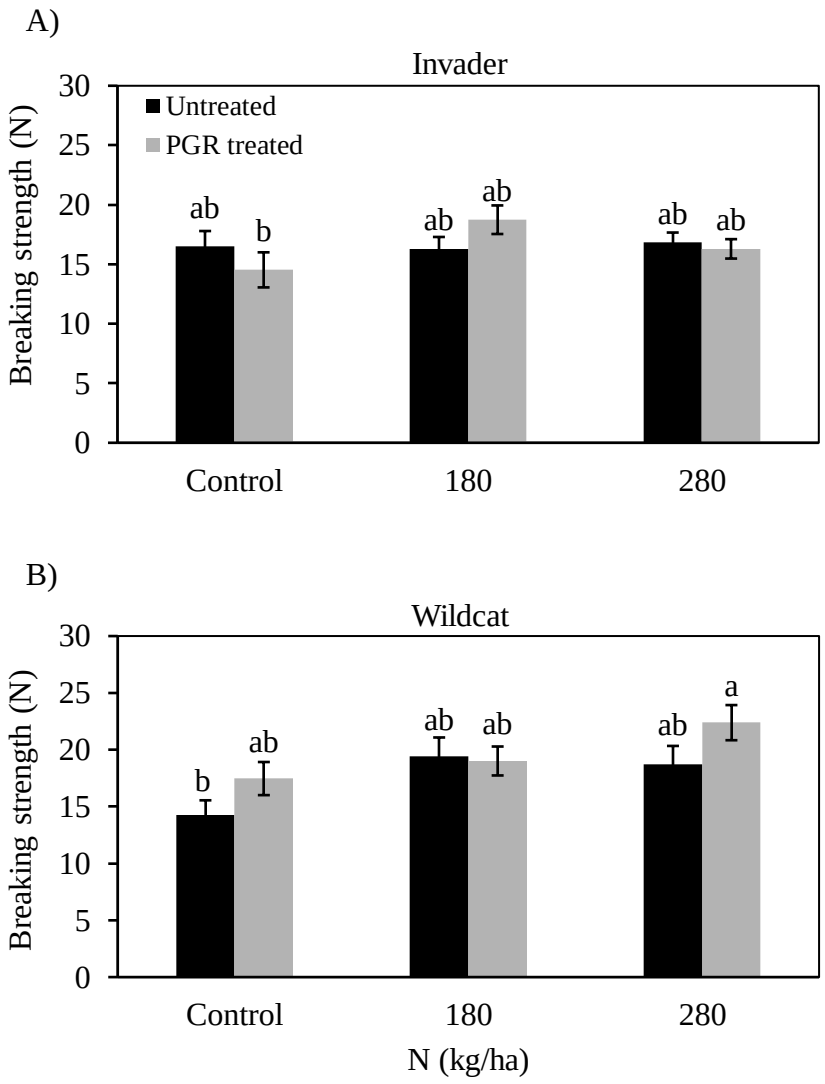

Figure 3.12. Breaking strength of PGR treated and untreated Invader (A) and Wildcat (B) plants grown under three nitrogen (N) rates (control, 180 kg/ha and 280 kg/ha). Data are means of 15 plants ± SE. Different letters indicate statistically significant differences among treatments at $P<0.05$ (LSD test).

3.3.3.7 Effect of PGR on lodging index under different nitrogen rates

The LI of the PGR untreated Invader plants increased by 14% and ~12% as the N rate increased from control to 180 kg/ha, and from 180 kg/ha to 280 kg/ha N rate (Figure 3.13A). The LI of PGR treated Invader plants also significantly increased by ~ 17% and ~25% in response to 180 kg/ha and 280 kg/ha N rate, respectively. On the other hand, the LI of PGR untreated Wildcat plants

decreased by 9% and ~13% as N rate increased from control to 180 kg/ha and 280 kg/ha N rate, respectively (Figure 3.13B). Whereas LI of PGR treated Wildcat plants showed no significant difference among the N rates.

Application of PGR significantly reduced the LI of Invader plants irrespective of the N rate (Figure 3.13A). PGR treatment of Invader plants decreased the lodging index from 3354.2 g cm/N to 2541.8 g cm/N (~24%), at control N rate; from 3886.8 g cm/N to 3077.1 g cm/N (~21%) at 180 kg/ha N rate, and from 4421.4 g cm/N to 3384.9 g cm/N (23%) at 280 kg/ha N rate. In the case of Wildcat plants, PGR application caused significant reduction (25%) in LI only at the control N rate, from 3702.6 g cm/N to 2771.2 g cm/N (Figure 13A). PGR treatment of the Wildcat plants decreased the LI only by 7%, from 3363.5 g cm/N to 3123.5 g cm/N, at 180 kg/ha N rate, and by 12%, from 3214.3 g cm/N to 2818.9 g cm/N, at 280 kg/ha N rate.

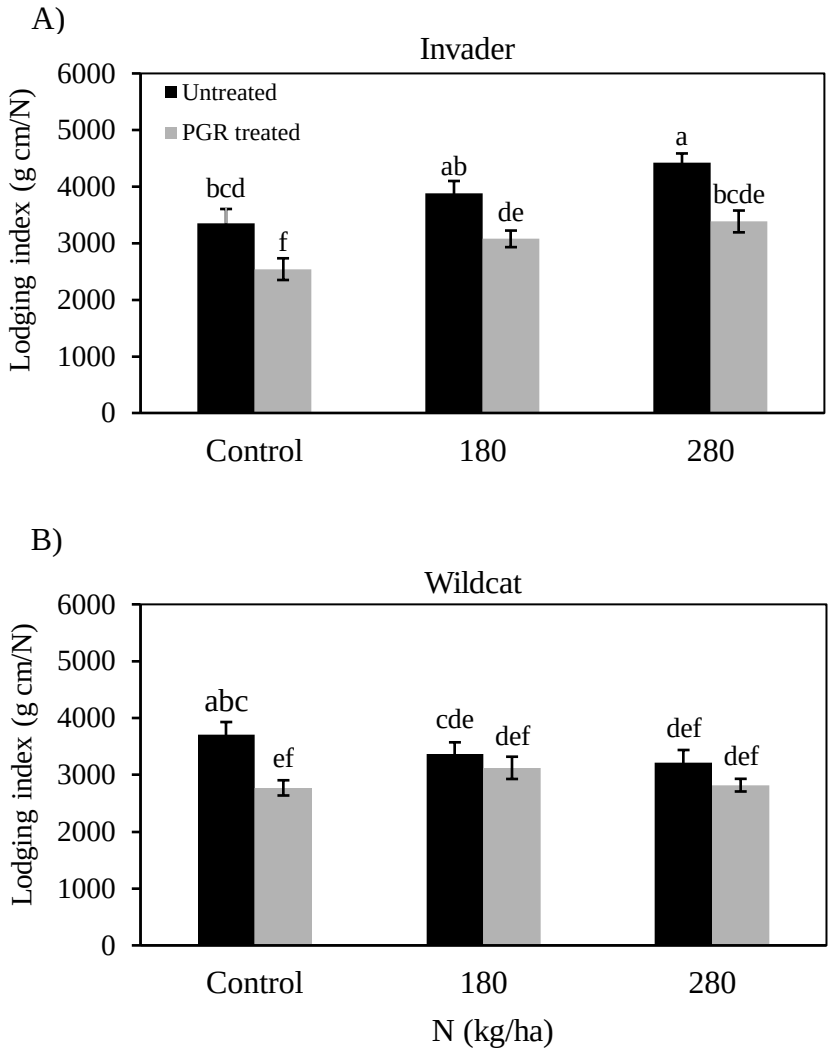

Figure 3.13. Lodging index of PGR treated and untreated Invader (A) and Wildcat (B) plants grown under three nitrogen (N) rates (control, 180 kg/ha and 280 kg/ha). Data are means of 15 plants ± SE. Different letters indicate statistically significant differences among treatments at $P<0.05$ (LSD test).

3.4 Discussion

Lodging is one of the major constraints that limits yield and quality of wheat. It is a complex phenomenon associated with various morphological traits, crop management practices and environmental conditions. This study was aimed to understand the effect of N fertilization as well as PGR treatment on lodging via investigating several lodging-related morphological traits in two spring wheat cultivars, namely Invader and Wildcat, with contrasting plant heights. In addition, the study performed comparative analysis of the expression patterns of GA metabolism genes in response to different rates of N fertilization.

This study revealed that N fertilization has a positive effect on plant height in both cultivars. Our data indicated that higher fertilization results in a significant increase in plant height in both cultivars, particularly at N rate of 280 kg/ha. This finding is consistent with previous studies that demonstrated that higher N levels stimulate stem elongation/plant height in cereal crops such as rice (Li et al., 2013; Wu et al., 2022; Yang et al., 2009; Zhang et al., 2013) and winter wheat (Kubar et al., 2022; Wang et al., 2012). The observed increase in plant height in response to increased N rate could be attributed to enhanced cell division and elongation induced by the increased dose of N. It has been reported previously that high levels of N increase stem length, thereby increasing the 'self-weight' moment transmitted into the ground that results in weakening of the stems (Crook and Ennos, 1995).

In addition to the stem elongation, nitrogen fertilization promotes vegetative growth, leading to biomass accumulation (Leghari et al., 2016). In the current study, plant fresh weight increased in response to N application and a more pronounced effect was evident in Invader at 280 kg/ha N rate. The increase in biomass due to an increase in N rate is consistent with the reports of Kashiwagi et al. (2010) and Li et al. (2022), who showed similar results in rice and winter wheat,

respectively. The increased fresh weight due to N application might be due to enhanced water and nutrient uptake aided by N, which supports vigorous growth and higher photosynthetic capacity. In agreement with this, N deficiency has been shown to affect canopy leaf area, protein and chlorophyl contents and thereby photosynthesis in wheat (Lawlor et al., 1989; Shangguan et al., 2000).

Lodging index (LI), a metric to evaluate lodging susceptibility, is considered as an indicator for assessment of the lodging resistance of crops. Higher LI values are associated with higher lodging risk in the crop (Ookawa and Ishibara, 1992). In this study, the LI was found to be higher in the plants grown at both moderate (180 kg/ha) and high (280 kg/ha) N rates in both cultivars. Similarly, previous studies showed that wheat (Tripathi et al., 2003) and rice (Yang et al., 2009, Zhang et al., 2016) genotypes provided with higher N levels exhibit high LI. In addition, Zhang et al. (2014) found that with increment in N from 0 to 300 kg/ha N rate, the LI of rice increased from 62.7% to 91.5%. They attributed the higher LI to higher plant height, fresh weight, dry matter of upper three leaves, and dry weight per unit length of the culm in response to higher N application. Since LI is calculated using BM and BS, our data suggests that either increased BM or/and decreased BS contributes to an increase in LI. Consistently, BM showed an increasing trend with an increase in N rate in both cultivars, suggesting that a higher rate of N, particularly the 280 kg/ha or more rate, leads to significantly higher BM, which reflects the contribution of increased plant height and fresh weight. It has been shown by several studies that excessive N increases plant height and weight of the upper portion of the plant that leads to rise in the potential for lodging as well as yield loss (Islam et al., 2007; Liu et al., 2015; Zhang et al., 2014). On the other hand, we observed no effect of N rate on another parameter that contributes to LI, which is BS of basal second internode. In contrast to this finding, high N rate has been reported to induce a

decrease in lodging resistance by weakening the physical strength of the lower internodes (Wu et al., 2012; Zhang et al., 2016). For example, high N rate has been shown to decrease BS of culms in japonica super rice (Zhang et al., 2014). The absence of effect of N on BS in the present study can be attributed to multiple factors such as genetic predisposition of the studied genotypes, role of other limiting factors associated with stem strength such as lignin, cellulose etc. as well as the influence of environmental conditions during the trial period.

Moreover, mechanical strength of the culm/stem is a very complex trait that is influenced by its morphological, chemical and anatomical traits. Morphological characteristics of the culm such as basal internode length and diameter, stem wall thickness, thickness of leaf sheath contribute to culm strength and lodging resistance (Islam et al., 2007; Muhammad et al., 2020; Zhu et al., 2008). These traits are further dependent on the accumulation of plant cell wall components, which are known as structural carbohydrates (SC), such as lignin, cellulose and hemicellulose that provide plant stem with mechanical support, elasticity and robustness (Niu et al., 2022; Raessler et al., 2010). In addition, non-structural carbohydrates (NSC) such as soluble sugars and starch, which are normally stored in the basal part of the culm, provide rigidity and breaking resistance to plants, enabling culms to recover more easily from damage induced by strong winds and stress conditions (Ishimaru et al., 2001; Kong et al., 2013; Peng et al., 2014).

Furthermore, the correlation analysis showed that LI was found to have high positive correlation with the BM ($r = 0.85$) while having very low correlation with BS ($r = -0.08$), indicating that plant height and fresh weight are the key factors determining LI in this study (Appendix 5). In support of this, both plant height ($r = 0.86$) and fresh weight ($r = 0.68$) showed a positive correlation with LI independent of N rate as well as cultivar, indicating the role of these traits in controlling LI under our study condition (Appendix 5).

It has been shown previously that stem lodging mainly occurs at the 2nd internode (Peng et al. 2014; Zhang et al. 2017a), indicating the importance of morphological traits of this basal internode for lodging resistance. Generally, culm breakage at the lower basal internodes of wheat is associated with high N rate (Kong et al., 2013). High rate of N enhances elongation of lower culm internodes and increases vegetative growth, leaf area index, biomass and tiller count, leading to the formation of a plant canopy that limits light interception to the base of plant and thereby inducing the development of thin and weak stems with decreased BS (Pinthus, 1974; Zhang et al., 2017a).

In our study, the length of second basal internodes increased at both 180 kg/ha and 280 kg/ha N rates in Wildcat plants, but no significant change in the second internode length was observed in Invader due to N fertilization. A previous study in winter wheat showed that higher N rates increase the length of the first and second basal internodes (Zhang et al., 2017a). While the diameter of the second internodes decreased with an increase of nitrogen level, the difference was not statistically significant. Several other studies reported that reduction of basal N rates reduced the length of the lower internode but increased its diameter, wall thickness and BS, and thereby reducing the LI (Lu et al., 2015; Wei et al., 2008). The ineffectiveness of N fertilization on the second internode traits (diameter and BS) can be attributed to the fact that culm characteristics can vary depending on genotype, timing of N application and environmental conditions irrespective of the rate.

Reduction of plant height has been the focus of research to improve lodging resistance (Berry and Spink, 2012; Peng et al., 2014). The plant hormone GA acts as a major regulator of internode elongation and plant height, and mutant plants defective in key GA metabolic and signaling genes generally exhibit dwarf or semi-dwarf phenotype (Cheng et al., 2019). Mutations

that compromise GA level and signaling were key in developing semi-dwarf and high yielding rice and wheat cultivars during the Green Revolution, which improved lodging resistance and productivity in both crops (Hedden, 2003). Consistently, treatment of Invader plants with chlormequat chloride (CCC; Manipulator), a PGR that inhibits the early steps of GA biosynthesis (Rademacher, 2000), at the elongation phase led to reduction of plant height and LI under all rates of N fertilization. Reduction in plant fresh weight and BM was observed at the control and 180 kg/ha N fertilization rates in response to the PGR treatment; however, this was not the case in Wildcat, the shorter cultivar. These findings suggest that the effect of PGR on lodging related traits varies with genotype regardless of N rates. In agreement with our results, application of another GA biosynthesis inhibitor, namely trinexapac-ethyl, as a PGR caused reduction in plant height in in both taller and shorter cultivars; however, the effect was more pronounced on taller than shorter cultivar (Spolidorio and Lollato, 2019). PGRs that inhibit GA biosynthesis have been shown to induce decreased cell division and elongation, which reduces plant height and improves stem mechanical strength, and thereby mitigate lodging (Bridgemohan and Bridgemohan, 2014). Previous studies in wheat have shown that the application of chlormequat chloride causes plant height reduction (Clark and Fedak, 1977; Espindula et al., 2009; Zhang et al., 2017a). Similar effects of this PGR were also reported in barley and oat, although the rate of reduction was species specific, with wheat showing the strongest response while oats were least responsive to chlormequat treatment (Clark and Fedak, 1977). Application of other GA biosynthesis inhibitor than chlormequat chloride, such as paclobutrazol also cause significant reduction in plant height, for example, in winter wheat (Peng et al., 2014).

Our data also showed that PGR application reduced the fresh weight of Invader plants at two of the N rates studied (control and 280 kg/ha). Similarly, application of trinexapac-ethyl, has

been reported to cause a significant decrease (23 to 27%) of above-ground biomass in wheat and barley (Rajala and Pelyonen-Sainio, 2001). In addition, tobacco plants expressing a *GA2ox* gene encoding GA inactivating enzyme exhibited reduction in stem growth and biomass accumulation compared to wild-type plants (Biemelt and Sonnewald, 2004). In contrast to these reports, Nagar et al. (2021) showed that application of paclobutrazol had a statistically non-significant effect on total biomass and plant height in all four wheat cultivars they tested under both field and controlled sowing conditions.

The BM of Invader, the taller cultivar, was found to reduce in response to PGR application at least at two of the N rates, control and 280 kg/ha. This reduction in the BM is due to the observed reduction in its plant height and fresh weight, factors that determine plant BM. Consistently, a significant reduction in LI was observed at all N rates, although no variation in BS, one of the determining factors of LI, was evident due to PGR application in either cultivar. These findings are in agreement with reports from previous studies. For example, uniconazole was reported to reduce the plant height, culm fresh weight as well as LI in common buckwheat (Wang et al., 2015). In addition, Peng et al. (2014) found that treatment with paclobutrazol leads to a considerable reduction in LI through decreased length of second basal internodes and plant height in winter wheat. In general, PGR treatment in cereals has been reported to cause reduced plant height and increased stem diameter (Crook and Ennos,1995; Tolbert, 1960). Although reduction in the length of third internode and increases in the diameter of both second and third internode in wheat were observed in response to ethephon, a PGR that releases ethylene (Tripathi et al., 2003), no effect of PGR treatment on the length, diameter and breaking strength of the second basal internode was evident in both cultivars we studied. This can be attributed to the possibility of compensatory roles by other growth hormones or culm structural components. In addition, extreme dry conditions

observed at the time of sowing might have resulted in limiting the internodal growth which resulted in non-significant effect of PGR application.

To gain insights into the transcriptional regulation of GA level in the basal internode of the two cultivars in response to N fertilization, this study examined the expression patterns of GA biosynthetic and catabolism genes in the second basal internodes of plants of both cultivars grown at the three N rates (control, 180 kg/ha and 280 kg/ha). In the present study, the expression levels of GA biosynthesis genes (*GA20ox1, GA20ox3, GA20ox4, GA3ox3*) were found to be upregulated at the highest N rate (280 kg/ha) in the second internodes of Wildcat; however it was not statistically significant. Upregulation of *GA20ox2* was also observed in Invader plants grown under 180 kg/ha nitrogen rate, suggesting an increase in GA biosynthesis. This is supported at least partly by the increases in plant height and fresh weight, which determine BM and LI, in plants grown under the same conditions. These results are in agreement with a previous study which demonstrated that nitrate promotes stem elongation and shoot branching in Arabidopsis and leaf sheath elongation in wheat in part through a DELLA-dependent mechanism. In response to nitrate supply, the synthesis and accumulation of bioactive GAs in the first internodes of wheat increased, particularly through its influence on the final stages of GA biosynthesis (Camut et al., 2021). In addition, the treatment of pea seedlings with N containing compounds such as potassium nitrate (KNO_3) and nitric oxide (NO) was shown to increase the expression level of GA biosynthesis genes and bioactive GA level, and the biomass of pea seedlings, indicating the positive influence of N treatment on GA synthesis and overall growth (Vidal et al., 2018). Moreover, Bethke et al. (2007) demonstrated a connection between NO and GAs in Arabidopsis, highlighting that NO promotes the transcription of GA biosynthetic genes, *GA3ox1* and *GA3ox2*, which in turn leads to the production of bioactive GAs. Similarly, downregulation of *GA20ox1* and *GA20ox4*, was

observed in the roots of maize under low nitrogen conditions (Wang et al., 2020), suggesting reduction in N fertilization leads to a decrease in bioactive GA levels. It has been reported that treatment of a medicinal plant, *Dendrobium officinale,* with calcium nitrate increases nitrate concentration in the tips of young stems, and this effect was associated with upregulation of key GA biosynthesis genes, *GA20ox*s and *GA3ox1*, leading to increased production of bioactive GA_1 and GA_4 and promotion of stem elongation (Du et al., 2023).

In the current study, the expression of GA catabolic gene, *GA2ox3* was downregulated in the second internode of Invader and Wildcat plants at 180 kg/ha and/or 280 kg/ha N rates, suggesting a decrease in the catabolism of bioactive GAs and therefore an increase in bioactive GA level. Therefore, the increases in plant height and fresh weight observed under elevated N rates may imply an increase in GA level. A previous study also showed enhanced expression of *GA2ox1*, *GA2ox5*, *GA2ox6*, *GA2ox7* and *GA2ox12* genes and thereby reduced bioactive GA level in maize roots grown under low N conditions (Wang et al., 2020).

4.0 GENERAL DISCUSSION AND CONCLUSION

Wheat is one of the most widely cultivated commercial crop. It serves as a staple food for over two-third of the global population owing to its potential to make versatile food products and a shift in the dietary habits that has resulted in higher consumption of wheat. The demand for wheat is increasing worldwide due to an increase in global population, entailing the need to increase its production. However, the production of wheat is limited by several factors including lodging, which is the state of permanent displacement of plants from their vertical position due to a combination of genetic factors that influence its morphological and anatomical traits as well as environmental and crop management factors. Most of the plant morphological traits that influence lodging, such as plant height, are regulated by the plant hormone gibberellin (GA). Thus, it is crucial to have a better understanding of the role of GA biosynthesis inhibiting plant growth regulators (PGRs) in reducing the risk of lodging by controlling the lodging-related traits. In addition, crop management/agronomical practices such as nitrogen (N) fertilization can enhance the lodging risk when applied at high rates. This thesis project therefore conducted a field trial to understand the effect of N fertilization rates as well as PGR treatment on several lodging-related morphological traits in two spring wheat cultivars, namely Invader and Wildcat, with contrasting plant height, and characterized the expression patterns of GA metabolism genes in the second basal internodes of the two cultivars in response to N fertilization rates.

Nitrogen, being an essential nutrient for plants, plays a critical role in plant growth and development. Our results indicated that high N fertilization rates positively influence plant height and fresh weight in both cultivars. The increased availability of N likely contributes to enhanced cell division and enlargement, and improved photosynthetic capacity that supports vigorous growth. The increase in plant height and fresh weight in response to high N rates were effective in

increasing the bending moment (BM) in both cultivars, thereby, resulting in enhanced lodging index (LI), which reflects higher lodging risk. Furthermore, an increase in the length of second basal internode was observed in response to the high N rate in Wildcat plants, but not in Invader plants, which can be due to the genetic differences between the cultivars that might influence nitrogen uptake and assimilation, and photosynthetic efficiency and thereby development of plant internodes. On the other hand, the N fertilization rate was found to have no significant effect on the diameter and breaking strength (BS) of the second basal internode of both cultivars. This might be due to the inherent genetic characteristics of the cultivars under study, the presence of limiting nutrients other than N that have an effect on these culm traits, vegetative growth of other plant morphological parts at the expense of these traits in response to N or the role of environmental factors.

GA biosynthesis inhibitors reduce the level of GA and thereby inhibit plant developmental processes regulated by GA such as stem elongation. Accordingly, application of a GA biosynthesis inhibiting PGR resulted in the reduction of plant height, fresh weight and BM of Invader plants at all N rates except for no decrease in fresh weight and BM was found at 180 kg/ha N rate. In agreement with these results, the LI of Invader plants decreased in response to PGR treatment regardless of the N rate. No significant effect of PGR on all morphological traits related to lodging was observed in the Wildcat plants irrespective of the N rates, highlighting the influence of genotypic differences in their response to GA level. Moreover, PGR treatment did not have effect on the length, diameter and BS of the second basal internodes in either cultivar. This may be due to the compensatory role of other plant hormones or contribution from stem structural components such as vascular tissues, parenchyma, sclerenchyma, and cell wall components including lignin, cellulose and hemicellulose. Alternatively, the dry conditions that prevailed

during the growing season could have limited the internodal growth and therefore the effect of PGR.

In order to assess the regulation of GA metabolism in response to N fertilization, this study examined the expression patterns of GA biosynthetic and catabolism genes in the second basal internodes of both cultivars grown at different N rates (control, 180 kg/ha and 280 kg/ha). Our results indicated that the expression pattern of genes involved in GA metabolism varies with N rates. One or more GA biosynthesis genes exhibited elevated expression level in the second basal internodes of the two cultivars at 180 kg/ha or 280 kg/ha N rates. This is in agreement with the increased plant height and fresh weight observed at the two N rates as compared to the control N rate, implying the role of N fertilization in enhancing GA synthesis and overall plant growth. On the other hand, the GA catabolism gene, *GA2ox3*, was downregulated in the Invader and Wildcat at both 180 kg/ha and/or 280 kg/ha N rates, suggesting a decrease in the catabolism of bioactive GAs and therefore an increase in bioactive GA levels. Given this, it is necessary to measure GA levels in the basal internodes of the two cultivars grown under different N rates. Furthermore, it is important to study other wheat cultivars to determine if different N rates induce a similar change in the expression patterns of GA metabolism genes.

In summary, the findings of this study provided insights into the effect of N rates and PGR on lodging in wheat. Elevated N rates enhance lodging while GA biosynthesis inhibiting PGR represses it through altering lodging-related plant morphological traits, indicating the importance of using optimum N fertilization rates and/or a PGR that limit GA synthesis to control the potential for lodging. However, the effects of these factors can vary depending on cultivars as well as environmental conditions during the growing season. Analysis of the expression patterns of the

GA metabolism genes suggest the role of both GA biosynthesis and catabolism genes in regulating GA levels and thereby lodging-related morphological traits in response to N fertilization in wheat.

This study offers important insights into the effect of N fertilization and PGR treatment on lodging-related traits in wheat; however, further study is needed to better understand the impact of both N fertilization and PGR treatment on stem structural components such as levels of lignin and cellulose, cell wall thickness and arrangement of vascular bundles, and association of these traits with stem strength and lodging. It is also important to examine how yield-related parameters and end-use quality of the studied cultivars is affected by the different rates of N fertilization and/or PGR treatment. Furthermore, investigating the expression pattern of lignin biosynthesis genes in response to N fertilization is crucial to understand the molecular bases regulating the relationship between N fertilization and stem lignin content. Genetic studies involving different mapping populations can also be applied to identify new markers and QTLs that can help to develop lodging resistant cultivars. Finally, integrating the findings of this study into comprehensive lodging risk prediction models that consider multiple traits and management practices would provide valuable tools for farmers and breeders to minimize lodging-related yield losses in high-yielding wheat cultivars.

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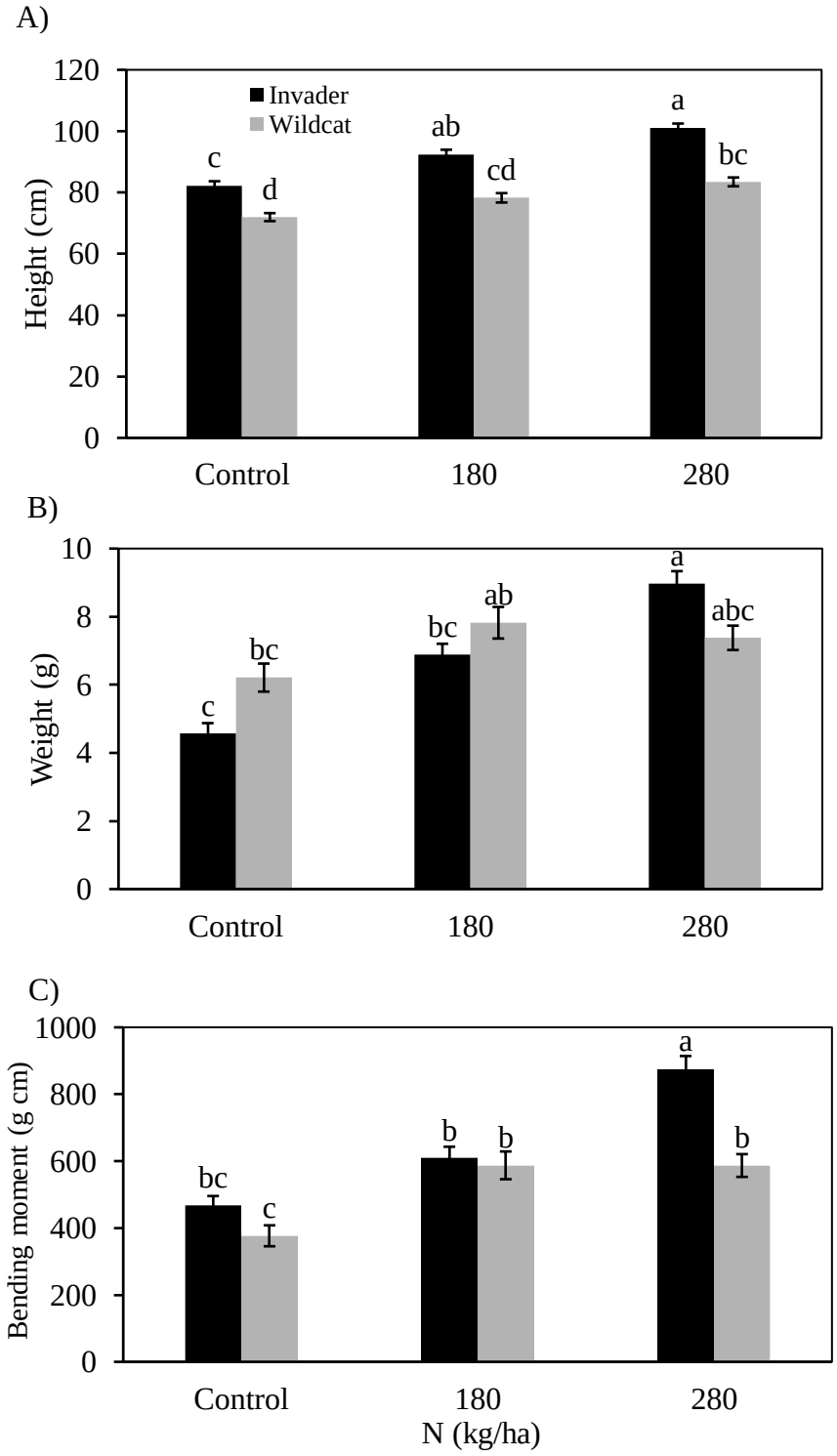
APPENDIX

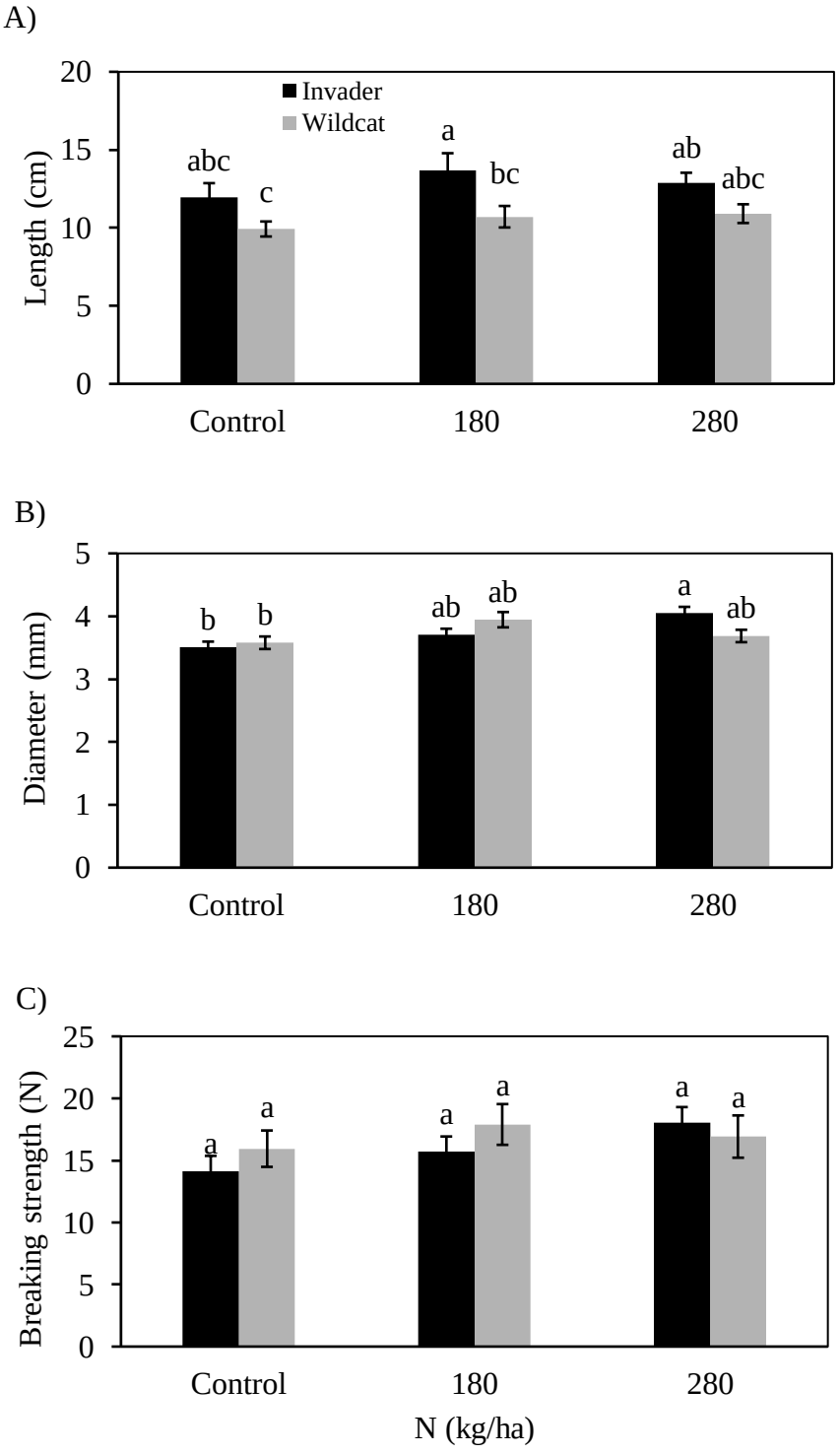
Appendix 1. Primer sequences used for qPCR

Gene	Type	Primer sequence (5' to 3')
TaGA20ox1	FP	CCGCCAGGACATGATGGATT
	RP	CGATCGACGGATGACGATGT
TaGA20ox2	FP	AAGGTGTCGCCGATGTTGAT
	RP	ATGCGGTGCAACTACTACCC
TaGA20ox3	FP	ACCGTGTCCTTCAACTGCTC
	RP	ATGTCACGGTACTCTTCGCC
TaGA20ox4	FP	TCTCCTTCAGCCACAACCAC
	RP	AGCACCTCCATTATCGCCAG
TaGA3ox2	FP	AGGTCGCCGCCGTCGAGTCC
	RP	CAGTTGAGGTGCATTGTGGC
TaGA3ox3	FP	GTGATGCAGAGCCACGTC
	RP	TGAGGATCTGGAAGAGGTCA
TaGA2ox3	FP	GACCCCGGGGACTACTTCT
	RP	TTGACGAGCTTGAAGAACCC
TaGA2ox6	FP	GTTCGACACCAAGGAGAAGG
	RP	GAGCTGAGCTTCCCGTAGTC
TaGA2ox9	FP	TCTCAACGACTCCTACCGCT
	RP	ATGGAAGGCCTCAGACCAG
Taβ-actin	FP	ACCTTCAGTTGCCCAGCAAT
	RP	CAGAGTCGAGCACAATACCAGTTG

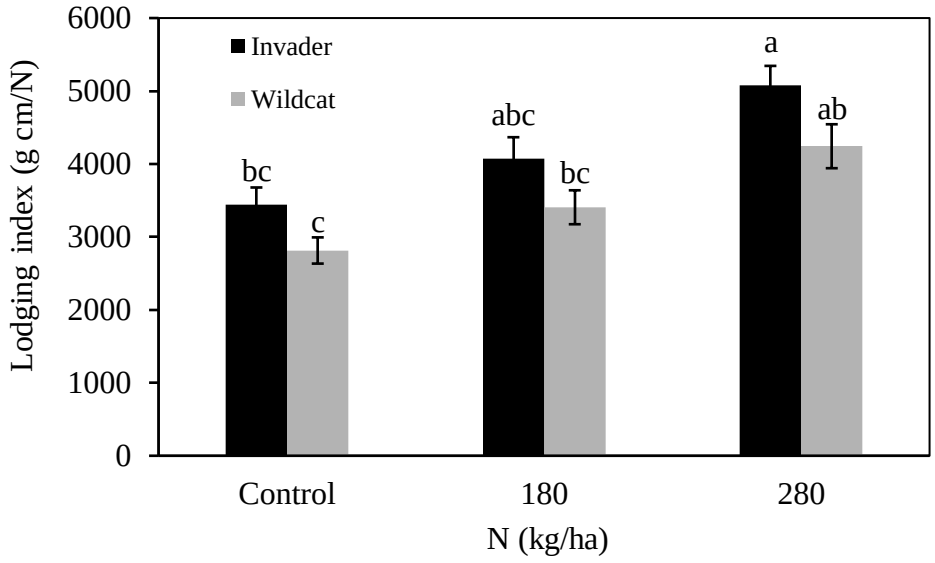
Appendix 2. Plant height (A), fresh weight (B) and bending moment (C) of Invader and Wildcat plants over two growing seasons at three nitrogen (N) rates (control, 180 kg/ha and 280 kg/ha). Data are means of 15 plants ± SE. Different letters indicate statistically significant differences among treatments at P<0.05 (LSD test).



Appendix 3. Length (A), diameter (B) and breaking strength (C) of the second basal internode of Invader and Wildcat plants over two growing seasons at three nitrogen (N) rates (control, 180 kg/ha and 280 kg/ha). Data are means of 15 plants ± SE. Different letters indicate statistically significant differences among treatments at P<0.05 (LSD test).



Appendix 4. Lodging index of Invader and Wildcat plants over two growing seasons at three nitrogen (N) rates (control, 180 kg/ha and 280 kg/ha). Data are means of 15 plants ± SE. Different letters indicate statistically significant differences among treatments at P<0.05 (LSD test).



Appendix 5. Correlation among morphological traits influencing lodging.

	PH	FW	Int2_BS	BM	LI
PH	1.00***	0.71**	0.12	0.88***	0.86***
FW	0.71**	1.00***	0.64**	0.84***	0.68**
Int2_BS	0.12	0.64**	1.00***	0.19	-0.08
BM	0.88***	0.84***	0.19	1.00***	0.85***
LI	0.86***	0.68**	-0.08	0.85***	1.00***

PH, plant height; FW, plant fresh weight; Int2_BS, second internode breaking strength; BM, bending moment, LI, lodging index

Significance of correlation among the traits was determined using F-test. * Indicating a p-value of < 0.05, ** indicating a p-value of < 0.01 and *** indicating a p-value of < 0.001.

Abbreviations

2ODDs	2-oxoglutarate-dependent dioxygenases
4CL1	*4-coumarate:CoA ligase1*
BM	bending moment
BP	before present
BS	breaking strength
C3H1	*p-coumarate 3-hydroxylase1*
CCR2	*cinnamoyl-CoA reductase2*
cDNA	complementary DNA
CE Feed	CE Feed
CEAD	Canada Eastern Amber Durum
CERS	Canada Eastern Red Spring
CESRW	Canada Eastern Soft Red Winter
CEWHRW	Canada Eastern Hard Red Winter
CEWW	Canada Eastern White Winter
CNHR	Canada Northern Hard Red
CPS	*ent*-copalyl diphosphate synthase
CPSR	Canada Prairie Spring Red
CPSW	Canada Prairie Spring White
CWAD	Canada Western Amber Durum
CWES	Canada Western Extra Strong
CWHWS	Canada Western Hard White Spring
CWRS	Canada Western Red Spring

CWRW	Canada Western Red Winter
CWSWS	Canada Western Soft White Spring
EUI	elongated uppermost internode
F5H2	*ferulate 5-hydroxylase2*
GA	gibberellin
GA13ox	GA13-oxidase
GA20ox	GA 20-oxidase
GA2ox	GA 2-oxidase
GA3ox	GA 3-oxidase
GAI	GA insensitive
GAMTs	gibberellin methyltransferases
GGDP	geranylgeranyl diphosphate
GID1	gibberellin insensitive dwarf 1
ha	hectares
KAO	kaurenoic acid oxidase
kg	kilograms
KO	kaurene oxidase
KS	kaurene synthase
LI	lodging index
Mya	million years ago
N	nitrogen
P450s	cytochrome P450 monooxygenases
PAL	phenylalanine ammonia lyase

PGR	ABA deficient 2
POD	peroxidase
qPCR	phytochrome-interacting factor 6
Rht-1	Reduced height-1
SLN1	SLENDER1
SLR1	SLENDER RICE1
TAL	tyrosine ammonia-lyase
TPSs	terpene synthases
UK	United Kingdom
USA	Unites States of America
RNA	ribonucleic acid