THE ROLE OF PLANT GROWTH REGULATORS IN INFLUENCING LOWEST POD HEIGHT IN SOYBEAN

By

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ABSTRACT

Thapar, Ankita. M.Sc. The University of Manitoba, Jan, 2021. The role of plant growth regulators in influencing lowest pod height in soybean. M.Sc. Supervisor: Dr. Belay T. Ayele.

Soybean production is negatively affected by certain morphology related factors such as the occurrence of short lowest pod height which results in stubble losses since the pods lower than the reach of the combine cutter are lost during harvest. Therefore, it is necessary to develop strategies that can promote the elongation of lower internodes such that the first pods can be set at a harvestable height. Plant hormones such as gibberellins (GA) and cytokinins play important roles in regulating stem elongation through promoting cell elongation and cell division, respectively. Therefore, plant growth regulators (PGRs) containing GAs and cytokinins can promote internode elongation and therefore the height of lowest pod bearing nodes. Using a soybean cultivar characterized by a short lowest pod height, this thesis investigated the effects of GA and/or cytokinin based PGRs, applied as a seed treatment, on enhancing the elongation of lower internodes and thereby lowest pod height. The findings show that the GA based PGR was effective in increasing the lengths of lower internodes and height of the lowest pods. In order to gain insights into the molecular basis of GA metabolism in the regulation of pod heights, this thesis examined the expression patterns of GA biosynthetic and catabolism genes in lower internodes of three different soybean cultivars with contrasting pod heights. The results indicate that the three cultivars exhibit differential expression patterns of the genes analyzed.

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

Soybean (*Glycine max* [L.] Merrill) is an important leguminous crop grown world-wide. It contains the highest protein and second highest oil content as compared to other legumes (Liu, 2004). Besides for human consumption, it is used to produce soybean meal which serves as feed for livestock (Hartman, 2011). Soybean has been grown in Canada for over 70 years and it ranks fourth, on the basis of acreage, among the principal crops grown (Soy Canada, 2020). However, the yield and quality of soybean is adversely affected by many biotic stress factors such as diseases, weeds and pests, and abiotic stress factors such as drought, flooding, heat and salinity stress (Miransari, 2015). Apart from these factors, certain morphological factors of the plant such as low pod height also impose challenges to soybean production mainly in regions such as Manitoba where the growing season is short and production is dependent on the cultivation of early maturing varieties (MPSG, 2016). Early maturing varieties are often shorter as compared to late maturing varieties, as a result such varieties tend to bear their first/lower pods close to the ground (Mislevy *et al*., 1988). This gives rise to a problem of harvest losses that represent a considerable reduction in soybean yield.

Soybean is commonly harvested using combines that have a header, consisting of a cutting platform, a reel, and a cutter bar at the edge of the cutting platform. Approximately 80 to 85% of the total harvest losses in soybean are results of the soybean pods that fail to get gathered inside the combine (Butzen, 2013). The primary cause for losses at the header is stubble losses, which is defined as pods that remain attached to the stubble when the cutter bars cut the soybean stalk above the height of the lowest pods. A study conducted in Manitoba concluded that the presence of a height of at least 12 cm between the base of the plant and the node bearing the lowest pod is suitable to prevent losses at the combine cutters (MPSG, 2016). Although attempts have been made

to prevent the problem of harvest losses through lowering the cutter bars, such a practice increases the soil and rock influx to the machinery (Ramteke *et al*., 2012). An alternative and long-term solution to avoid these losses is through breeding soybean cultivars that exhibit a higher lowest pod height and at the same time possess early maturity behaviour. However, breeding and selection of such cultivars takes several years. The use of agronomic practices that are under the control of the growers such as modifying row spacing, planting date, and plant population, was found to have no significant impact on lowest pod height (Tkachuk, 2017). Therefore, application of other approaches such as the use of plant growth regulators (PGRs) that can raise lowest pod heights can be considered as alternative solutions to combat the problem.

Plant growth regulators are defined as chemicals that mimic plant hormones and influence various plant developmental processes at low dosages (Rademacher, 2015). Out of all hormones, gibberellins (GA) play a major role in regulating stem elongation. Gibberellins induce internode elongation by promoting cell elongation (Reid *et al*., 1983). The level of bioactive GAs in plants depends mainly on the balance between the activities of the biosynthetic (GA20ox and GA3ox) and catabolic (GA2ox) enzymes (Yamaguchi, 2008). Genes encoding these enzymes are encoded by multigene family play important roles in determining GA levels and therefore stem elongation (Sun, 2008). For example, higher expression of the GA biosynthetic genes such as *GA20ox1* and *GA3ox1* resulted in internode elongation and therefore longer stems in different species (Coles *et al*., 1999; Oikawa *et al*., 2004; Radi *et al*., 2006) while loss of function mutation in these genes such as *GA3ox1* results in GA deficiency and dwarf phenotype (Ross *et al*., 1989). On the other hand, the loss of function mutations in *GA2ox* have been shown to result in reduced internode lengths (Rieu *et al*., 2008). In addition to GA, other hormones such as cytokinins play roles in stem elongation via promoting cell division. For instance, exogenous application of cytokinin has been

found to enhance the plant height and yield in several crop species such as cotton (*Gossypium hirustum*), rice (*Oryza sativa)* and cowpea (*Vigna unguiculata*) (Kapgate *et al*., 1989; Kaur and Singh, 1991; Khalil and Mandurah, 1989). In addition, cytokinin deficient plants have been reported to have shorter shoots with smaller apical meristems (Werner, 2001). Cytokinins also play an important role in enhancing the number of pods and total seed yield in soybean (Nagel *et al*., 2001; Yashima *et al*., 2005).

This thesis tested the hypothesis that GA and cytokinin related PGRs have the potential of increasing the cotyledonary and first internodes and therefore lowest pod heights in soybean. To this end, the study examined the effects of treatment with different GA and cytokinin related PGRs on internode length and pod height. Furthermore, the study determined the expression patterns of GA metabolism related genes in soybean cultivars with different pod height.

2.0 LITERATURE REVIEW

2.1. Importance of soybean

Soybean (*Glycine max* (L.) Merrill) is one of the most valuable crops in the world. This important leguminous plant serves as an important oilseed crop as well as a good source of protein, feeding human and livestock populations (Masuda and Goldsmith, 2009). The protein content of a soybean seed is about 35% of its dry weight which is higher than any other animal or plant protein (Asbridge, 1997) due to which it is sometimes called "poor man's meat" (Karande *et al*., 2008). Besides high levels of protein, dry soybean constitutes 35% carbohydrates (17% of total is dietary fibre), 19% oil, 5% minerals and vitamins (Hassan, 2013). Owing to its high protein, vitamin and insoluble fibre contents, soybean products have been added to the most recent health dietary guidelines by the World Health Organization (CropLife Canada, 2020). The most popular soy products consumed globally include miso, tempeh, tofu, soy sauce, soymilk, mayonnaise and many more (Endres, 2001). The high protein fibre in soybean also serves as a feed for farm animals such as poultry, pork, and cattle. In the United States of America (USA), nearly 97% of the total soybean meal is used for feeding livestock (NC soybean, 2019).

Soybeans have been characterized as functional foods due to the presence of compounds called isoflavones and phenolic compounds (Young, 1991). The estrogen-like activity of soy isoflavones reduce the risk of breast cancer, prostate cancer, and osteoporosis whereas phytosterols in soybean have a role in lowering the cholesterol levels. The anti-oxidative activity of soy isoflavones is known to reduce the risk of other major diseases such as atherosclerosis and diabetes (Kumar *et al*., 2010). Therefore, the demand for this crop is rising because of its use as an important component in many food formulations and industrial products (Wilson, 2008). Soybean is processed for its oil for human consumption and as an alternative to petroleum oil (Carlsson, 2009).

The soy vegetable oil constitutes saturated fatty acids (15.65%), monosaturated fatty acids (22.78%) and polyunsaturated fatty acids (57.74%) (Wolke, 2007). The industrial uses of soybean oil include production of paints, lubricants, plastics, crayons, and candles (Endres, 2001).

Another feature that makes soybean an important crop is its ability to fix nitrogen through the formation of a symbiotic association with bacteria, specifically *Bradyrhizobium* (Chung and Singh, 2008). The bacterial nitrogen fixation property has enabled soybean to be used as an important component in both, mixed cropping, and sequential cropping systems, thereby resulting in elevated soil biological health (Singh and Shivakumar, 2010).

2.2. Origin and History of soybean

The cultivated soybean belongs to the family *Leguminosae*, subfamily *Papilionoideae* and tribe *Phaseoleae*, genus *Glycine* and the subgenus *Soja*. China is the home of soybeans since it was first domesticated by Chinese farmers as a food crop in the eastern half of the country between 17th and 11th century BC (Hymowitz, 1970; Dupare *et al*., 2008). There are other various evidences that prove China as the origin of soybean. The distribution of wild soybean is limited to China, Korea and Japan, but it is extensively distributed mainly in China. In addition, historical records show the use of soybean in Chinese characters as 'Shu', in many Chinese books (Qiu and Chang, 2010). It is believed that soybeans were introduced to the other parts of the world from China directly or indirectly. With the development of trade overseas, soybeans were distributed to the neighbouring countries like Burma (now Myanmar), Japan, India, Malaysia, Indonesia, Nepal, and Thailand between first century AD and 1100 AD (Mishra and Verma, 2010). In 1765, Samuel Bowen introduced soybean to North America (Hymowitz and Harlan, 1983), and it gained popularity among the soybean farmers and extension workers between mid-19th to early 20th centuries. During

World War II, the increased demand for oils and lubricants resulted in an increased soybean demand. Since then, the US has been the leading producer of soybean in the world (Chang *et al.,* 2015). In 1855, T.V.P of Mount Carmel, Ohio, first reported soybean distribution in Canada from Texas whereas Zavitz in 1893 introduced soybean into Canada (Beversdorf, 1995). From 1961 till 1986, soybean was predominately grown in Ontario region, and it was 20 years later that the crop was successfully introduced to other provinces like Quebec and Manitoba (Statistics Canada, 2017).

2.2.1. History of Soybean in Canada

In 1893, Professor Charles Georgeson from Kansas agricultural experiment station, provided soybean seeds to Charles A. Zavitz of Ontario Agricultural College (Shurtleff and Aoyagi, 2019). The director of the central experimental farm in Ottawa began conducting soybean trials and sent seeds to the experimental stations in other provinces for testing in the year 1897 (Saunders, 1899). In the following year, soybeans were first cultivated in British Columbia, Saskatchewan, Nova Scotia, and Manitoba whereas in Quebec and Alberta its cultivation started in 1910 and 1922 respectively (Shurtleff and Aoyagi, 2019). The production of soybean increased from 18,000 to 820,000 hectares (ha) in Canada from 1945 to 1995. In 1995, Ontario accounted for 90% of the total production of soybean in Canada (Canadian Grains Council, 1995).

A soybean variety named ‘Manitoba Brown’, suited for the short growing season of the Prairies was developed at Manitoba Agricultural College in 1922 (Shurtleff and Aoyagi, 2010). In 1950s, University of Manitoba began research on soybeans and resulted in the introduction of high-yielding soybean varieties suited for Manitoba’s climate (MPSG, 2018b). In 1970 onwards, extended soybean research at the University of Manitoba, eastern Canada and the US have led to

the introduction of soybean varieties with high protein levels and early maturity, that has made cultivation possible in northern and western regions of the province (MPSG, 2018b) which earlier was only limited to the traditional Red River Valley region of Manitoba (Dick and Taylor, 2007).

2.3. Soybean crop development

Soybean is an erect, annual, herbaceous, bushy legume that generally attains a height of 40 to 100 cm. Warm temperature and short-day conditions are generally favourable for soybean growth and development. Temperature between 20°C and 30°C is ideal for soybean development (Liebenberg, 2012). Higher temperature results in reduction of pods/plant, seeds/pod and hence the grain yield (Puteh *et al*., 2013) whereas low temperatures inhibit the growth, flowering and seed formation in soybean (Borowski and Michalek, 2014).

Soybean can be classified as determinate, indeterminate and semi-determinate, depending on its growth habit (Bernard and Weiss, 1973). Determinate varieties are characterized by cessation of the vegetative growth when the terminal bud terminates into a cluster of mature pods soon after flowering begins, whereas the indeterminate type continues to grow throughout their reproductive period such that they form leaves and flowers at the same time. Semi-determinate varieties are characterized by indeterminate stems but at the flowering stage, the vegetative growth is terminated (Bernard and Weiss, 1973). The first leaves are unifoliate, oval and opposite whereas all other leaves are trifoliate and alternate (Hicks, 2012). Approximately two-thirds of the self-pollinating flowers produced on the racemes form small pods which bear 1-4 seeds per pod (Acquaah, 2009). The shape of pods is usually straight or curved and seeds are oval or spherical.

Soybean development is classified into two growth stages named as vegetative (V) and reproductive (R) stages. Emergence (VE) of cotyledons above the soil surface marks the beginning

of vegetative stage which is followed by the appearance of two unifoliate leaves aligned opposite to one another at the first node on the main stem and is termed as cotyledon stage (VC). The appearance of first trifoliate leaves at the node above the unifoliate leaves is achieved at V1 stage. Basically, the subsequent V stages after VC are numbered by fully developed trifoliate leaves (V1 – Vn). When the first flower appears at any of nodes on the main stem, the plant is considered to have entered the reproductive stage 1 (R1) followed by the full bloom stage (R2). The succeeding stages mark the development of pods (R3, R4) and seeds (R5, R6) and the final stage constitutes the maturation of pods and seeds (R7 and R8) (Endres and Kandel, 2015).

2.4. Production of soybean

The production of soybean around the globe in the year 2019 was 358.65 million metric tonnes. Brazil ranks first in the production of soybean followed by United States, Argentina, China, Paraguay and India (USDA, 2019). Overall, Canada ranked as the seventh largest soybean-producing country, producing 1.3% of the global production (BASF guide, 2019). On average, soybean in Canada has a yield of 42.5 bushes/acre (Soy Canada, 2019). The total seeded area under soybean in the year 2019 was recorded to be 2.31 million ha. Ontario ranks first in the production followed by Manitoba, Quebec and Saskatchewan (Soy Canada, 2019). In Manitoba, the area under soybean production increased from 210,400 to 594,700 ha from 2010 to 2019 (Soy Canada, 2019). The highest production in Manitoba (2,245,300 metric tonnes) was recorded in 2017 whereas the production in 2018 was 1,731,600 metric tonnes, which accounted for 22 percent of the total soybean production in Canada. Soybeans have become a success story over the last 10 years owing to the introduction of early maturing and glyphosate-resistant varieties across Northern Great Plains (MPSG, 2018b). Nearly 200 soybean varieties are registered in Canada that

have tolerance against specific diseases, pests, and environmental conditions and about 80% of all soybean varieties grown in Canada are tolerant to herbicides (CropLife Canada, 2020).

2.5. Factors affecting soybean production

2.5.1. Abiotic stress factors

Soybean production is exposed to several biotic and abiotic stress factors world-wide, and these factors suppress the soybean yield by 20% annually (Soy Canada, 2018). Abiotic stress factors have a huge impact on world's agricultural production (Bray *et al*., 2000). Abiotic stress factors that affect soybean production include excess soil moisture, drought, heat, chilling and salinity stress, ozone exposure, metal toxicity and UV-B irradiation (Wang *et al*., 2003; Mittler, 2006). Short or long-term exposure to such stresses incur huge losses in quality and yield (Shah *et al*., 2017). These adverse climatic conditions have an impact on soybean's growth starting from seed germination until flowering, but the seedling stage, out of all developmental stages, is most vulnerable to drought and flooding stress (Hossain *et al*., 2013).

Drought stress results in dehydration of the tissues which is the consequence of altered water relations, membrane structures, physiological and biochemical processes (Yordanov *et al*., 2003; Mutava *et al*., 2015). Drought and heat stresses negatively impact the number of pods/plant; number of seeds/pod, weight of seeds, seed yield and the total biomass in soybeans (Lafet and Ahmad, 2015). The impact on seed yield is amplified if the plants are exposed to stress at the beginning of seed filling stage specifically, R5 (Maleki *et al*., 2013), which may even lead to reduction in soybean yield by 46% (Shou *et al*., 1991) due to decline in photosynthetic rate and stomatal conductance (Ohashi *et al*., 2006).

Economic losses due to flooding are likely to be the second largest after drought (Zhou, 2010). The diffusion rate of oxygen is lower in water than in air, therefore, flooding stress leads to hypoxic growth conditions and limits the availability of energy required to support the overall growth of the plant. Flooding stress results in an imbalance between the production and utilization of sugars due to limited rates of oxidative phosphorylation (Colmer and Voesenek, 2009). Further, high soil moisture content affects the permeability of root cells to water by altering the osmotic balance due to a reduced hydraulic conductivity, which impairs root functions (Patel *et al*., 2014). Flooding stress also targets the mesophyll cells by inhibiting their photosynthetic activity as a result of starch accumulation and inhibits photo-assimilate translocation in the plants (Sachs and Vartapetian, 2007; Mutava *et al*., 2015). Flooding stress accounts for a 17-40 % reduction in the grain yield during vegetative stage whereas a 40-57 % decline is seen during the reproductive stage of soybean development (Nguyen *et al*., 2012).

Another major abiotic stress affecting the productivity worldwide is salinity stress, which is caused when the level of salt in the soil exceeds the level in the cells, resulting in plants becoming incapable of water and nutrient uptake, specifically, K^+ and Ca^{2+} (Khan *et al*., 2007). Many studies have reported the negative impact of high salt concentrations on the soybean yield parameters particularly number of branches, number of pods and seed weight of 1000-seeds (Katerji *et al*., 2003; El-sabagh *et al*., 2015).

2.5.2. Biotic stress factors

The biotic factors that affect soybean production include diseases, weeds and insect pests which can result in huge economic losses. Phytophthora root rot, pod and stem blight, brown spot and bacterial blight being few of the major diseases that affect soybean production in Manitoba

(MPSG, 2018c). However, plants are often susceptible to more than one disease at the same time or consecutively under natural growing conditions (Miransari, 2015).

Stem and root diseases cause more damage to soybeans than the foliar diseases. Their symptoms appear during the reproductive stages and thereby have direct impact on the pods and seed production. Phytophthora root rot and, pod and stem blight are the most prevalent root and stem diseases in Canada (MPSG, 2019b). Phytophthora root and stem rot are caused by *Phytophthora sojae*, which is a fungal pathogen that is most active in wet compact soils in form of oospores (Malvick, 2018). The symptoms include appearance of brown lesions on roots and brown discoloration of the stem. The leaves of infected plants turn yellow and ultimately wilt but remain attached to the plant. This disease can affect soybeans at any of the development stages and can be managed by adopting proper drainage measures and use of resistant cultivars. The fungus *Diaporthe phaseolorum* var. *sojae* causes pod and stem blight. Warm and wet conditions are highly favourable for the occurrence of this disease. Appearance of black dots, termed as pycnidia, on the stems, fallen petioles and pods are the key signs of infection. Pod and stem blight can be controlled by rotating the crop, use of high-quality seeds and use of seed treatment fungicides (Malvick, 2018).

The causative agents of soybean foliar diseases widespread in Manitoba, namely, bacterial blight and brown spot are *Pseudomonas syringae* and *Septoria glycines*, respectively. Bacterial blight is characterized by small, yellow water-soaked spots that turn darker with time. This disease can be managed effectively by adopting strategies such as crop rotation and avoiding any cultivation practices when the foliage is wet (MPSG, 2019a). Brown spot is a fungal disease that thrives in wet, rainy conditions. Symptoms include formation of reddish-brown lesions on young leaves whereas development of irregular spots on older leaves. It usually affects the lower canopy

first and moves upward if the wet conditions prevail. Use of foliar fungicides, rotating crops and incorporation of the crop residue are ways to control occurrence and spread of brown spot (MPSG, 2019b).

2.6. Lower first pod height in Soybean

Apart from the biotic and abiotic stress factors, other factors that are related to the morphology and anatomy of the soybean plant also contribute to yield and quality losses in soybean production. One of these concerns is the occurrence of short pod height which causes seed loss during harvest, since pods lower than the reach of the combine cutter are lost at the time of harvesting (Ramteke *et al*., 2012).

First pod height (FPH) is considered an important agronomic trait for the harvesting of soybeans mechanically (Kang *et al*., 2017). Although mechanical harvesting reduces labour costs, it results in seed losses. There are six reasons for seed loss, namely pre-harvest loss, shatter loss, stubble loss, lodged loss, cylinder loss and separation loss (Charles *et al.*, 1993). Pre-harvest loss accounts for the loss of pods before harvesting due to lodging or shattering; shatter loss is due to the improper header operations; stubble loss is the term used for the pods that remain on stubble because they are below the combine cutters at the time of harvesting; lodged losses describe the loss due to the lodged or loose stalks; cylinder loss is the loss of beans that are not threshed because of high moisture content; and separation loss is the term that describes the loss due to incorrect blower and sieve settings (Charles *et al.*, 1993; Kang *et al.*, 2017). Among the stated losses, pre-harvest losses, shatter losses and lodged losses could be reduced by the development of lodging and shattering resistant soybean cultivars whereas the proper concave-clearance adjustment of the combine cutter could minimize the cylinder and separation losses. However, cutter bars failure to

capture the lowest pods at harvest is a major concern for soybean varieties, especially those grown in the Prairies (Kang *et al*., 2017). It is estimated that about one to two bushels per acre (67.25 kg/ha) are lost during harvest due to short FPH. The numbers may increase in cases where the plants are short or lodged (Staton, 2014). Previous studies have proved the reduction in yield loss from 94.1 Kg/ha to 27 Kg/ha by lowering the cutter bars of combines by 0.1 cm (Kang *et al.*, 2017). But as the cutter bars are lowered, the mechanical damage due to digging of the bars into the soil also increases (Kowalczuk, 1999). Low FPH also causes other problems like the lowest pod touches the ground and this makes the pod vulnerable to rotting in humid conditions and colour changes in dry conditions. The lowest pods also tend to catch diseases easily and the seeds in these pods tend to germinate before harvest, thereby adversely affecting seed production (Zdravkovic, 2005).

Therefore, it is necessary to develop soybean cultivars with longer internodes such that they bear first pods at a higher height (Kang *et al.*, 2017).

2.7. Factors affecting the first pod height in soybean

The first node's height in soybean plants is a genetically determined quantitative trait that also depends on the environmental conditions. Previous studies have reported the significant effect of genotype (G), environment (E) and their interactions (G × E) on different parameters including plant height, FPH, seed yield and number of branches per plant in soybeans (Ngalamu *et al*., 2013).

2.7.1. Genetic factors

Stubble loss can be mitigated by making genetic advancements by selection of soybean varieties that set their lowest pods at a higher height (Kang *et al*., 2017). It has been reported that FPH is

positively correlated with the plant height but negatively correlated with the seed yield including the number of pods, number of seeds/pod and weight of seeds (Oz *et al*., 2009). Another study reported that higher FPH to plant height ratio may result in negative correlation between FPH and seed yield, that is, with a higher FPH/ plant height ratio there was a significant decrease in the number of pods (Ghodrati *et al*., 2013; Kang *et al*., 2017). Since soybean's seed yield is determined by the number of pods (Oz *et al*., 2009), the results of these studies suggest that selection for higher FPH may lead to reduction in the soybean yield. Therefore, development of cultivars with higher FPH through genetic advancements may not serve the purpose of mitigating stubble loss (Tkachuk, 2019).

2.7.2. Environmental factors

Environmental factors affecting lower pod height mainly include temperature, precipitation and moisture (Oz *et al*., 2009). For soybeans, a temperature between 15-20°C is suitable at emergence, 20-25°C at flowering stage and 15-22°C at maturity (Liu *et al*., 2008). A warm climate generally favours good growth and thereby enhances the plant height whereas a cold climate decreases or slows the growth of plant, hence resulting in lower FPH (Fehr *et al.,* 1977). Previous studies revealed that lower temperatures in Manitoba during the early growth stages of soybean development decreases the FPH (Skrudlik *et al*., 1996). In contrast, increasing temperatures between the range of 18/14 °C, 22/18 °C and 26/22 °C (day/night temperatures) has been shown to result in an increase in the number of mainstems, height and mean internode length (Thomas and Raper, 1977).

On the other hand, day/night temperatures as high as 34/26°C to 42/34°C resulted in reduced internode length (Allen *et al*., 2018). Such high temperatures also result in reduction of

overall growth of the plant, number of flowers and seeds/pod (Canci and Toker 2009; Wheeler and Von Braun, 2013). Higher temperatures (21°C to 26°C) during night-time leads to wasteful respiration and hence reduced accumulation of dry matter in the plants. The sugars produced by photosynthesis during the day, otherwise required for grain filling, are expended at night-time due to the enhanced rate of respiration. As a result, less assimilated carbon is available for the kernels/seeds thereby reducing the potential grain yield (Sato and Ikeda, 1979). Moreover, higher night temperatures result in faster accumulation of heat units, leading to a reduced grain filling period and earlier maturation, adversely affecting the grain yield (Thomas and Raper, 1978; Zheng *et al*., 2002). Overall, different soybean cultivars differ in their sensitivity to higher temperatures (Sapra and Anaele, 1991).

Both extreme dry or wet conditions as a result of below/above the average precipitation cna result in a reduction in plant height, FPH and seed yield (Tkachuk, 2019). Low or excess soil moisture negatively impact the processes that leads to cell elongation (Farooq *et al*., 2009), hence reducing the FPH in soybeans. Therefore, both average precipitation and good rainfall distribution patterns are required during the vegetative and reproductive developmental stage of soybean for attaining good yield (Sontos *et al*., 2015).

2.7.3. Management practices

Crop management practices can also influence the FPH. These practices include managing seeding rate, plant density, planting date, row spacing, tillage operations and altering the harvest equipment (Tkachuk, 2017).

2.7.3.1. Effect of seeding rate and plant density on lower pod height in soybean

Plant density is defined as the number of live plants per unit area and serves as an important management tool that enhances the overall soybean yield. However, it depends on environmental factors, seeding depth, soil conditions and planting date. To achieve the target plant density, appropriate seeding density must be calculated (Tkachuk, 2017). In Manitoba, the average targeted planting density is around 395,000 plants/ha (Mohr *et al*., 2014). To achieve this, the seeding density falls between the range of 470,000 to 519,000 seeds/ha and 420,000 to 445,000 seeds/ha using air drill and planters, as seeding equipment, respectively (MPSG, 2016). It has been reported previously that low seed densities (148,000 seeds/ha) result in a greater number of branches, reduced plant height and reduced FPH (Beuerlein, 1988). Another study conducted in Northern USA found that the lowest pods were set lower when lower seed density of 350,000 seeds/ha was used as compared to higher seed density of 650,000 seeds/ha (Beaver and Johnson, 1981). In contrast, higher seed densities result in plants with fewer branches, increased height, lower number of pods and seeds/plant (Weber *et al*., 1966) but higher FPH (Beuerlein, 1988). Plant population density was found to be positively correlated with plant height and FPH but negatively correlated with combine harvest losses (Beikufner *et al*., 2019). It has been shown by Aphalo and Ballare (1995) that lower plant densities reduce internode elongation and adversely impact the FPH. However, tall plants grown as a result of high plant densities are inclined to have weak stems that are susceptible to lodging and disease development (Weber *et al*., 1966; Beuerlein, 1988). Similar results of the effect of lower plant densities on FPH were observed by other studies (Gulluoglu *et al*., 2017).

2.7.3.2. Effect of planting date on lower pod height in soybean

Delayed planting date adversely affects the growth and development of soybean and impacts the seed quality as it results in a changed oil and protein content (Hu and Wiatrak, 2012). Therefore, a negative correlation exists between the planting dates and yield response in soybean in which delayed planting dates cause decrease in yield. While late planting dates resulted in reduced plant height, higher seed-weight and seeds per pod (Heatherly and Elmore, 2004; Pederson and Lauer, 2004), higher yields due to early planting were attributed to increased number of pods, seeds, nodes and dry matter (Pederson and Lauer 2004). Planting dates also have impact on the onset of vegetative and reproductive stages of soybean development (Hu and Wiatrak, 2012). However, a study conducted in Manitoba revealed that planting dates ranging from April 27 to June 12 do not have significant effect on the FPH of soybeans (Tkachuk, 2017). On the contrary, a study conducted in Turkey reported May 15 as the best planting date with respect to FPH as compared to other planting dates studied (15th April, 1st May, 15th May, 1st June, 15th June and 30th June) in which lower FPH's were recorded (Karaaslan *et al*., 2012).

2.7.3.3. Effect of row spacing on lower pod height in soybean

A study conducted in north-eastern US showed variation in the response of soybean to different row spacings (Seiter *et al*., 2004). Soybean was found to have a higher yield, lower plant height and reduced lodging when planted in rows spaced 18 cm apart, whereas at 76 cm row spacing, it experienced lower yields, higher plant heights and increased lodging (Seiter *et al*., 2004). Another study conducted in south western Ontario also reported higher yield with narrower row spacing (18 cm vs. 35 cm) and highest seeding rates (395,000 vs 593,000 seeds/ha). Similar results were demonstrated by Rosa *et al.* (2016) in Brazil where narrow row spacing promoted higher FPH in

soybean. However, the effect of row spacing and seeding rates on FPH is of little practical significance such that the largest difference in pod height in a given cultivar with different seeding rates was only 0.4 cm (McLaren *et al*., 1984). Moreover, in South and East of Carman, Manitoba, row spacing and seeding rates are found to have no significant effect on FPH (MPSG, 2012).

2.7.3.4. Effect of tillage operations on lower pod height in soybean

Previous study has shown that conventional plough tillage significantly increases FPH as compared to no tillage (Gaweda *et al*., 2014). Conversely, another study reported that soybean plants cultivated under no tillage set their pods at a higher height than those grown under conventional tillage (Lopes *et al*., 2007). However, a study in Brazil which involved different treatments including no tillage, reduced tillage and two conventional tillages (disking with disk plowing and disking with moldboard plowing) found that tillage treatments did not have significant effect on lowest pod height (Sontos *et al*., 2015). Therefore, tillage effects may be site specific.

2.7.3.5. Effect of altering harvest equipment settings on lower pod height in soybean

A study conducted by Prairie Agricultural Machinery Institute (PAMI) on the effect of harvest speed, header type and harvest angle on stubble loss indicated that the speed of the combine at the header (2mph – 5mph) does not significantly affect the stubble loss but had an impact on other losses resulting from lodged stalks, dropped pods and loose seeds (Simundsson, 2017). The type of header, with or without air reel, also did not have a significant effect on stubble losses but affected other harvest losses. For example, the use of an auger header with air reel can reduce the losses by almost half as compared to using an auger header with no air reel (Simundsson, 2017).

Therefore, the problems associated with lower pod heights can be resolved more efficiently by using cultivars that can set their pods higher. However, since developing cultivars takes long time, increasing the FPH through other approaches such as use of plant growth regulators (PGRs) may be applied as alternatives in the mitigation of problems associated with soybean yield loss due to low pod heights.

2.8. Plant Hormones and Plant Growth Regulators

Plant hormones play significant roles in plant growth and development as they enable plants to respond to internal and external stimuli at low dosages. Plants regulate and coordinate growth and stress tolerance by modifying the production or signal transduction of its hormones (Colebrook *et al*., 2014). The five major classical plant hormones are auxin, cytokinin, gibberellin (GA), abscisic acid (ABA), and ethylene, with each having specific functions (Santner *et al.,* 2009).

Plant growth regulators (PGRs) are the chemicals synthesized artificially by humans using natural or synthetic ingredients that play a key role in growth and development of plants by affecting hormonal balance through inhibiting the biosynthesis of endogenous hormones or their translocation to the site of action and blocking the hormone receptors (Rademacher, 2015). PGRs also have roles in enhancing the source-sink relationship and promotes the translocation of photo-assimilates, thereby aiding fruit and seed development and overall crop productivity. In field crops, growth regulators aid in partitioning of photoassimilates from the source to sink tissues (Solaimali *et al*., 2001). They act as regulators of several developmental processes in cultivated plants such as stimulating or reducing shoot elongation, enhancing or delaying seed germination, inducing flowering, breaking dormancy, delaying the senescence of leaves, reducing or enhancing fruit set, and accelerating fruit ripening and defoliation (Fishel 2006; Rademacher, 2015).

In 1930s, ethylene and acetylene were known to induce flowering and formation of fruit in pineapple; it was since then that the uses of PGRs became systematic (Bartholomew *et al.*, 2014). Later, many PGRs were detected and today they have become a significant part of modern agriculture. Plant processes can be actively regulated using PGRs as they enable faster solutions to various problems associated with crop production. PGRs are applied to crops mainly via foliar sprays although other PGR application methods such as drenching, dipping and seed treatment are also practised. Factors such as the active ingredient used, weather conditions during the treatment, plant stage of development and use of proper formulants determine the success of PGRs. There are two types of PGRs - true and atypical. True PGRs are the compounds that directly interfere with the plants natural hormonal status whereas atypical PGRs are the compounds that do not interfere directly with the hormonal system of plants but act by being phytotoxic (Rademacher, 2015).

2.8.1. Types of plant hormones

2.8.1.1. Auxins

Charles Darwin in 1880 observed the bending of plants towards sunlight and in his book 'The Power of Movement in Plants' he determined that the tip of coleoptile of etiolated canary grass seedlings shows growth towards the light source (positive phototropism), when illuminated from one side and that "some influence is transmitted from the upper to the lower part, causing the latter to bend". These experiments led to the discovery of the first plant hormone, auxin, in 1926 by Fritz Went (Hopkins, 2004a).

The main precursor of auxin synthesis is Tryptophan (Trp) which is first converted to indole-3-pyruvate (IPA) by the tryptophan aminotransferase (TAA1) family of Trp amino

transferases (Stepanova *et al*., 2008) and subsequently indol-3-acetic acid (IAA) is produced from IPA by the YUCCA family of flavin monooxygenases (Davies, 2010a; Mashiguchi *et al*., 2011). The natural auxin present in most plants is IAA, which is synthesised mainly in the leaf primordia and developing seeds (Laxmi *et al*., 2013). Besides IAA, indole-3-butyric acid (IBA), 4-chloroindole-3-acetic acid (4-CI-IAA) and phenylacetic acid (PAA) with similar structure and activity as IAA have been found in plants (Sauer *et al*., 2013). Synthetic auxins including IAA, 1-naphthaleneacetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D), 2-Methoxy-3,6-dichloro-benzoic acid (dicamba), carbaryl and many others (Hopkins, 2004a, Davies, 2010a).

Auxins plays major role in the regulation of plant growth via cell enlargement and have role in cell expansion, cell division and cell elongation. Auxins promote root initiation, apical dominance and gravitropism (Takatsuka and Umeda, 2014; Gana, 2011) and maintains shoot apical dominance in plants (Estelle, 1992). They also have a role in fruit setting and leaf senescence (Davies, 2010a). Carbaryl is used for fruit thinning and other synthetic auxins like mecoprop, dicamba, are used as herbicides. Application of IAA exogenously to crops such as cowpea (*Vigna unguiculata*) (El-Saeid *et al*., 2010), mung bean (*Vigna radiata*) (Quaderi *et al*., 2006), faba bean (*Vicia faba*) (Ibrahim *et al*., 2007) and okra (*Abelmoschus esculentus*) (Khandaker *et al*., 2018), results in increased plant height, number of flowers and overall yield of the crop. NAA and IAA are known to be used for stimulation of root growth and prevention of fruit drop in orchards. IAA is also found to enhance the total plant height, fruit size and seed yield in rice (Kaur and Singh, 1991) and cotton (Kapgate *et al*., 1989). In soybeans, IAA and tryptophan have been reported to induce root nodule formation (Sudadi, 2012). Auxins mainly 2,4-D and triclopyr marks their use in improving the fruit setting or preharvest fruit retention (Rademacher, 2015).

2.8.1.2. Gibberellins

Gibberellins (GAs) belong to the class of tetracyclic diterpene plant hormones that are formed as a result of complex pathways (Yamaguchi, 2008). Gibberellins were discovered before World War II in Japan, but it was known much later in the west. While searching for the cause of bakanae disease of rice, which is characterized by elongated stem, yield reduction, sterility and pale green texture, GAs were discovered. Hori (1918) and Kurosawa (1926) discovered the fungus *Gibberella fujikuroi* which was known to be responsible for abnormal elongation in rice. In 1938, a water-soluble purified compound with high biological activity produced by the fungus was obtained and named as gibberellin by Yabuta (Hedden and Sponsel, 2015).

Gibberellins are synthesised in young shoot tissues, developing seeds and apical regions of roots and transported to the sink organs through xylem, phloem or via cell to cell (Hopkins 2004c). About 140 GAs are known to occur in higher plants to date but a very few are biologically active such as GA_1, GA_3, GA_4 and GA_7 (Sun, 2008). The most common form of bioactive GA in plants is GA_1 and the most commercially produced form is GA_3 (Gupta and Chakrabarty, 2013). The active GAs plays a key role in a number of plant developmental processes including shoot elongation, seed germination, leaf expansion, fruit setting and flower development (Maske *et al*., 1997; Haba *et al*., 1985; Yamaguchi and Kamiya, 2000). They also have role in induction of bolting in long day plants and fruit setting. It is well established that GA_3 plays an important role in various physiological effects such as photosynthesis, seed germination, stem elongation, flowering, and cell expansion (Yuan and Xu, 2001; Taiz and Zeiger, 2012). It has a wide range of applications in agriculture, viticulture, and horticulture for example, in the production of seedless grapes, in berry thinning, increasing fruit size in sweet cherries and pears and enhancing seed germination. On the other hand, $GA_{4/7}$ has a role in reducing fruit resetting in apples and improving

the quality of apple fruit. In terms of commercial value, inhibitors of GA biosynthesis represent an important group of PGRs. Inhibitors of GA biosynthesis help in keeping the fruit trees compact thereby reducing the cost involved in pruning and are also helpful in improving the quality of ornamental plants which also reduces the amount of space required in greenhouses for their production. Examples of GA biosynthesis inhibitors include chlormequat chloride, ancymidol, flurprimidol and triazole derivatives such as paclobutrazol. These PGRs are also widely used in cereal production mainly in triticale, wheat, rye and oats (Rademacher, 2018). Exogenous application of GA_3 as a foliar application has been reported to improve yield and related components in many plants (Ghodrat *et al*., 2012) through enhancing the use of photosynthates (Khan *et al.*, 2002).

2.8.1.3. Cytokinins

In 1892, Wiesner hypothesised the presence of some growth factors responsible for cell division in plants, and in 1913, Hamberlandt discovered a compound present in phloem that is responsible for inducing cell division (Chen, 1988), and milky endosperm of coconut was found to have similar ability (Overbeek, 1941). The work of Hamberlandt was extended by Skoog and co-workers at University of Wisconsin who demonstrated the presence of compounds promoting cell division in vascular tissues (Jablonski and Skoog, 1954). These studies led to the discovery of the first cytokinin named as Kinetin, from the herring sperm (Miller *et al*., 1955). Miller in 1961 isolated the first naturally occurring cytokinin, Zeatin, from corn kernels. Therefore, cytokinins were discovered as a result of efforts put together to find out factors possessing the tendency to enhance cell division in plants (Chen, 1988). There are two classes of naturally occurring cytokinins, isoprenoid and aromatic. The isoprenoid cytokinins have an isoprene-derived side chain whereas

the aromatic cytokinins possess an aromatic-side chain, both at the N_6 terminus (Mok and Mok, 2001). Both groups vary in terms of the presence or absence of hydroxyl group in their side chains. The most prevalent natural isoprenoid cytokinins are isopentenyl adenine (iP), trans-zeatin (tZ), cis-zeatin (cZ) and dihydrozeatin (dZ). Whereas ortho-topolin (oT), meta-topolin (mT), their methoxy-derivatives and benzyladenine (BA) are the adenine-derived aromatic cytokinins (Sakakibara, 2006; Yamburenko, 2017). Recent studies provide evidence that plants use both methylerythritol 4-phosphate (MEP) and mevalonic acid (MVA) pathways for isoprenoid biosynthesis. The MEP produces the precursors in plastids whereas the others are formed as a result of MVA pathway (Rodriguez and Boronat, 2015).

Cytokinins are a group of phytohormones that play key roles in plant growth and development by regulating cytokinesis in shoots and roots. These compounds are essential for regulation of apical dominance, leaf senescence, phyllotaxis and in nutrient acquisition and distributions (Hirose *et al.,* 2008). Cytokinins also have a role in regulating the response of plants to biotic and abiotic stress factors. For example, cytokinins mediate plant response to drought, salinity, temperature, and nutrient stress (Cortleven *et al*., 2019) Overall, this hormone contributes to fine tuning growth regulation in plant systems (Werner, 2009). Cytokinins can modify the geometry of shoot apical meristem and thus affect phyllotaxis by regulating the size of stem cell niche (Giulini *et al*., 2004) and has a role in formation of nitrogen-fixing nodules (Murray *et al.*, 2007; Frugier *et al.,* 2008). In addition, sink strength is enhanced by cytokinins as was evident from the transport of radioactive metabolites like amino acids and carbohydrates to the sites treated with cytokinins (Werner *et al.,* 2008). Cytokinins such as kinetin and 6-benzyladenine are highly valuable in tissue cultures (Jana *et al*., 2013) whereas thidiazuron is used as cotton defoliant (Jin *et al*., 2020). Both endogenous cytokinin and its exogenous application to soybean at reproductive

stages have been found to increase total plant height, total yield and seed weight in soybean (Kambhampati *et al.,* 2017). Exogenous application of cytokinin at flower initiation resulted in increased pod initiation in lupine (Atkins and Pigearie, 1993), and foliar application of BA resulted in increased translocation of assimilates from leaves to the reproductive parts of *Jatropha curcas* L, thereby resulting in increased seed yield (Pan and Xu, 2011). Soaking seeds of *Linum usitatissimum* for 10 h in a solution of kinetin at 10^{-6} M before planting was shown to lead to an increase in the number of capsules/plant, number of seeds/capsule and total yield (Ullah *et al*., 2010). A previous report has also indicated that application of exogenous application of Kinetin (10 mg/l) leads to an increase in seed yield in safflower (Dholekar *et al*., 2001). Kinetin in combination with GA_3 was found to induce proliferation in potato shoots whereas BA and iP did not show any positive results in this regard (Elliott, 1970). In pea, application of BA at 1µM increases rhizobial nodulation in roots but higher concentrations of BA lead to decreased nodule number (Lorteau *et al*., 2001). This group of hormones have also been firmly proven to regulate seed yield in cereals such as barley and wheat (Zalewski *et al*., 2010; Nguyen *et al*., 2020). On the contrary, studies in tobacco have demonstrated reduced root growth and biomass because of the inhibitory effect of cytokinin on both primary root elongation and lateral root formation (Werner *et al*., 2001). Therefore, factors such as the concentration applied, environmental conditions and the site and stage of application strongly influence the effect of exogenous application of cytokinins (Kinet *et al*., 1993).

2.8.1.4. Abscisic acid

After the discovery of growth-promoting hormone auxin in the first half of last century, it became evident that there are substances that restrict the auxin response and have an inhibitory effect on

growth (Hopkins, 2004b). Plant extracts were tested using paper chromatography along with bioassays, and as a result, inhibitor β, a biologically active substance with growth-inhibitory activity was discovered (Clark and Kefford, 1953). Some scientists named it as 'abscisin II' because of its involvement in abscission whereas other group of researchers named it as 'dormin' because of its role in bud dormancy. Both abscisin II and dormin were known to have similar chemical structure and was named as ABA (Srivastava, 2002; Hopkins, 2004b).

ABA is a terpenoid derived from a common precursor, isopentenyl diphosphate (IDP) (Nambara and Poll, 2005). Synthesis of ABA starts in plastids and is completed in the cytosol (Hirschberg, 2001).

ABA is known as a stress hormone as it protects the plant under stress conditions. It regulates the opening and closure of stomata during drought stress. It is also involved in inhibiting germination of seeds and known as a general inhibitor of growth and metabolic activities (Vishwakarma *et al*., 2017). Soybean plants sprayed with ABA at V6 stage every 6 days showed a decrease in stem length and lodging but exhibited increases in podding rate, number of pods/plant and stem diameter (Chung and Kim, 1989). ABA has been observed to enhance the overall yield in soybean in ways such as increasing the number of lateral roots, protecting the photosynthetic machinery, maintaining stable stomatal conductance (Reinoso *et al*., 2011), and enhancing carbon allocation and its partitioning into seeds (Travaglia *et al*., 2009).

2.8.1.5. Ethylene

The unintended presence of ethylene in the environment led to its discovery as a plant hormone. In 1800s, leakage of illuminating gas from streetlights resulted in premature shedding of leaves, abnormal stem thickening and twisting of plants (Chang, 2016). In 1901, the active compound

affecting plants was discovered and named as ethylene by Russian scientist, Dimitry Neljubow and it was discovered that the abscission in plants was stimulated by ethylene. Gane in 1934 reported that plants synthesize ethylene (Abeles *et al*., 2012) and in the following year, Crock proposed ethylene as a plant hormone causing inhibition of vegetative tissues and resulting in fruit ripening (Arshad and Frankenberger, 2012; Bakshi *et al*., 2015).

Ethylene, the first identified gaseous plant hormone, is responsible for plant processes such as induction of flowering, ripening of fruits, abscission layer formation leading to fruit fall, stimulation and inhibition of cell growth and development (Abeles *et al*., 2012). A study demonstrated that ethylene application in soybean results in the induction of reproductive structure abscission (Urwiler and Stutte, 1986). Ethephon, in the presence of acidic environment decomposes into ethylene, phosphoric acid and hydrochloric acid. Thus, ethephon is a widely used PGR that has a role in reducing stem elongation in barley, boll opener in cotton, and intensifying the flow of latex in rubber trees (Rademacher, 2015). Application of ethephon (280 g a.i. ha^{-1}) at V4, V6, or V4 and V6 growth stages of soybean has been shown to result in reduction of plant height without reducing the lower pod height (Grabau *et al*., 1991), and therefore, had potential to mitigate the stubble losses at harvest. Rhizobitoxin and aminoethoxyvinylglycine (AVG), are inhibitors of ethylene synthesis and work by blocking the conversion of S-adenosylmethionine to 1-aminocyclopropane-1-carboxylate (ACC) which is catalysed by enzyme ACC synthase. AVG is usually applied one to four weeks before harvest, to apples, peaches and plums, and has a potential role in enhancing the accumulation of soluble sugars, acidity reduction, delay in softening and flavour enhancement via production of ester volatiles (Bregoli *et al*., 2002; Jobling *et al*., 2003; Silverman *et al*., 2004). Other compounds such as trinexapac-ethyl and prohexadione-calcium are

also considered as inhibitors of ethylene and GA biosynthesis. These compounds act by displacing enzyme ACC oxidase responsible for converting ACC into ethylene (Rademacher, 2015).

2.9. Role of gibberellin-related plant growth regulators

2.9.1. Gibberellin metabolism

Bioactive GAs are produced from geranylgeranyl diphosphate (GGDP), which is a common precursor for diterpenoids (Yamaguchi, 2008). Depending on the type of enzymes involved and their site of action, the biosynthesis pathway can be broadly divided into three stages: terpene synthases (TPS's) associated with proplastids, cytochrome P450 monooxygenases (P450s) acting in the endoplasmic reticulum, and 2-oxoglutarate-dependent dioxygenases (2ODDs) located in cytosol (Hedden, 1999) (Figure 2.1). The first step in the biosynthetic pathway of GA synthesis is the formation of *ent*-kaurene from GGDP via the action of terpene synthases (TPSs) in the proplastids. The two TPSs namely, *ent*-copalyl diphosphate synthases (CPS) and *ent*-kaurene synthases (KS) are responsible for the formation of tetracyclic hydrocarbon intermediate *ent*-kaurene from GGDP (Olszewski, 2002). In the second step, GA_{12} is produced as a result of subsequent oxidations of *ent*-kaurene under the action of P450 monooxygenases located on the endoplasmic reticulum; *ent*-kaurene is first converted to *ent*-kaurenol and *ent*-kaurenal that is finally oxidized to *ent*-kaurenoic acid by the action of *ent*-kaurene oxidase (KO). After this, *ent*-kaurenoic oxidase (KAO) hydroxylates *ent*-kaurenoic acid into an intermediate *ent*-7α hydroxykaurenoic acid, which finally forms GA_{12} via GA_{12} aldehyde. The GA_{12} could be regarded as the first intermediate specific for GAs. Bioactive GA_1 is the first bioactive GA formed from GA_{12} by the action of soluble 2ODDs. These steps take place in the cytosol of the cell where GA 20-oxidase (GA20ox) acts on GA_{12} and results in loss of one CO_2 molecule at C-20 and then the

formation of GA_{20}. Then GA 3-oxidase (GA3ox) causes oxidation at C-3 and forms the bioactive GA_1 from GA_{20} (Yamaguchi, 2008).

Various inactivation mechanisms of GAs have been identified. Gibberellin inactivation, which converts bioactive GA to inactive GAs, involves three classes of inactivation enzymes; GA 2-oxidase (GA2ox), GA methyltransferase (GAMTs) and GA 16-17 oxidase. The enzyme GA2ox inactivates bioactive GAs by 2β-hydroxylation, and it is the dominant process of GA inactivation (Hedden and Thomas, 2016). Whereas GAMT1 and GAMT2 have been found to catalyse the methylation of C-6 carboxy group of GAs, resulting in the inactivation of bioactive GAs in Arabidopsis (Varbanova *et al.*, 2007). The gibberellin 16-17 oxidase catalyses 16α, 17-epoxidation of GA_{12}, GA_9, and GA_4 by a cytochrome dependent P450 monooxygenase encoded by the rice gene designated as *ELONGATED UPPERMOST INTERNODE (EUI*) (Zhang *et al.*, 2011).

The steps catalysed by the 2 ODDs including GA20ox, GA3ox and GA2ox play important roles in regulating GA levels, and genes encoding these enzymes have been identified from different plant species and form multigene families (Yamaguchi 2008). Previous studies have demonstrated that mutations in *GA20ox* and *GA3ox* genes confer dwarf phenotypes in different plant species such as rice (Itoh *et al*., 2001; Oikawa *et al*., 2004) and Arabidopsis (Chiang *et al*., 1995; Luo *et al*., 2015). On the other hand, functional deficiency of *GA2ox* genes has been found to cause slender and longer stems in many plants (Schomburg *et al*., 2003, Reinecke *et al*., 2013). Feedback regulation of *GA20ox* (Xu *et al*., 1995) and *GA3ox* (Yamaguchi *et al*., 1998) genes and feedforward regulation of *GA2ox* (Thomas *et al*., 1999) genes, is known to maintain GA homoeostasis in plants. Different members of the gene families encoding the 2 ODDs are known to exhibit different functions that could be organ, tissue, or developmental stage specific and have unique expression patterns (Han and Zhu, 2011). For example, in Arabidopsis, *GA3ox1* is highly

expressed during vegetative growth (Phillips *et al*., 1995) whereas *GA3ox2* is responsible for GA biosynthesis in young seedlings and has a role in seed germination (Yamaguchi *et al*., 1998). Furthermore, *GA3ox3* and *GA3ox4* have been reported to have roles in reproductive organ development in Arabidopsis (Mitchum *et al*., 2006). A total of five *GA20ox* genes have been reported in Arabidopsis, out of these, loss-of-function in *GA20ox1*, *GA20ox2* and *GA20ox3* resulted in reduced germination, anther developmental arrest and dwarf phenotypes. On the other hand, *GA20ox4* and *GA20ox5* have been reported to have minor impact on the above stated phenotypes (Plackett *et al*., 2012). It has also been reported that genes encoding 2 ODDs have distinct expression patterns and functions in other species such as rice. For instance, the loss-of-function in the *GA20ox2* gene of rice has been shown to lead to dwarf phenotype (Han and Zhu, 2011).

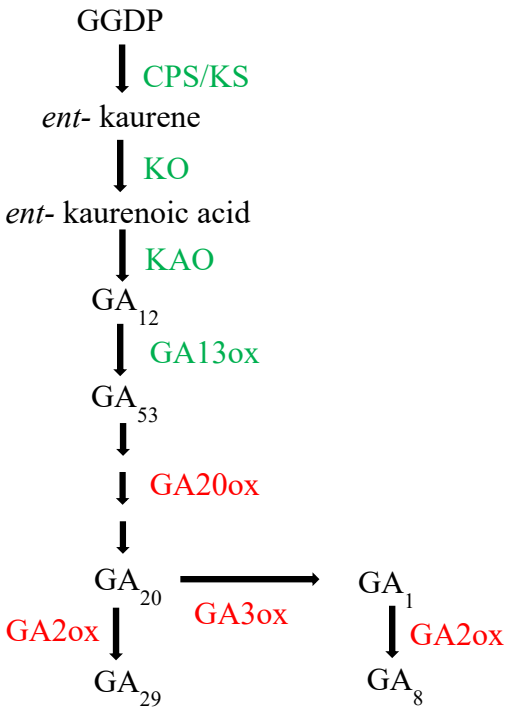

Figure 2.1. A simplified diagram of GA metabolic pathway in plants. GGDP, geranyl diphosphate; CPS, *ent*-copalyl diphosphate synthase; KS, *ent*-kaurene synthase; KO, *ent*-kaurene oxidase; KAO, *ent*-kaurenoic acid oxidase; GA_{12}-aldehyde, a precursor for other GA's; GA_{53}, GA_{20}, GA_{29}, GA_1 and GA_8 are different forms of gibberellins; GA13ox, GA 13-oxidase; GA20ox, GA 20-oxidase; GA3ox, GA 3-oxidase; GA2ox, GA 2-oxidase.

2.9.2. Inhibitors of GA biosynthesis

Inhibitors of GA biosynthesis inhibit the formation of bioactive GAs and thereby affect many GA regulated plant developmental events including stem elongation. Since inhibitors of GA synthesis reduce bioactive GA levels in plant tissues, they cease cell elongation and cell division, as a result of which plants become more compact and therefore the effect of these inhibitor chemicals is beneficial in the crop production industry (Rademacher and Brahm, 2010). Since their effect is inhibiting stem/internode elongation and growth, these compounds are also termed as growth retardants and form an important group of PGRs. Inhibitors of GA biosynthesis can be categorised into four groups: quaternary ammonium compounds, compounds with an N-containing

heterocycle, structural mimics of 2-oxoglutaric acid, and the most recent 16, 17 dihydro-gibberellins. All these inhibitors act by inhibiting a specific step in the GA biosynthetic pathway (Rademacher, 2018) (Figure 2.2).

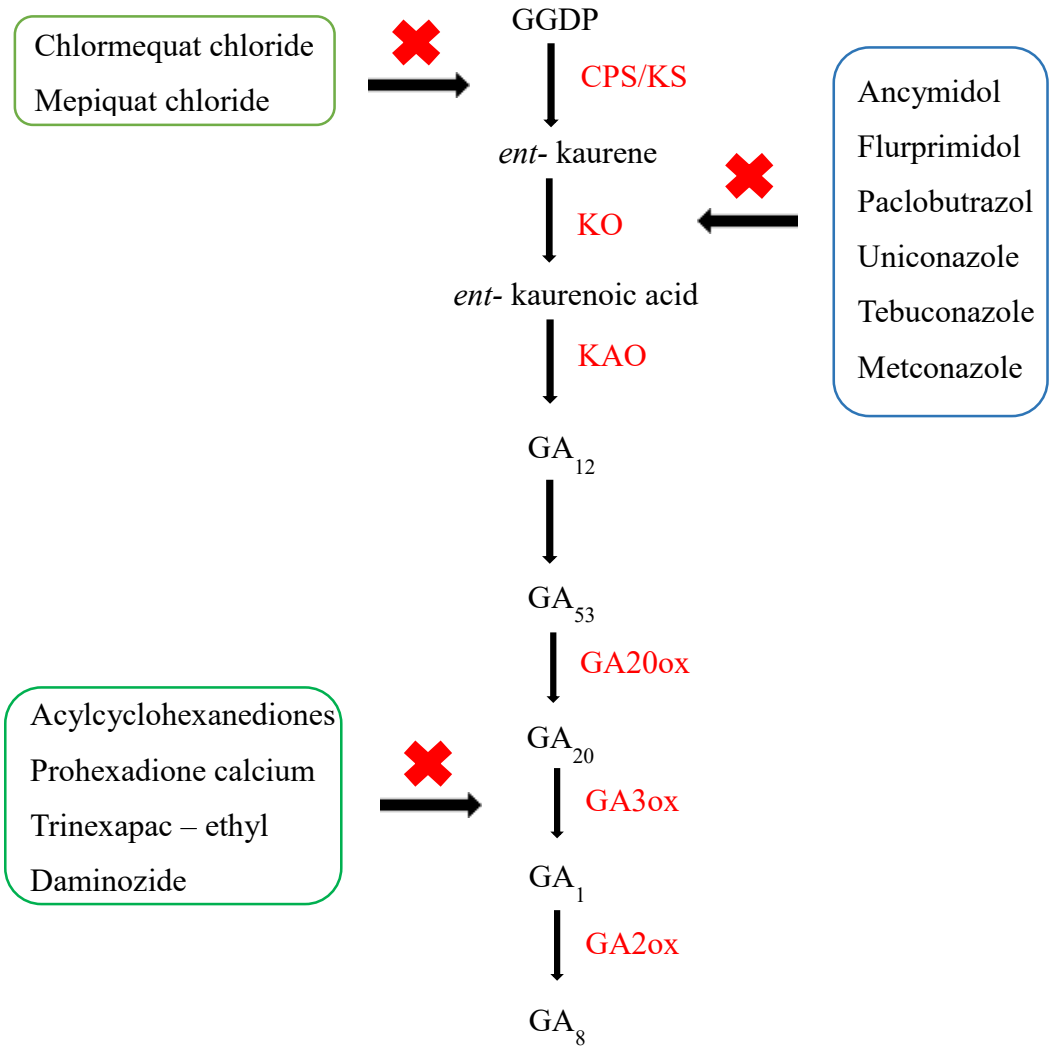

Figure 2.2. Inhibitors of GA biosynthesis and their main targets (Rademacher, 2018). GGDP, geranyl diphosphate; CPS, *ent*-copalyl diphosphate synthase; KS, *ent*-kaurene synthase; KO, *ent*-kaurene oxidase; KAO, *ent*-kaurenoic acid oxidase; GA_{12}-aldehyde, a precursor for other GA's; GA_{53}, GA_{20}, GA_1 and GA_8 are different forms of gibberellins; GA20ox, GA 20-oxidase; GA3ox, GA 3-oxidase; GA2ox, GA2-oxidase.

2.9.2.1. Quaternary ammonium compounds

These compounds inhibit synthesis of *ent*-kaurene which marks the first step in the biosynthesis of GA. They carry a positively charged ammonium, phosphonium or sulfonium group that inhibit the action of terpene cyclases. Chlormequat chloride and mepiquat chloride are the most practically relevant quaternary ammonium class-based GA synthesis inhibitors. Chlormequat chloride inhibits the production of GAs by interrupting the activity of copalyl-diphosphate synthase and *ent*-kaurene synthase, which are the terpene synthases required for the conversion of GGDP to *ent*-kaurene (Rademacher, 2018). As a result, this plant growth regulator reduces the stem height and risks of lodging (Shekoofa and Emam, 2008) and increases stem strength (Miranzadeh *et al.*, 2011). This PGR was first discovered in 1950s by Dr N.E Tolbert who was a chemistry professor at Michigan State University. He applied chlormequat chloride to wheat plants which resulted in reduced height and thicker stems. Today, this is the most widely used PGR around the globe, particularly in wheat, rye and oats. BASF is its major supplier and producer; it is sold under the commercial name Cycocel (Rademacher, 2018). Mepiquat chloride is another widely used plant growth regulator that acts by inhibiting the biosynthesis of GA by blocking the conversion of GGDP to copalyl-pyrophosphate and formation of *ent*-kaurene (Rademacher, 2000). It prevents lodging, stabilises the stem, stimulates flowering and ultimately results in higher crop yield and became a popular PGR after its introduction in 1979, particularly as Pix^{R} in cotton. Mepiquat chloride application results in reduced leaf growth, height, and dry matter but increases the shoot/root ratio in castor plants (Souza *et al*., 2018). The effects of mepiquat chloride on plant height are related to its ability to suppress GA synthesis which shortens stem internodes (Wang *et al.*, 2014).

2.9.2.2. Compounds with nitrogen-containing heterocycle

The conversion of *ent*-kaurene to *ent*-kaurenoic acid is catalysed by cytochrome P450 dependent monooxygenases. Compounds with nitrogen-containing heterocycle inhibit the formation of these enzymes. These compounds include ancymidol, flurprimidol, paclobutrazol, uniconazole, tebuconazole and metconazole (Rademacher, 2018). All these inhibitors have a sp^2-hybridised nitrogen atom in common. The nitrogen atom is situated at the periphery of the molecule in their heterocyclic ring. It seems likely that the lone electron-pair on this molecule interfaces with the cytochrome P450's central iron particle, resulting in inhibition of their activity (Rademacher, 1987).

Ancymidol and flurprimidol are structurally related, commercially relevant pyrimidines that are mainly used in ornamentals. Elanco Products Co. introduced ancymidol in 1971 and flurprimidol in 1989. Both are used to inhibit growth in various monocotyledonous and dicotyledonous species, ornamental herbaceous and woody species, ornamental cover species, perennial turf grasses, coniferous and deciduous trees (Rademacher, 2000). Tebuconazole and metconazole are also considered as efficient inhibitors of GA biosynthesis. These two fungicides are of practical relevance in oilseed rape, where they inhibit the formation of fungal ergosterol by inhibiting the functioning of P450-dependent monooxygenases (Rademacher, 2015). Paclobutrazol and related uniconazole have been found to be highly useful in rice and ornamentals. Based on presence or absence of a double bond, they possess one or two carbon atoms respectively (Sugavanam, 1984). Paclobutrazol structure has two enantiomers, the 2S, 3S form possess a high plant growth-regulatory activity and blocks GA biosynthesis more specifically, whereas the 2R, 3R enantiomer plays a role in sterol biosynthesis inhibition. The 2S, 3S form has a structure similar to that of *ent*-kaurene which is an intermediate in GA biosynthetic pathway. On the other hand,

the 2R, 3R form has structural similarity with lanosterol, which is an important intermediate in sterol biosynthesis (Sugavanam, 1985). Paclobutrazol and closely related uniconazole have an average life of 6 months, which makes them highly persistent in plants and soil. They are used to control vegetative growth in fruit trees such as litchis and mangos in countries exhibiting warm climates. Another use of these inhibitors is lodging control in rice and aid in the production of compact ornamentals (Rademacher, 2018).

2.9.2.3. Structural mimics of 2-oxoglutaric acid

This category of GA inhibitors targets mostly the later steps in GA biosynthesis. The main compounds under this group include acylcyclohexanediones, prohexadione calcium and trinexapac-ethyl (Rademacher, 2018). They block soluble 2-ODDs that are required to carry out steps after GA_{12} synthesis in GA biosynthetic pathway (Kamiya *et al.,* 1992). Acylcyclohexanediones target GA3ox and GA2ox that are responsible for formation of GA_1 from GA_{20} and GA_8 from GA_1, respectively. These inhibitors compete with 2-oxaloglutarate which is the substrate for these enzymes (Hedden *et al.,* 1991). With application of these inhibitors, it has been shown that the level of biologically active GAs decrease along with their inactive metabolites whereas the concentration of GA_{20} and its precursors increases considerably (Na *et al.,* 2011). Acylcyclohexanediones also inhibit the anthocyanin formation in the flowers (Rademacher *et al.,* 1992) and reduce ethylene formation in cell suspensions of sunflower (Grossmann, 1992).

2.9.2.4. 16, 17-Dihydro-gibberellins

This group of GA biosynthesis inhibitors just like acylcyclohexanediones also target the last steps of GA biosynthesis, that is, inhibiting dioxygenases. They are considered as the most recent group

of growth retardants (Rademacher, 2018). In *Lolium temulentum*, the level of GA_1 declined and GA_{20} elevated with the application of these retardants, resulting in reduced shoot elongation Growth of cool season grasses, *Festuca arundinacea*, *Poa pratensis*, *Lolium perenne* was effectively inhibited by 16, 17 dihydro GA_5 (Junttila *et al.*, 1997). After testing systematically with suitable formulations, exo-16, 17–dihydro-$GA_{5\text{-}13}$ acetate was found to be the best growth retardant for graminaceous plants as they compete very effectively for their enzymatic sites with the natural GA substrates. However, its synthesis in large quantities from GA_3 is not cost effective for commercialization (Rademacher *et al.*, 1999).

2.9.3. Role of GA as a potential PGR in soybean

Gibberellin plays a major role in promoting stem elongation (Yang *et al*., 1996). Some studies have reported that GA induces internode elongation as a result of cell elongation (Brian *et al*., 1954; Weller *et al*., 1957) whereas other studies have reported cell division to be primarily responsible for inducing internode elongation in GA treated plants (Greulach and Haesloop, 1958; Leivonen, 1958). Yet there are other reports that highlight both cell elongation and cell division together play role in the elongation of soybean internodes (Bradley and Crane, 1957; Reid *et al*., 1983). In case of tall pea plants, the internodes have a greater number of cells and increased length of cells as compared to their dwarf counterparts. It has been reported previously that internode elongation in deep water rice is a result of two growth processes, cell elongation and cell division (Kende *et al*., 1998). Exogenous application of GA in deep water rice resulted in an increased internode length, which implicated GA in internode elongation (Raskin and Kende, 1983; Hattori *et al*., 2008).

GAs promote stem elongation by increasing the mechanical extensibility of cell wall and enhancing cell wall loosening in the stem cells (Behringer *et al*., 1990). It has been found that GA induces the activity of cell wall loosening enzymes in Arabidopsis namely, xyloglucan endotransglycosylase/hydrolases (XTH), pectin methylesterases (PME), expansins, pectin lyases and aquaporins (Park *et al*., 2017; Gazara *et al*., 2019). The xyloglucans of cell wall are hydrolysed by the enzyme xyloglucantransglycolases (XETs) that causes molecular rearrangement by cleavage and re-ligation of the xyloglucan polymers in the cell wall matrix, resulting in cell wall extension. In addition, XETs enhance the penetration of extracellular proteins called expansins that result in the cell wall loosening by disrupting the polysaccharide adhesion (Sun, 2010). A study with deep water rice also showed the role of GA in upregulation of the expression of genes encoding expansins and XETs in elongating internodes (Kende *et al*., 1998). Therefore, growth-promoting PGRs enhance the activity of XETs thereby resulting in cell elongation whereas growth-inhibiting PGRs inhibit the activity of these enzymes, thus inhibiting growth (Smith *et al*., 1996). Cell division, another cause of stem elongation, is also stimulated by GAs. It has been shown previously that the expressions of genes encoding for cyclin dependent kinases (CDKs) and M-cyclins that are responsible for phase transition in mitotic cycle are elevated by GA (Sauter and Kende, 1992).

Genetic studies of GA signal transduction in Arabidopsis have resulted in the identification of its signalling components (Sun and Gubler, 2004). Among these are nuclear proteins of GRAS family of transcriptional regulators termed as DELLA proteins, which are responsible for suppression of GA signalling (Weiss and Ori, 2007). The transcription factors that are designated as GA insensitive (GAI) and repressor of *ga1-3* mutant RGA inhibit the transcription of genes that result in stem elongation. Spindly (SPY) is another repressor that acts upstream of GAI and RGA

as a second messenger and prevents the transcription of GA responsive genes by enhancing the functions of RGA and GAI (Figure 2.3). But in the presence of GA, these inhibitors get deactivated and transcription of the genes results in stem elongation. Therefore, GA induces stem elongation by deactivating the GA repressors – GAI, RGA and SPY such that the growth-inducing genes induced by GA are transcribed and thereby lead to stem elongation. Under the application of the GA biosynthesis inhibitor paclobutrazol, the level of RGA in the nucleus increases whereas in the presence of GA, the levels of RGA decreases which proves that RGA protein gets degraded in the presence of GA (Davies, 2010b). Overall, the degradation of DELLA proteins under the effect of GAs promotes stem elongation (Sun, 2010). GA biosynthetic mutants exhibit dwarfism due to lower levels of endogenous GA, leading to accumulation of the growth repressing DELLA protein and thereby reduction of internode elongation (Cheng *et al*., 2019).

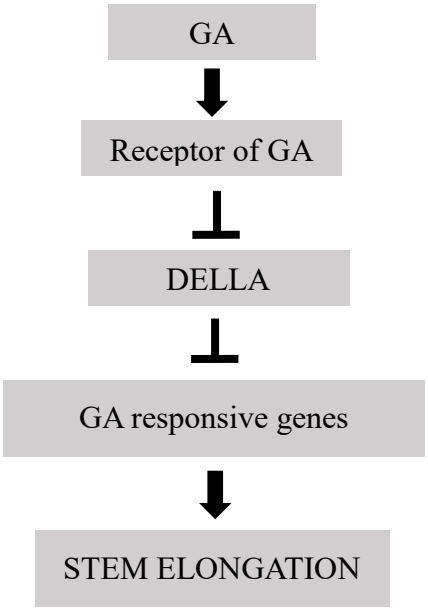

Figure 2.3. A simplified diagram of GA signalling and its effect on stem elongation (Silverstone *et al*., 2001). GA, Gibberellin; DELLA, growth repressor DELLA proteins such as GAI and RGA in Arabidopsis.

Given that GA affects shoot elongation, PGRs with GA activity or that reduces GA level can be used to modify the internode length, FPH and yield of soybean plants. Seeds treated with GA_3 at a concentration of 100 mg/l showed a significant increase in germination as compared to the untreated control seeds of soybean. The treatment positively impacted seed yield, particularly the number of pods/plant, number of seeds/pod and weight of seeds (Agawane and Parhe, 2015). Exogenous application of GA_3 (20 mg/l) at bud initiation stage, was also shown to lead to an increase in seed yield and harvest index in soybean (Upadhyay and Ranjan, 2015). Furthermore, foliar application of GA_3 (100 mg/l) to soybean plants at V3 stage followed by another spray after 15 days, has been reported to have positive effects on first node height, plant height, thickness of the stem, leaf area and dry matter accumulation (Leite *et al*., 2003). Similar results were observed in response to foliar spray of soybeans with GA_3 (100 mg/l) at three different times during plant development (Sarkar *et al*., 2002). Overall, different studies have shown that GA_3 significantly increases internode length in soybean (Rai *et al*., 2006; Naeem *et al*., 2004).

On the contrary, no significant effect on germination, plant height and yield related parameters was observed when seeds of soybean are soaked in solution of GA_3 (10 mg/l) followed by spraying at the vegetative and flowering stages. However, these treatments resulted in an increase of production by 0.90 tonnes/ha with respect to the control (Domingo, 1981). Similarly, no significant effect of GA_3 application (300 g/l) at the R2 physiological stage was observed on number of pods. In contrast to the reports of previous studies, the treatment led to decreased number of seeds/pod and protein content but increased internode lengths and oil content. The studies therefore suggest that exogenous GA_3 exerts different effects when applied at different concentrations and at different growth stages.

2.10. Role of cytokinin-related plant growth regulators

2.10.1. Cytokinin metabolism

The first step in cytokinin biosynthesis is the formation of iP ribotide using dimethylallyl diphosphate (DMAPP) in the presence of ATP/ADP (Figure 2.4). This step is catalysed by adenosine phosphate-isopentyl transferase (IPT) (Sakakibara, 2006). Cytochrome P450 mono-oxygenase family 375, subfamily A (CYP375A), hydroxylates iP ribotides to tZ ribotides. Whereas the formation of cZ is brought about by the prenylation of tRNA using DMAPP, and this reaction is catalysed by tRNA IPTs (Sakakibara, 2006). The enzyme responsible for cis hydroxylation is still unknown. The nucleotides are converted to their nucleobase forms via dephosphorylation. The ribotides are then converted to their active forms via two pathways, the Lonely Guy (LOG) and the two-step pathway (Kudo *et al.*, 2010). In the LOG pathway, the cZ ribotide forms are directly converted to their active free base cytokinins since LOG has phosphoribohydrolase activity, whereas in the two-step pathway, the ribotides are first converted to ribosides and then to their active free base cytokinins (Kurakawa *et al.*, 2007). This step is followed by inactivation of the active forms via degradation, which is catalysed by cytokinin oxidase or dehydrogenase (CKX). This catabolic enzyme is a FAD-containing oxidoreductase and has a key role in regulating the level of cytokinin in plant tissues (Werner *et al.*, 2001). Conjugation of glucose at 3rd, 7th and 9th carbon of the purine ring or in the hydroxyl group of the side chain also results in inactivation of the active cytokinin. Inactivation of cytokinin has an important role in the regulation of its activity (Sakakibara, 2006).

Two types of N-conjugation namely, 7 and 9 glucosylation and alanine conjugate formation can deactivate cytokinins. Compounds named as 6-benzylamino-2-(2-hydroxyethylamino)-9-methylpurine, 3-isobutyl-l-methylxanthine, papaverine; theophylline, caffeine and theobromine

are found to be effective in inhibiting the conversion of cytokinins to 7 and 9 glucosides, which otherwise deactivate cytokinins (Tao *et al.*, 1991). Various compounds that antagonize the effects of cytokinins are known today and these compounds are termed as anticytokinins; they inhibit the progression of cell cycle and result in structural abnormalities in microtubules, which suggest that they inhibit cyclin dependent kinase enzymes (Spichal *et al.*, 2007).

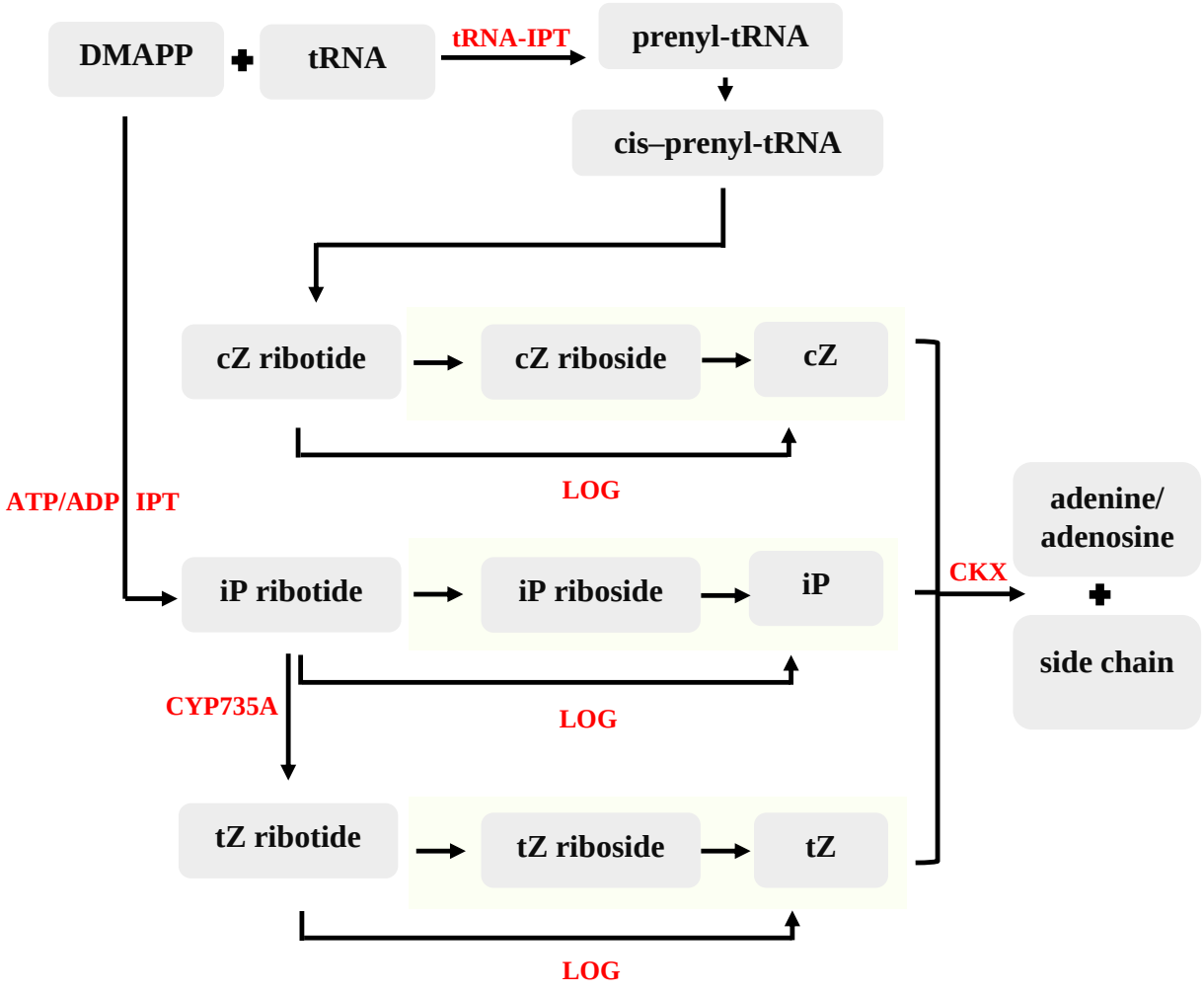

Figure 2.4. A simplified schematic diagram of cytokinin metabolic pathway (Sakakibara, 2006 and Cai *et al.*, 2018). DMAPP, dimethylallyldiphosphate; IPT, adenosine phosphate-isopenenyltranferase; iP, isopenteyl adenine; cZ, cis-zeatin; tZ, trans-zeatin; Z, zeatin; LOG, LONELY GUY; tRNA-IPT, t-RNA-isopentenyltransferase; CKX, cytokinin oxidase/dehydrogenase.

2.10.2. Role of cytokinin as a potential PGR in soybean

In soybeans, not all flower buds grow and form pods, most of them abort during development. The rates of abscission are usually higher at top nodes and lower parts of the main stem (Kokubun, 2011). It has been found that cytokinins take part in the regulation of flower and pod development in soybeans (Reese *et al*., 1995). There is a direct correlation between the endogenous cytokinin levels and number of flowers set and aborted (Carlson *et al*., 1987; Nooden *et al*.,1990; Basuchaudhuri, 2016). Therefore, the rate of pod abortion can be substantially decreased, and thereby the sink strength can be increased by application of cytokinin-related growth regulators (Nagel *et al*., 2001; Nonokawa *et al*., 2007; Passos *et al*., 2011).

Previous reports have shown that exogenous application of BA to soybean (3.4×10^{-7} moles) showed an increase by 79% induces an increase in seed yield, suggesting the important role of cytokinin in determining total seed yield (Nagel *et al*., 2001). Similarly, reduction in pod abortion and an increase in seed yield was observed in response to application of BA (300 mg/l) at the end of flowering (Borges *et al*., 2014). Application of kinetin on determinate soybean genotypes was also shown to improve seed yield (Kumar *et al*., 2002). Furthermore, BA application at reproductive stages of soybean development has been reported to stimulate flowering and increase number of pods/plant and total seed weight in soybean (Crosby *et al*., 1981; Mosjidis *et al*., 1993).

In contrast to the above findings, cytokinin has also been reported to have no significant effect on any of the plant growth parameters when leaves were sprayed at V3/V4 stage followed by another spray 15 days later (Leite *et al*., 2003). Another study reported a reduction in number of pods when cytokinin is applied to the racemes, but it led to an increase in the percentage of pods set if applied 7 days following anthesis (Nonokawa *et al*., 2007). It has been reported that application of Kinetin reduces shoot length of morning glory but increases stem diameter and plant

fresh weight (Chaudhry and Khan, 2000). Foliar application of kinetin was also found to decrease plant height in soybean (Khatun, 2016). Moreover, application of BA as a seed treatment agent led to delayed emergence and reductions in leaf, stem and root growth but an increase in the number of leaf cells in soybean (Riedell *et al*., 1985).

Overall, the effect of exogenous application of cytokinin as a PGR on soybean plants is dependent on a number of factors including its concentration (Nascimento and Mosquim, 2004), the cultivar used (Cho *et al*., 2002), and nutrition status of the plant (Passos *et al*., 2011).

2.11. Crosstalk between gibberellin and cytokinin

Gibberellins and cytokinins are growth-promoting hormones but demonstrate opposite effects on plant developmental processes including root and shoot elongation, shoot regeneration in culture, meristem activity and cell differentiation when applied in combination (Jasinski *et al*., 2005; Weiss and Ori, 2007). They are known to interact at the biosynthetic, catabolic and signalling levels although the mechanism underlying their interaction is not very clear yet (Jasinski *et al*., 2005; Maekawa *et al*., 2009). GA and cytokinin together induce male reproductive development in Arabidopsis and tobacco (Huang *et al*., 2003) whereas cell differentiation in cultures which is induced by cytokinin is inhibited by the action of GA (Flick *et al*., 1983). In agreement with this, an enhanced ability of shoot meristem regeneration has been reported to occur in mutants with reduced GA levels (Ezura and Harberd, 1995).

Cell elongation and maturation requires a high GA/cytokinin ratio and their interaction in which GA inhibits cytokinin response has been demonstrated (Wainberg *et al*., 2005). The phenotypes of *spy* (GA signalling repressor) mutants in Arabidopsis resembled that of GA treated wild-type plants while overexpression of *SPY* in Arabidopsis resulted in phenotypes with

decreased GA activity (Swain *et al*., 2001). These results suggest the role of *SPY* as a negative regulator in GA signalling (Wainberg *et al*., 2005). It has also been demonstrated that loss of *SPY* function results in cytokinin resistance in plants, which clearly suggests the role of *SPY* as a positive regulator in cytokinin signalling (Wainberg *et al*., 2005). Gibberellin therefore inhibits cytokinin response with SPY as a mediator, but cytokinin was found to have no effect on GA signalling (Wainberg *et al*., 2005). Another study in tomato reported that GA inhibits the initial stages of cytokinin signalling pathway while cytokinin affects the later steps of GA signalling pathway (Fleishon *et al*., 2011). A recent study has shown that upregulation of the negative regulators of cytokinin signaling is the cause for antagonistic action of GA with respect to cytokinin during soybean germination (Gazara *et al*., 2019).

3.0. INVESTIGATING THE RELATIONSHIP BETWEEN PLANT GROWTH REGULATORS AND LOWEST POD HEIGHT IN SOYBEAN

Abstract

Height of the first pods is one of the major concerns in soybean production as it causes harvest losses. This research investigates the effects of seed treatment with plant growth regulators (PGRs) on the heights of lowest pod bearing nodes in soybean by using three different PGRs containing gibberellin (GA) and/or cytokinin and a soybean cultivar with lowest pod height namely, TH37004. Our results indicate that seed treatment with GA containing PGR promoted cotyledonary and first node heights and therefore the height of lowest pod bearing nodes. The treatment however did not affect the thickness and bending strength of lower internodes, lengths of the internodes beyond first internode, total plant height, and the yield parameters considered in the study. On the other hand, cytokinin containing PGR or a PGR containing both cytokinin and GA had negative effects on the cotyledonary and first node heights. To understand the molecular bases for variations in the height of lowest pods/pod bearing nodes, the study analysed the expression patterns of the GA biosynthetic *GA20ox* and *GA3ox* genes and GA catabolic *GA2ox* gene in the lower internodes of three cultivars that exhibit contrasting lowest pod height at three developmental stages (V3, V6 and V9). Our results showed variation in the expression patterns of these genes across the three cultivars. Higher expression levels of *GA20ox2, GA20ox3, GA3ox2* and *GA3ox3* were observed in the lower internodes of the cultivar with a higher lowest pod height than the cultivar with shorter lowest pod height. On the other hand, lower expression levels of the GA catabolic genes were observed in the cotyledonary (*GA2ox4*) and first internodes (*GA2ox1*, *GA2ox2-1, GA2ox4* and *GA2ox8*) of the cultivars with higher lowest pod heights.

3.1. Introduction

Soybean (*Glycine max* (L.) Merril) is a crop with great economic value, owing to its high protein and oil content (Stagnari *et al*., 2017). This leguminous plant is an important oilseed crop all around the world (Datta *et al*., 2017). Due to the high value of soybean in the food industry and for animal consumption, its demand is rising annually (Recknagel *et al*., 2015). The introduction of herbicide-tolerant varieties increased soybean production in Canada (CropLife, 2020). Historically, soybean cultivation in Canada was limited to the eastern region and it was later introduced to western Canada because of the challenges such as lower temperature, shorter and drier growing seasons of the Prairies (Dick and Taylor, 2007). Soybean production is affected by various biotic and abiotic stress factors (Gupta *et al*., 2016). Besides these, factors related to plant morphology, such as the occurrence of lower first pod height is a major issue impacting soybean production in the Prairies (Ramteke *et al*., 2012; Kang *et al*., 2017).

First pod height (FPH) is a prominent agronomic trait for efficient harvesting of soybeans using mechanized combine harvesters (Kang *et al*., 2017; Jiang *et al*., 2018). Although mechanized harvesting offers a labour and cost-effective option, it results in seed losses especially in cases where FPH is short (Kang *et al*., 2017). Stubble losses are one of the major sources of seed loss, wherein the pods beneath the reach of combine cutters are lost during harvest (Charles *et al*., 1993). Studies have been conducted to reduce these losses by lowering the cutter bars to catch the lowest pods. However, lowering of the cutter bars causes the cutter to dig into the soil and causes equipment as well as soil damage (Kowalczuk, 1999). Therefore, it is important to develop soybean cultivars with a higher FPH such that the nodes which bear the lowest pods at a height where they can be easily harvested using combines to solve the problem of stubble loss. Since FPH is a quantitative trait that is genetically determined (Kang *et al*., 2017), breeding soybean cultivars

that are early maturing and have a higher FPH will significantly benefit soybean producers in areas where the growing seasons are very short (MPSG, 2019). However, breeding takes several years and other methods such as the use of plant growth regulators may be used as short-term solutions to reduce the problem of lower FPH in soybean.

Plant hormones have a major role in regulating plant growth and development as they enable the plants to respond to the internal and external stimuli at low dosages (Colebrook *et al*., 2014). The five major classical plant hormones include auxin, cytokinin, GA, ABA, and ethylene, each having specific functions (Santner *et al.,* 2009). Plant growth regulators (PGRs) are the chemicals synthesized using either natural or synthetic ingredients that affect hormonal balance via inhibition of the formation of endogenous hormones or their transmission to the site of action (Rademacher, 2015). When applied in low concentrations, PGRs have the ability to regulate various physiological processes in plants such as stimulating or reducing shoot elongation, enhancing or delaying seed germination, inducing flowering, breaking dormancy and delaying the senescence of leaves. PGRs are applied to crops via foliar sprays, drenching, dipping or seed treatments. Factors determining the success of PGRs include, the type of active ingredient used, weather conditions during the treatment, developmental stage of the plant, concentration applied and use of proper formulants (Rademacher, 2015).

Some PGRs such as those containing GAs are involved in increasing the length of internodes in various plant species by enhancing the functioning of the enzymes responsible for cell elongation (Behringer *et al*., 1990), namely xyloglucan endotransglycosylase/hydrolases, pectin methylesterases, expansins, pectin lyases and aquaporins. These enzymes/proteins function by enhancing the cell wall plasticity and loosening in stem cells (Gazara *et al*., 2019). The balance between its biosynthesis, which is regulated by GA 20-oxidase (GA20ox) and GA 3-oxidase,

(GA3ox), and catabolism, which is regulated primarily by GA 2-oxidase (GA2ox) determines the bioactive GA levels in plants (Yamaguchi, 2008), and genes encoding these three enzymes, namely the *GA20ox, GA3ox* and *GA2ox* , form a multigene family (Hedden and Phillips, 2000). Bioactive GAs regulates vegetative as well as reproductive growth in plants by degrading the negative regulators of growth called DELLA proteins (Sun, 2010). Several studies reveal that mutations in the GA biosynthetic genes result in reduced endogenous levels of GA reducing the internode length in plants which leads to dwarfism due to accumulation of DELLA protein (Cheng *et al*., 2019). For example, loss of function mutations in *GA20ox* and *GA3ox* of Arabidopsis result in a dwarf phenotype (Luo *et al*., 2015). Whereas functional deficiency in the GA catabolic gene, *GA2ox*, resulted in formation of thinner and longer stems in Arabidopsis (Rieu *et al*., 2008).

Previous studies have reported the role of GA related PGRs and its influence on various plant responses in different crop species (Kumar *et al*., 2001; Lien *et al*., 2016; Jaques *et al*., 2019). Seed priming with GA enhanced embryo growth and weakened the endosperm layer, which therefore resulted in overcoming seed dormancy and enhanced seed germination in the perennial grass, *Leymus chinensis* (Ma *et al*., 2018). Priming of chickpea seeds with bioactive GA_3 as a PGR was shown to result in higher seed yield, protein content and a greater number of pods per plant (Mazid, 2014). GA containing PGRs have been used to enhance the petiole length and delay petal abscission by hydrolysing starch and sucrose to their simple forms (Khan and Chaudhry, 2006). Previous studies conducted on plant species such as *Codiaeum variegatum* (Shedeed *et al*., 1991), *Polianthes tuberose* (Chang *et al*., 2001) and *Zantedeschia* (Brooking and Cohen, 2002) concluded the role of GA in plant growth via increasing cell elongation and cell division. In addition to regulating plant growth, GA containing PGRs applied as foliar sprays, are also reported to be able to reverse the effects of environmental stress such as drought stress by enhancing the

photosynthetic as well as transpiration rate in cotton (Kumar *et al*., 2001). In soybeans, foliar spray of GA at 20 and 42 days after planting has been shown to result in increases in the number of leaves, branches, flowers, pods, and 100 seed weight and seed yield/plant (Sarkar *et al*., 2002). In addition, seeds primed with GA_3 were shown to result in plants with increased number of pods/plant, pods/seed, seed weight and overall yield (Agawane and Parhe, 2015). Seed treatment followed by two foliar applications of GA_3, first at V3 stage and second application at 15 days after the first application has been reported to increase first node height (Leite *et al*., 2003). It has also been shown that application of GA_3 to the hypocotyl of soybean after its emergence out of the soil causes an increase in length of first two internodes, however total plant height remained the same as in untreated plants (Mislevy *et al*., 1989). However, the studies that examined changes in internode length/node height of soybean due to PGRs used either different varieties that are not commonly grown in the Prairies/Western Canada and/or application of PGRs were at the post-emergence or later stages of development.

In addition, cytokinin containing PGRs play a key role in the growth and development of plants by promoting cell division (Sakakibara, 2006). Cytokinins along with other phytohormones such as auxins and gibberellins are believed to influence stem extension, that is, increase in the length of internodes (Aloni *et al*., 1979). Cytokinins are reported to shorten the phases of mitotic cycle and result in enhanced mitotic activity (Houssa *et al*., 1994). It has been shown previously that cytokinin deficient mutants exhibit smaller shoot apical meristems and dwarf phenotypes (Werner *et al*., 2001). Different factors such as the amount/concentration applied, environmental conditions and the site and stage of application determine the effectiveness of exogenously applied cytokinins (Kinet *et al*., 1993). For instance, application of benzyladenine (BA), a form of cytokinin, leads to increased rhizobial nodulation in roots, however, higher concentrations of BA

decreased nodule number in pea (Lorteau *et al*., 2001). Another study on maize has shown that foliar application of BA enhances plant height, stem diameter, leaf area index and plant dry matter (Amin *et al*., 2007). Total number of pods/plant is one of the major seed yield determining factors in soybean (Oz *et al*., 2009). Although the number of flowers set by soybean plant is high, approximately 75% of the flowers and pods undergo abscission naturally during plant development (Peterson *et al*., 1990). It has been reported that exogenous application of BA can reduce pod and flower abortion and therefore has a role in increasing the soybean yield (Nonokawa *et al*., 2007), and can enhance overall yield by nearly 3% (Nagel *et al*., 2001).

This thesis project investigated the potential of GA and cytokinin related PGRs in increasing the cotyledonary and first node, and lowest pod height in soybean and therefore mitigate the problem of stubble losses in soybean. The study further characterized the expression patterns of GA metabolic genes (*GA20ox*, *GA3ox* and *GA2ox*) to get insights into the cause of variation in the lowest pod heights of three different cultivars with contrasting pod heights.

3.2. Materials and Methods

3.2.1. Plant material and growth conditions

Three soybean cultivars, namely TH37004, Lono and Notus were used in this study, and they were chosen based on their contrasting characteristics with respect to lowest pod height. Mature dry seeds were surface sterilized using 70% ethanol for one minute followed by gentle shaking with 1.2% sodium hypochlorite for 20 minutes using a benchtop orbital shaker (Thermofisher Scientific, Waltham, MA, USA). Seeds were then rinsed thoroughly with sterile water. Twenty seeds were then placed in Petri-plates between layers of Whatman #1 filter paper (GE Healthcare, Little Chalfont, UK) moistened with 10 ml sterile water. The plates were then incubated in

darkness in a growth cabinet maintained at 22^{o}C for four days. On the fourth day, germinated seeds were transplanted into one-gallon plastic pots filled with LA4 sunshine mix (Sungro Horticulture, Bellevue, WA, USA) and supplemented with slow release fertilizer at the rate 9 g/pot (ACER®nt 13-12-12 consisting of 13% N, 12% P_2O_5, 12% K_2O and micro elements). Plants were grown in growth chamber at 22/18°C (day/night temperatures) and 16/8 h photoperiod. The light intensity of the growth room was 178.17 ± 3.82 µmol/m^2/s. Plants were watered regularly and supplied with N-P-K (20:20:20) at the rate 1 g/pot, 30 days after planting.

3.2.2. Treatment with plant growth regulators

Three different PGRs were investigated in this study and their effect on lowest pod height was tested using cv. TH37004, which was shown to have the lowest pod height among the three cultivars studied in this project. The PGRs considered in this study contain GA (referred hereafter as PGR #1), cytokinin (referred hereafter as PGR #2) and GA plus cytokinin (referred hereafter as PGR #3) as active ingredients. The PGR treatments were undertaken in the form of seed treatments. To this effect, mature dry seeds were surface sterilized according to the same protocol described above. The seeds were then imbibed with four different concentrations of each PGR (0 µM, 10 µM, 50 µM and 100 µM) under darkness at 22^{o}C for three days. Subsequently four germinated seeds from each treatment were transplanted into 3 L pots and grown under the same condition as described above. The pots were arranged in a completely randomized design in a growth chamber. A total of 20 plants were grown for each treatment.

3.2.3. Parameters studied and tissue collection

Different parameters including cotyledonary and first node heights, were recorded. The measurements were taken every week from the cotyledon stage (VC) after planting until the heights of the nodes were stabilized and did not grow any further. The lengths of the second, third and fourth internodes and total plant height were measured regularly from vegetative stage 3 (V3) stage until their elongation was stabilized did not increase any further. Lowest pod height was measured in two forms - as the height from the base of the plant to the tip of the lowest pod or the node bearing the lowest pod every week from reproductive stage 4 (R4) until full maturity (R8) stage. The cotyledonary and first internodes of plants were collected at harvesting for determination of their thickness and bending strength; the thickness of the internodes was determined using Vernier calliper whereas the bending strength of the internodes was measured using an Instron 3366 universal testing machine (Instron, Norwood, MA). The bending strength data was calculated using Instron Bluehill® software (Instron, Norwood, MA) based on the maximum compressive load that the internodes can bear before breaking. Yield-related parameters including number of pods per plant, number of seeds per plant, weight of seeds per plant, were recorded at harvest.

Cotyledonary and first internode tissues were collected from all three cultivars (TH37004, Lono and Notus) at V3, V6 and V9 stage of soybean development. The tissues were immediately frozen in liquid nitrogen upon collection and stored at -80°C until further use for RNA extraction.

3.2.4. RNA extraction

Total RNA was isolated from the cotyledonary and first internode tissue samples of all three cultivars using TRIzol (Invitrogen, Carlsbad, CA, USA). The tissues (~100-150 mg) were ground

into fine powder using liquid nitrogen in a mortar and pestle. Immediately after transferring the fine powder into fresh sterile tubes, 1 ml of TRIzol was added to each tube containing the fine powder. Addition of TRIzol was followed by vortexing and then incubation at room temperature for 10 minutes. The mixture was centrifuged at 16,200g at 4°C for 10 minutes to facilitate separation of nucleoprotein complexes. The supernatant obtained was transferred to new tubes and 0.2 ml chloroform was added per 1ml TRIzol followed by 15 seconds of vigorous shaking and incubation at room temperature for 3 minutes. The mixture was centrifuged again for the purpose of phase separation at 16,200g at 4°C for 15 minutes. The aqueous upper phase was transferred to new tubes and 0.5 ml isopropanol per 1 ml TRIzol was added for precipitation of RNA. After incubating the mixture for 10 minutes at room temperature, it was centrifuged at 16,200g for 10 minutes at 4°C to obtain the pellets. The supernatant was discarded, and the pellet containing the RNA samples was washed using 1 ml of 75% ethanol. After vortexing, it was centrifuged at 7500g for 5 minutes at 4°C. The supernatant was removed, and the pellets were subjected to air-drying at room temperature for approximately 5 minutes. The RNA pellets were then dissolved in 50 µl diethyl pyrocarbonate (DEPC) water followed by incubation at 55°C in a water bath for 10 minutes and stored at -80°C until further use.

3.2.5. DNase treatment

The total RNA samples were digested with DNase (DNA-*free* kit; Ambion, Austin, TX) in order to remove the genomic DNA contaminants. To this end, 5 µl (0.1 volume of total RNA) 10X DNase I buffer and 1 µl rDNase I enzyme were mixed gently with 50 µL of total RNA (10 µg) to a total reaction volume of 56 µL. The mixture was incubated for 20 to 30 minutes at 37°C with gentle mixing every 10 minutes. To stop the reaction, 5 µl (0.1 volume of total RNA) of re-

suspended DNase inactivation reagent was added and mixed well. This was followed by incubation at room temperature for 2 minutes with occasional mixing. The sample was centrifuged for 2 minutes at 10,000g and the supernatant was separated for further use. The integrity of the isolated RNA was verified by gel electrophoresis whereas the purity and concentration of the RNA was checked using the epoch multi-sample spectrophotometer (Biotek Instruments, Winooski, VT, USA).

3.2.6. cDNA synthesis

The DNase digested RNA samples were used for the synthesis of complementary DNA (cDNA) samples that were used for qPCR assays using the iScript reverse transcription supermix (Bio-Rad, Hercules, CA, USA). Briefly, the reaction mixture contained 4 µL of 5X iScript reverse transcription supermix, total RNA (1 µg) and nuclease free water to a total reaction volume of 20 µl. The complete reaction mix was incubated in a thermal cycler using a protocol that included priming for 5 minutes at 25°C followed by reverse transcription for 30 minutes at 42°C and finally RT (reverse transcriptase) inactivation for 5 minutes at 85°C. The cDNA was diluted 20X and stored at -20°C until further use for qPCR analysis.

3.2.7. Primer designing

Available sequences of GA metabolism genes in Arabidopsis were used to search the corresponding soybean homologs against the soybean genome sequence data in Phytozome v12.1 (https://phytozome.jgi.doe.gov/) using the basic local alignment search tool (BLAST). The resulting sequences of soybean GA metabolism genes were confirmed by BLAST searches with respective orthologs in the NCBI database. Soybean GA metabolism genes were named base on

their orthologs in Arabidopsis. Since sequences of some the *GA20ox*, *GA3ox* and *GA2ox* genes from soybean share very high degree of similarity, primers were designed from a region that is conserved between/among the nucleotide sequences using Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/) and their gene specificity was verified by RT-PCR (Appendix 1). The target genes identified are the soybean *GA20ox* genes including *GmGA20ox1* (GeneID: Glyma03g02260), *GmGA20ox2* (GeneID: Glyma07g08950), *GmGA20ox3* (GeneID: Glyma09g27490), *GmGA20ox5* (GeneID: Glyma13g09460), *GmGA20ox6* (GeneID: Glyma14g25280), *GmGA20ox7* (GeneID: Glyma16g32550), *GmGA20ox8* (GeneID: Glyma20g29210); soybean *GA3ox* gens including *GmGA3ox1* (GeneID: Glyma04g07520), *GmGA3ox2* (GeneID: Glyma06g07630), *GmGA3ox3* (GeneID: Glyma13g43850), *GmGA3ox4* (GeneID: Glyma14g16060), *GmGA3ox5* (GeneID: Glyma15g01500) and *GmGA3ox6* (GeneID: Glyma17g30800); and GmGA2ox genes including *GmGA2ox1* (GeneID: Glyma02g01330), *GmGA2ox2* (GeneID: Glyma10g01380), *GmGA2ox3* (GeneID: Glyma10g24270), *GmGA2ox4* (GeneID: Glyma11g00550), *GmGA2ox5* (GeneID: Glyma13g28970), *GmGA2ox6* (GeneID: Glyma13g33290), *GmGA2ox7* (GeneID: Glyma13g33300), *GmGA2ox8* (GeneID: Glyma15g10070) and *GmGA2ox9* (GeneID: Glyma15g39750). *Gmβ-actin* was used as a reference gene as described previously (Sidhu *et al.*, 2020). Primer information of the target genes is summarized in Table 3.1.

Table 3.1. Primers sequences used for expression analysis of gibberellin metabolic genes.

Gene	**Type**	**Primer sequence (5' to 3')**
GmGA20ox1	FP	TCCTTGTCAAAAGCCTGACC
	RP	CCCACTTGGTCTTGGTGAAG
GmGA20ox2	FP	TCCGCAGAAAATCATGACAA
	RP	TGCTGACACAGCTTGTGGAT
GmGA20ox3	FP	CATCATGTCAGCAGCCAAGT
	RP	AACACATCAAGCCCTCCAAC
GmGA2ox1	FP	AGGCTTGTGAGGAGTTTGGA
	RP	TCCCCATTGTGTCCAATTTT
GmGA2ox2-1	FP	AACCACAGCCACAAAAGGAC
	RP	AAATTTGCTGGGTTTTGCTG
GmGA2ox2-2	FP	GCCTGCAAATCTGTCTCACA
	RP	TTTCACTCAAGGGTGCTCCT
GmGA2ox4	FP	CGTTGATGGCCTTCAGATTT
	RP	GTGTTCGTCAACACCCTGTG
GmGA2ox8	FP	CACGGTCTCATGCCACATAC
	RP	ACTCTTGTACACCCCGTTGC
GmGA3ox2	FP	AAACAACATAAGCGGGTTGC
	RP	GTTCACTAAGACCCGGTGGA
GmGA3ox3	FP	TAGATGTGCACTGCATCGTG
	RP	GGCAAGGAATCAAGAACCAA
GmGA3ox4	FP	TATGTGGCACGAAGGATTCA
	RP	CGGGACAACTTGGGTAGAAA

FP: Forward Primer; RP: Reverse Primer

3.2.8. Real-time qPCR assay

Quantitative qPCR assay was conducted following a protocol described in Sidhu *et al* (2017). Each qPCR reaction mixture consisted of 5 µl of 20X diluted cDNA, 1.25 µl forward primer (5 µM; final concentration 300 nM), 1.25 µl reverse primer (5 µM; final concentration 300 nM), 2.6 µl nuclease-free water and 10 µl of SsoFast Eva Green Supermix (BioRad, Hercules, CA, USA), making a total volume of 20 µl for each reaction. The reaction mixtures were subjected to the thermocycling conditions in the following order, using the CFX96 real-time PCR system (Bio-Rad, Hercules, CA, USA). The first step included initial denaturation and enzyme activation at 95°C for 5 minutes. This was 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). Normalization was performed using *Gmβ-actin* as a housekeeping gene.

3.2.9. Statistical analysis

Statistically significant differences between samples were examined using Student's t-test statistical function of excel at $P<0.05$.

3.3. Results

3.3.1. Selection of cultivar with the lowest height of the first pod

Preliminary experiments performed showed that the lowest pods originate at either the cotyledonary or first nodes. Thus, in order to assess the difference in lowest pod height among the three cultivars, namely TH37004, Lono and Notus, cotyledonary and first node heights were compared using plants grown at 22/18°C day/night under a 16/8 h photoperiod. The three cultivars

exhibited significant differences in the parameters recorded. Cultivar TH37004 exhibited the lowest cotyledonary and first node heights as compared to other two cultivars (Figure 3.1; Appendix 2, 3). The cotyledonary node height for cv. TH37004 was 23.8% and 34.6% lower than Lono and Notus, respectively (Figure 3.1A). In addition, the first node height exhibited by cv. TH37004 was 28.2% and 38.2% lower as compared to Lono and Notus, respectively (Figure 3.1B).

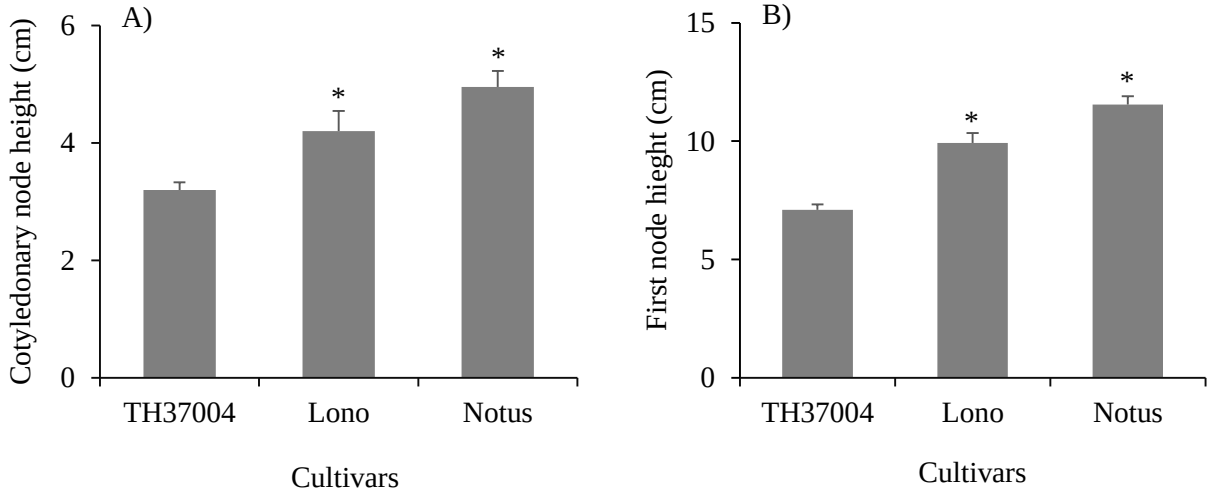

Figure 3.1. Cotyledonary (A) and first (B) node heights of cvs. TH37004, Lono and Notus. Data are means of 15 plants ± SE. Asterisks indicate statistically significant differences between cv. TH37004 and the other two cultivars (P<0.05; Student's t-test).

3.3.2. Plant growth regulators and their effects on cotyledonary node and first node heights

This project investigated the effects of three different PGRs, namely PGR #1, PGR #2 and PGR #3 on the cotyledonary node, first node and lowest pod heights using cv. TH37004, which was found to have the lowest cotyledonary node, first node and lowest pod heights as mentioned above and different concentrations of the PGRs were considered.

3.3.2.1 Effect of PGR #1

The effects of four different concentrations of PGR#1 (0 µM, 10 µM, 50 µM and 100 µM) on cotyledonary and first node heights of cv. TH37004 were tested through seed treatment (Figure 3.2; Appendix 4, 5). As the concentrations of PGR #1 increased, there was an increase in cotyledonary node height (Figure 3.2A). As compared to the control, seed treatment with 10 µM, 50 µM and 100 µM concentrations of PGR #1 caused significant increase in cotyledonary node height by 20.2%, 35.1% and 32.4%, respectively (Figure 3.2A, C, D). The height of the first node also increased as the concentration of PGR#1 increased (Figure 3.2B). Relative to the control, treatment with 10 µM, 50 µM and 100 µM concentrations of PGR#1 induced significant rise in first node height by 19.4%, 38.5% and 46.5%, respectively (Figure 3.2B). Therefore, PGR #1 had a growth-promoting effect on the cotyledonary and first node heights of cv. TH37004. However, our results showed that there was no significant difference in both cotyledonary and first node heights as the concentration of PGR#1 increased from 50 µM to 100 µM (Appendix 4, 5).

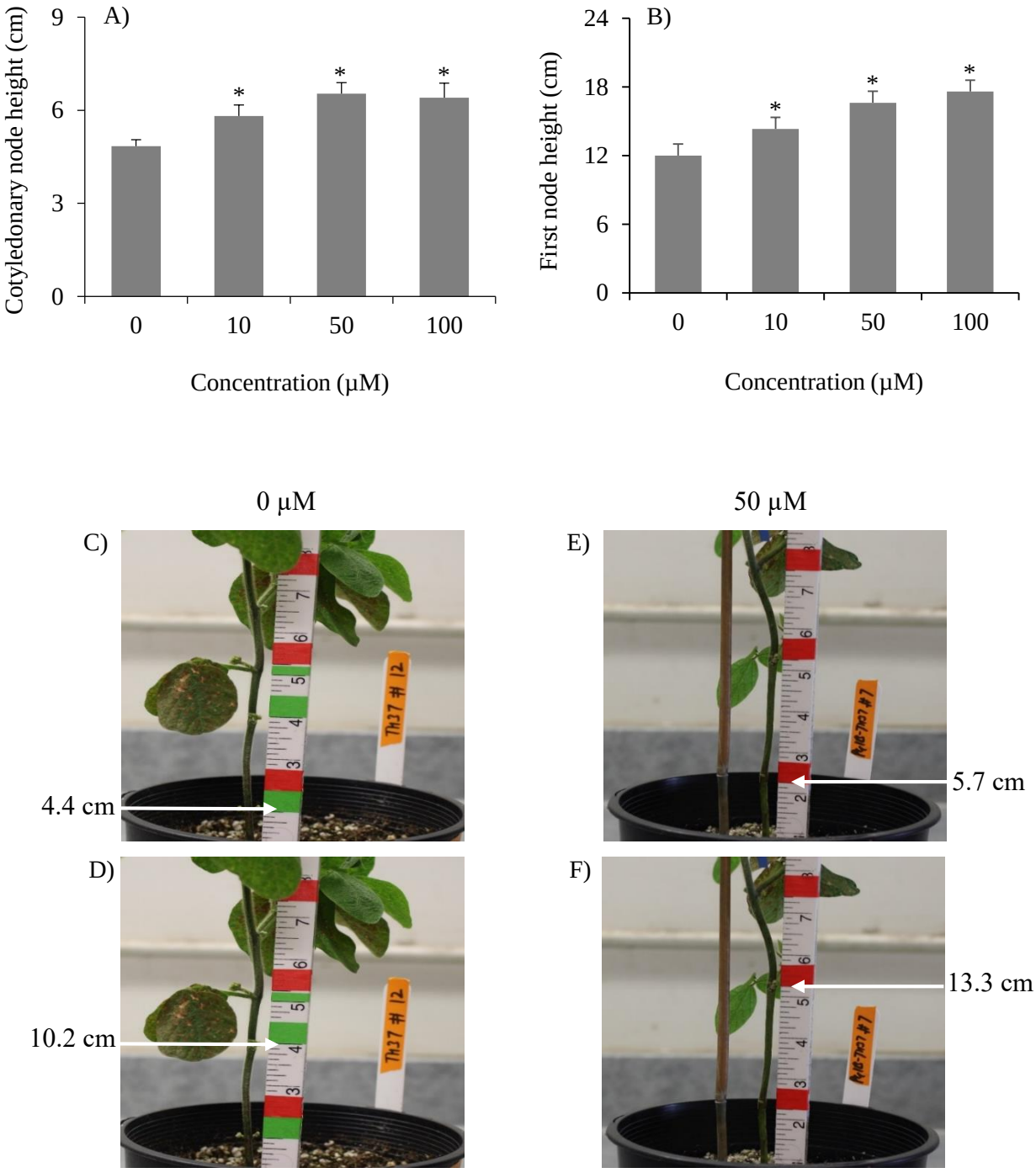

Figure 3.2. Effects of seed treatment with different concentrations of PGR #1 on cotyledonary (A) and first (B) node heights of cv. TH37004, and effects of 50 µM of PGR #1 cotyledonary (C, E) and first (D, F) node heights relative to the control (0 µM). Data are means of 20 plants ± SE. Asterisks indicate statistically significant differences between untreated control and treated plants (P<0.05; Student's t-test).

3.3.2.2. Effect of PGR #2 and PGR #3

Plants grown from seeds of cv. TH37004 treated with all concentrations of PGR #2 showed reduced cotyledonary and first node heights (Figure 3.3; Appendix 6, 7). Treatments with 10 µM, 50 µM and 100 µM of PGR #2 significantly decreased the cotyledonary node height by 38.3%, 61.0% and 68.9%, respectively (Figure 3.3A), as compared to the control untreated plants. Seed treatment with PGR #2 at 10 µM, 50 µM and 100 µM concentrations also significantly decreased first node height by 30.8%, 43.7% and 55.2%, respectively (Figure 3.3B).

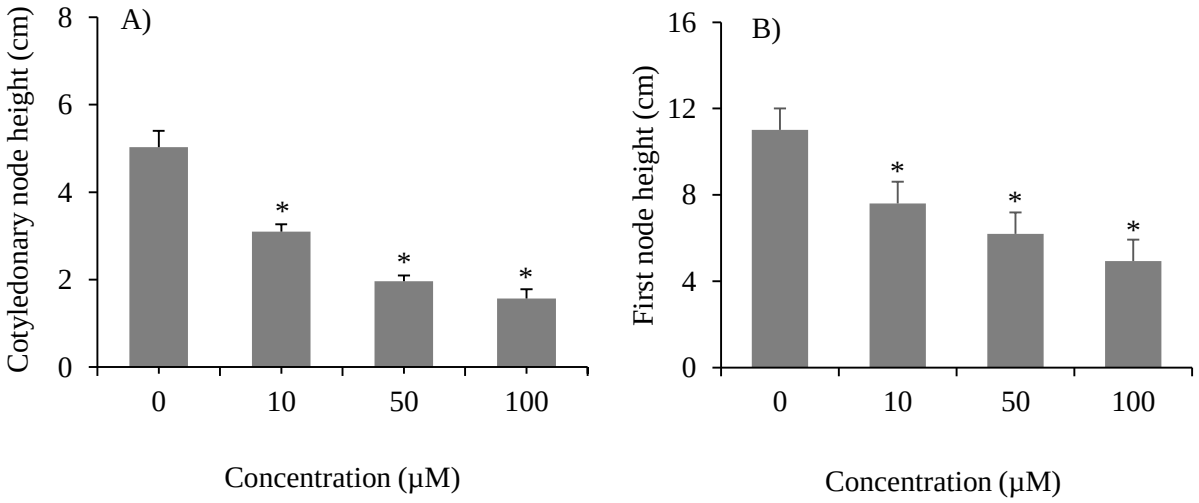

Figure 3.3. Effect of different concentrations of PGR #2 (A, B) on cotyledonary (A) and first (B) node heights of cv. TH37004. Data are means of 20 plants ± SE. Asterisks indicate statistically significant differences between untreated and treated plants (P<0.05; Student's t-test).

The results on the effect of treatment with PGR #3 on cotyledonary and first node heights were similar to that of PGR #2 (Figure 3.4; Appendix 8, 9). Relative to the untreated control, seed treatment with PGR #3 at 10 µM, 50 µM and 100 µM concentrations significantly decreased cotyledonary node height by 20.8%, 32.1% and 40.6%, respectively (Figure 3.4A). In addition,

the treatments with the same PGR and concentrations significantly reduced first node height by 21.1%, 46.2% and 58.6%, respectively (Figure 3.4B). These results indicated that both PGR #2 and #3 had inhibitory effects on cotyledonary and first node heights. As a result, PGR #1 was selected to further investigate the effect of PGR on lowest pod height.

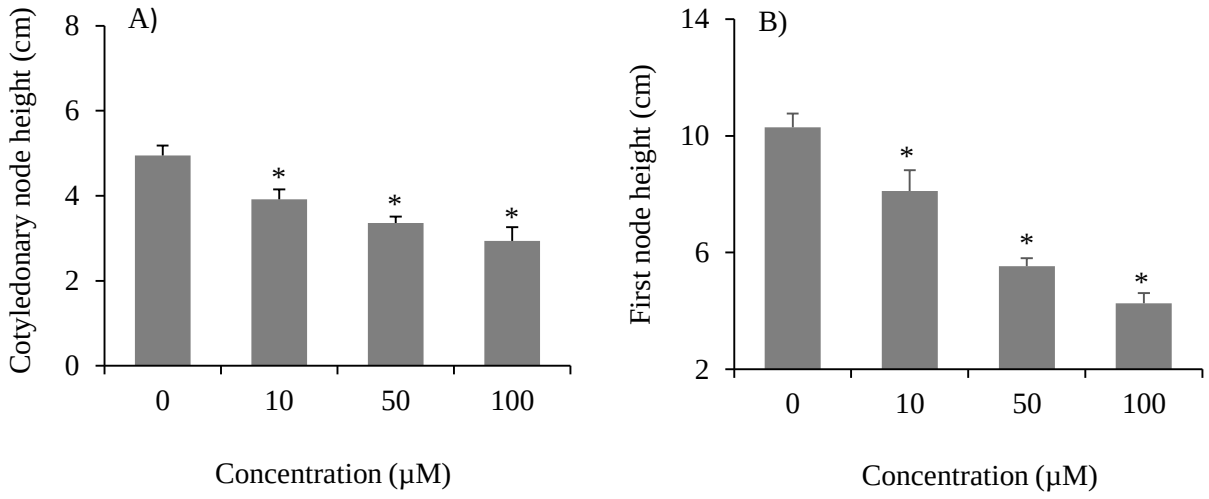

Figure 3.4. Effect of different concentrations of PGR #3 on cotyledonary (A) and first (B) node heights of cv. TH37004. Data are means of 20 plants ± SE. Asterisks indicate statistically significant differences between untreated and treated plants (P<0.05; Student's t-test).

3.3.3. Effect of PGR #1 on first pod height

The lowest pod height was determined in two ways, by measuring the height from the base of the plant to the tip of the lowest pod and the height from the base of the plant to the node bearing the lowest pod. The height of the tip of the lowest pod from the base of the control plants was found to be 10.7 cm, and seed treatment with 50 µM of PGR #1 induced the maximum increase of lowest pod height followed by 100 µM and then by 10 µM (Figure 3.5A; Appendix 10). Relative to the plants grown from untreated control seed, treatment with PGR #1 at 50 µM caused significant

increases in the lowest pod height by 29.1%. However, there was no significant difference in the increase of pod heights measured from the base to the tip of the lowest pod between treatments with 50 µM and 100 µM concentrations (Appendix 10).

Similarly, the increase in height of from the base of the plant to the pod bearing node in response to seed treatment with at 50 µM concentration of PGR#1 was the highest followed by 100 µM and then by 10 µM (Figure 3.5B; Appendix 11). As compared to the control, there was 5.6%, 19.4% and 12.2% increases of pod heights measured from the base to the node bearing the lowest pod by PGR #1 at 10 µM, 50 µM and 100 µM concentrations, respectively. No significant difference was found between the pod heights measured from base to node bearing the lowest pod between 50 µM and 100 µM concentrations (Appendix 11).

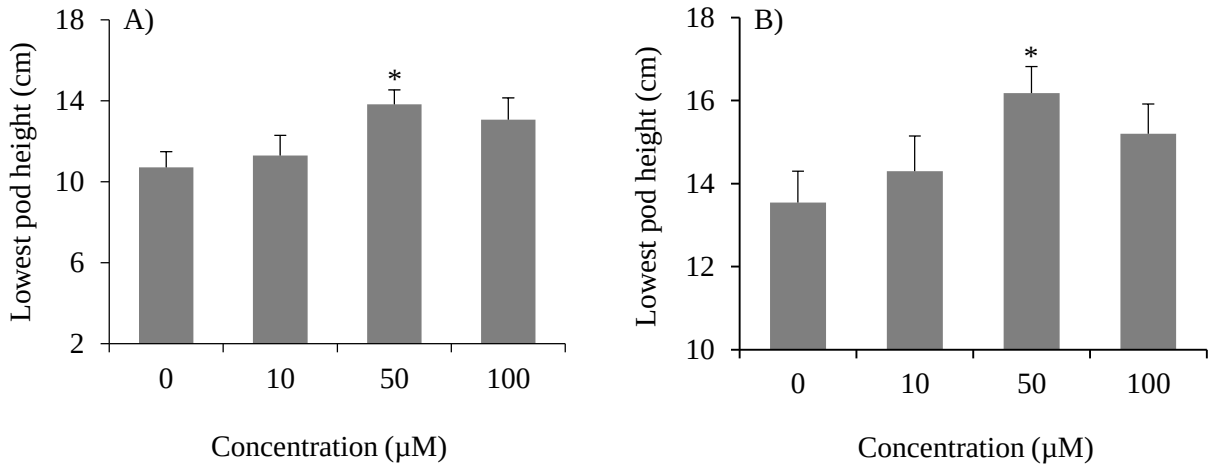

Figure 3.5. Effect of PGR #1 on the lowest pod height of cv. TH37004. Pod height determined by measuring the height from the base to the tip of lowest pod (A) and the node bearing the lowest pod (B). Data are means of 20 plants ± SE. Asterisks indicate statistically significant differences between untreated and treated plants (P<0.05; Student's t-test).

3.3.4. Effect of PGR #1 on the length of subsequent internodes and total plant height

The effect of treating seeds with PGR #1 on the lengths of second, third and fourth internodes was studied. No significant difference in these parameters was found between the control plants and those grown from seeds treated with PGR #1 at all concentrations (Figure 3.6A; Appendix 12). In addition, treatment with 10 µM, 50 µM and 100 µM concentrations of PGR #1 had no significant effect on total plant height (Figure 3.6B; Appendix 13).

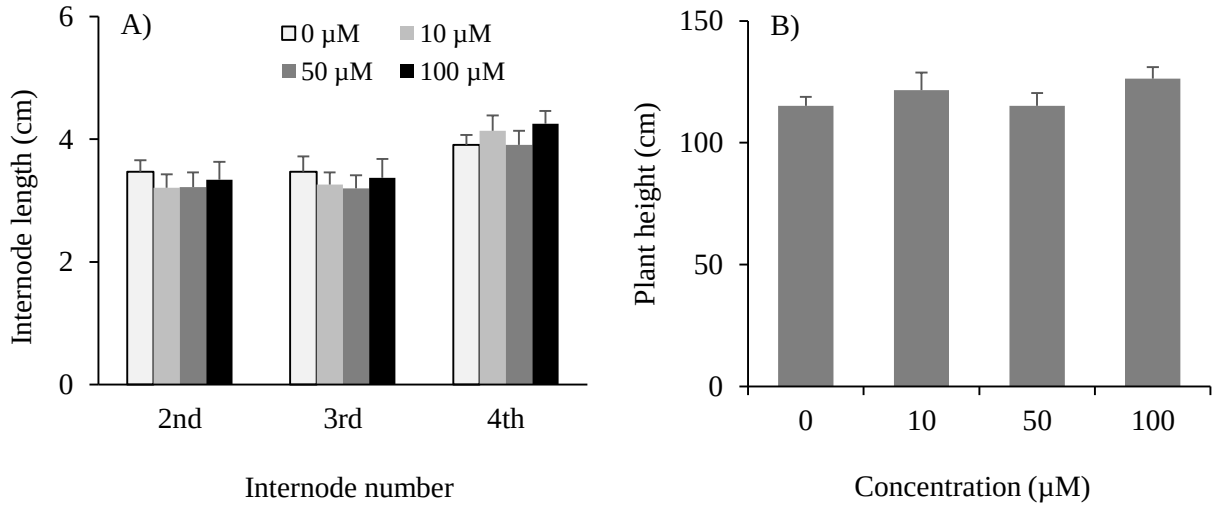

Figure 3.6. Effect of different concentrations of PGR #1 on the lengths of first, second and third internodes (A) and total plant height (B) of cv. TH37004. Data are means of 20 plants ±SE. Asterisks indicate statistically significant differences between untreated and treated plants (P<0.05; Student's t-test).

3.3.5. Effect of PGR #1 on the thickness and bending strength of cotyledonary node and first internodes

Since there was no significant difference between the total height of plants treated with 50 and 100 µM concentrations, the lower concentration i.e. 50 µM, was chosen for determination of the effect of treatment on stem strength. No significant difference in the thickness of cotyledonary and first

internodes was observed between treated and untreated plants (Figure 3.7A). Seed treatment with 50 µM PGR #1 also caused no significant effect on the bending strength of the cotyledonary and first internodes (Figure 3.7B).

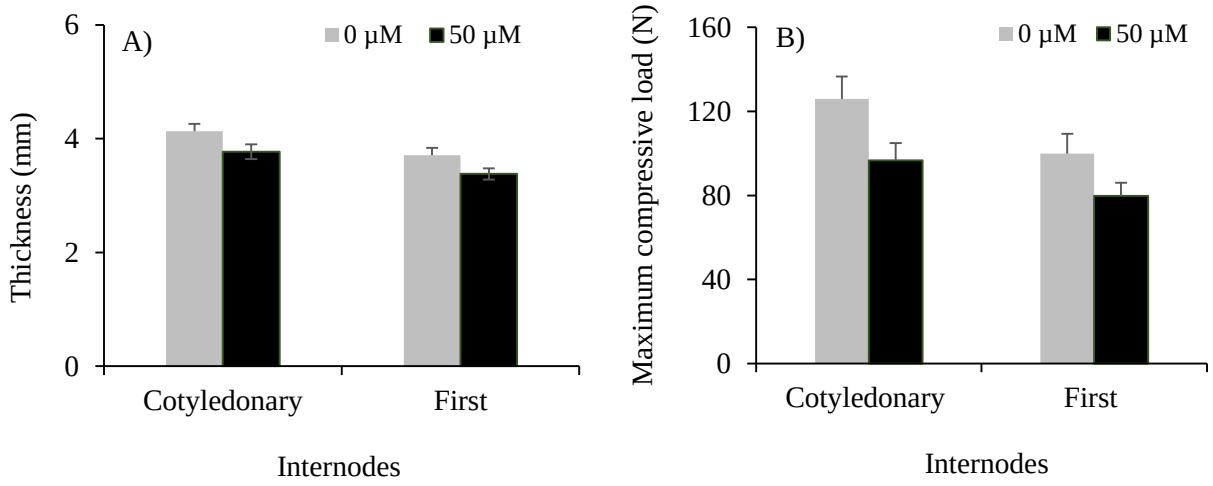

Figure 3.7. Effect of PGR #1 (50 µM) on the thickness (A) and bending strength (B) of cotyledonary and first internodes of cv. TH37004. Data are means of 20-25 plants ±SE. Asterisks indicate statistically significant differences between untreated and treated plants ($P<0.05$; Student's t-test).

3.3.6. Effect of PGR #1 on yield-related parameters

The effect of seed treatment with different concentrations of PGR #1 on different yield-related parameters was examined. As compared to the control, seed treatment with PGR #1 of different concentrations did not have any significant effect on all the yield-related parameters examined in this study including number of pods set per plant, number of seeds produced per plant and the weight of seeds harvested from each plant (Table 3.2, Appendix 14).

Table 3.2. Effect of different concentrations of PGR #1 on yield related parameters[a].

Concentration (μM)	No. of pods/plant	No. of seeds/plant	Seed weight (g)/plant
0	55.65 ± 1.99	118.13 ± 3.78	21.14 ± 1.03
10	53.56 ± 1.29	112.17 ± 3.46	20.48 ± 0.82
50	57.89 ± 3.31	119.65 ± 6.07	22.07 ± 1.30
100	63.19 ± 2.77	121.40 ± 4.51	23.89 ± 0.94

[a]Data are means of 20 plants ±SE

To assess if treatments with different concentrations of PGR #1 affected the ratio of lowest pod height (LPH), which was determined based on the distance between the base of the plant and the tip of the lowest pod or the node bearing the lowest pod, to the total plant height (TPH) was determined. Our results showed that this ratio between LPH and TPH varied between 0.09 to 0.14 which is very low to have any effect on the seed yield (Table 3.3).

Table 3.3. Effect of different concentrations of PGR #1 on lowest pod height to total plant height ratio[a].

Concentration (μM)	0	10	50	100
LPH/TPH[b]	0.09	0.09	0.12	0.10
LPH/TPH[c]	0.12	0.12	0.14	0.12

[a]Data are means of 20 plants

[b]Pod height measured from the base to the tip of the lowest pod

[c] Pod height measured from the base to the node bearing the lowest pod

3.3.7. Expression of gibberellin biosynthetic and catabolic genes

In order to assess differences in gibberellin (GA) biosynthesis and catabolism in the cotyledonary and first internodes in three cultivars that show difference in lowest pod height of cvs. TH37004, Lono and Notus, the expression levels of GA biosynthetic (*GmGA20ox* and *GmGA3ox*) and

catabolic (*GmGA2ox*) genes were studied in the two tissues at three different stages of soybean development including V3, V6 and V9 stages.

3.3.7.1. Expression of gibberellin biosynthetic *GmGA20ox* in the cotyledonary internode

The expression level of *GmGA20ox1* showed very slight increases from V3 to V6 and from V6 to V9 stages (Figure 3.8A). This gene exhibited lower level (2.1-fold) of expression in cv. Lono than cv. TH37004 at V3 stage. However, there was no significant difference in its expression levels in the cotyledonary internodes of the tested cultivars at other two stages.

The expression level of *GmGA20ox2* in all three cultivars increases as the developmental stage increases from V3 to V6 and from V6 to V9 (Figure 3.8B). No difference in the expression level of *GmGA20ox2* was found in the cotyledonary internode of the three cultivars at V3 and V6 stages. However, the expression level of *GmGA20ox2* in the cotyledonary internode at V9 stage was significantly higher (3.2-fold) in cv. Notus than that found in cv. TH37004 (Figure 3.8A). Even if it was not statistically significant, slightly higher expression level of the same gene was found in the cotyledonary internode of cv. Lono than cv. TH37004 at V9 stage.

The expression level of *GmGA20ox3* in the cotyledonary internodes of all the three cultivars also showed an increase as the developmental stage increased from V3 to V6 stage; however, the expression level of the same gene either remained at similar level or showed a decrease when the developmental stage changed from V6 to V9 (Figure 3.8C). The level of *GmGA20ox3* expression in the cotyledonary internode at V3 and V6 stages was significantly higher (> 2-fold) in cv. Notus than cv. TH37004. The same gene had higher level of expression in the cotyledonary internode of cv. Lono than cv. TH37004 at V6 stage. The three cultivars showed similar level of *GmGA20ox3* expression in the cotyledonary internode at V9 stage.

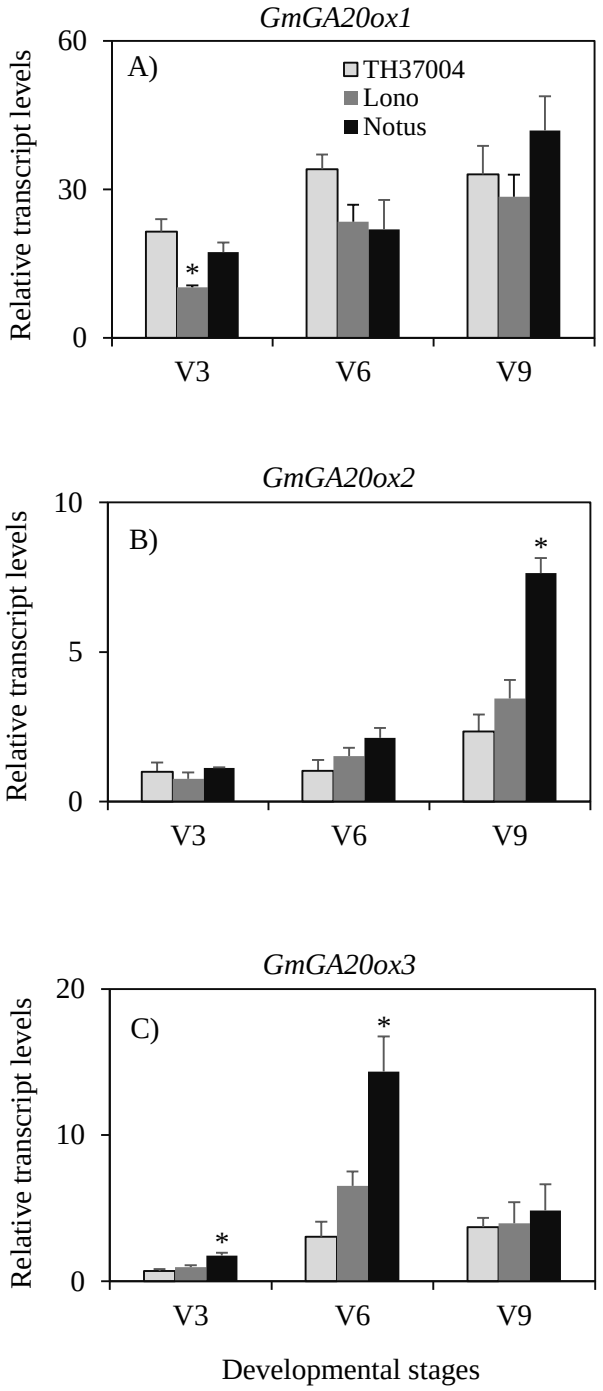

Figure 3.8. Relative expression levels of *GmGA20ox1* (A), *GmGA20ox2* (B) and *GmGA20ox3* (C) genes in the cotyledonary internodes of cvs. TH37004, Lono and Notus at three different developmental stages (V3, V6, and V9). The transcript level of each gene was determined relative to the transcript level of *GA20ox2* in cv. TH37004 at V3 stage which was set to 1. The data are means of 2-3 biological replicates ± SE. Asterisks represent statistically significant difference in expression levels relative to that found in cv. TH37004 within a specific developmental stage.

3.3.7.2. Expression of *GmGA20ox* in the first internode

An increase or similar expression level of the *GmGA20ox1* was found in the first internodes of the three cultivars (Figure 3.9A). However, its expression level in the first internodes of cv. Lono and cv. Notus was significantly lower (~2-fold) than in cv. TH370004 at V3 stage. But, no difference in the expression level of the same gene was found in the first internodes of cv. Notus and cv. Lono. In addition, the expression of *GmGA20ox1* in cv. Notus was significantly downregulated (2.7-fold) at V6 stage relative to its expression in cv. TH37004 at the same stage. There was no difference in the expression level of the same gene between the first internodes of cv. TH37004 and cv. Lono at V6 stage, and between the three cultivars at V9 stage.

The expression level of *GmGA20ox2* in the first internode of the three cultivars showed either a very slight increase or remained at similar level as the developmental stage increased from V3 to V6 and V6 to V9 (Figure 3.9B). Comparison among the three cultivars showed that its expression level in the first internode was higher in cv. Lono (1.6-fold) and cv. Notus (1.8-fold) than in cv. TH37004 at V3 stage. However, cvs. Lono and Notus showed similar expression level of *GmGA20ox2* in their first internodes at V3 stage. No significant difference in the expression level of first node *GmGA20ox2* was observed among the three cultivars at V6 and V9 stages.

A decrease or similar expression level of *GmGA20ox3* was found as the developmental stage increased from V3 to V6 and from V6 to V9 (Figure 3.9C). Similar to that found for *GmGA20ox2*, the expression level of *GmGA20ox3* in the first internode was significantly higher in cv. Lono (1.5-fold) and cv. Notus (2.3-fold) than in cv. TH37004 at V3 stage. However, cv. Notus showed higher expression level of *GmGA20ox2* in its first internodes at V3 stage than that found in cv. Lono. All the three cultivars displayed a similar expression level of *GmGA20ox3* in the first internode at V6 and V9 stages.

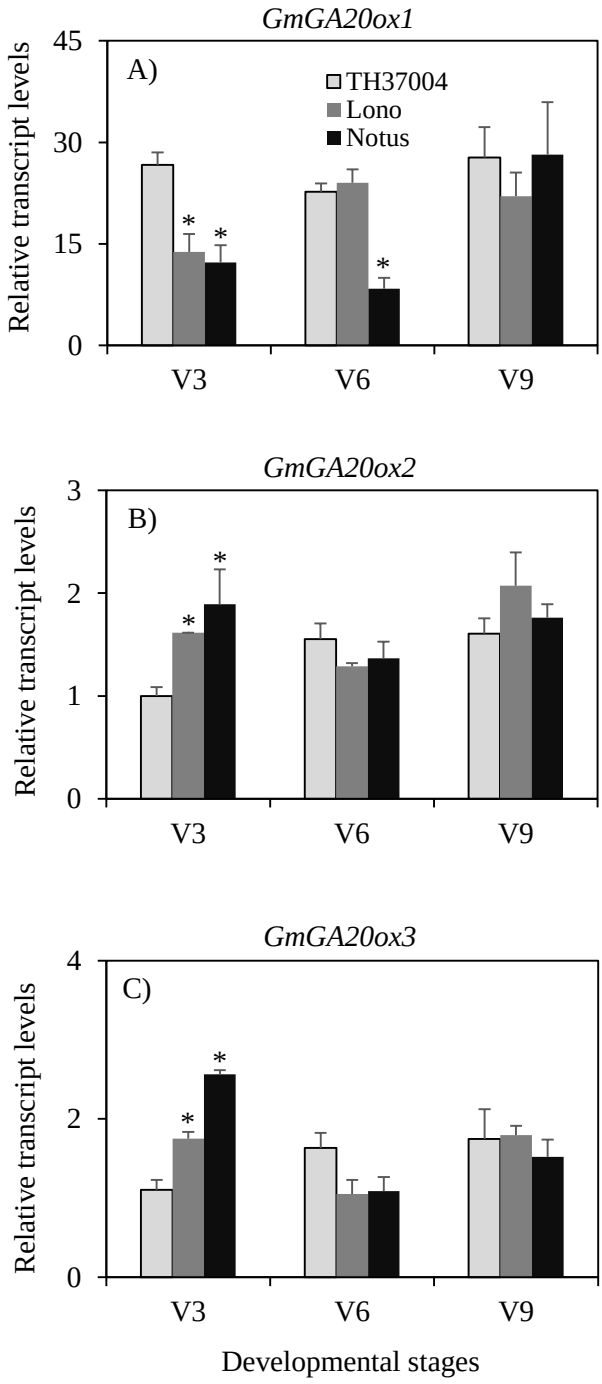

Figure 3.9. Relative expression levels of *GmGA20ox1* (A), *GmGA20ox2* (B) and *GmGA20ox3* (C) genes in first internodes of cvs. TH37004, Lono and Notus at three different developmental stages (V3, V6, and V9). The transcript level of each gene was determined relative to the transcript level of *GA20ox2* in cv. TH37004 at V3 stage which was set to 1. The data are means of 2-3 biological replicates ± SE. Asterisks represent the statistically significant difference in expression levels relative to that found in cv. TH37004 within a specific developmental stage.

3.3.8. Expression of gibberellin biosynthetic *GmGA3ox* genes

The expression levels of GA biosynthetic genes, *GmGA3ox2*, *GmGA3ox3* and *GmGA3ox4* were analysed in the cotyledonary and first internodes of the three cultivars at V3, V6 and V9 stages of soybean development (Figure 3.10).

3.3.8.1. Expression of *GmGA3ox* in cotyledonary tissues of selected cultivars

The expression of levels of both *GmGA3ox2* and *GA3ox3* genes in the cotyledonary internodes of the three cultivars were found to show increases as the developmental stage increased from V3 to V6 and from V6 to V9 (Figure 3.10A, B). The increase in the expression levels of both genes in the cotyledonary internode of cv. Notus was substantial as the developmental stages increased from V3 to V6 stage. When compared with the cotyledonary internode of cv. TH37004, cv. Notus had significantly higher expression levels of both genes (over 3-fold) at V6 and V9 stages. In addition, the cotyledonary internode of cv. Lono showed higher expression level of *GA3ox3* than that found in cv. TH37004 at both V6 and V9 stages. However, there was no difference in the expression level of *GmGA3ox2* between the internodes of the two cultivars at both stages. Both the *GmGA3ox2* and *GmGA3ox3* genes showed no difference in their expression levels in the cotyledonary internodes of the three cultivars at V3 stage.

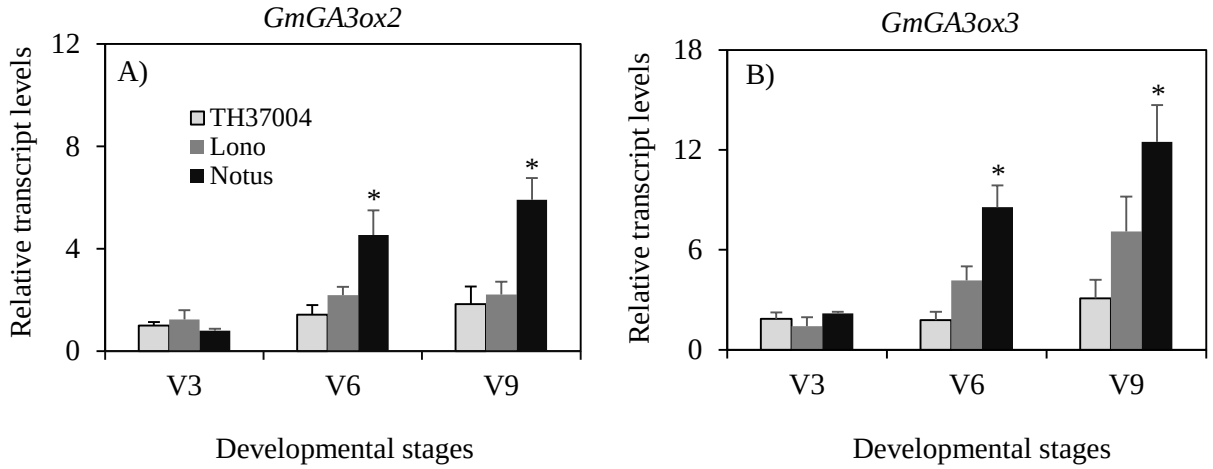

Figure 3.10. Relative expression levels of *GmGA3ox2* (A), *GmGA3ox3* (B) genes in the cotyledonary internodes of cvs. TH37004, Lono and Notus at three different developmental stages (V3, V6, and V9). The transcript level of each gene was determined relative to the transcript level of *GA3ox2* in cv. TH37004 at V3 stage which was set to 1. The data are means of 2-3 biological replicates ± SE. Asterisks represent statistically significant difference in expression levels relative to that found in cv. TH37004 within a specific developmental stage.

3.3.8.2. Expression of *GmGA3ox* in the first internode

The expression level of *GmGA3ox2* in the first internode showed a decrease from V3 to V6 stages and a similar level was maintained from V6 to V9 stage (Figure 3.11A). In the case of *GmGA3ox3*, its expression level in cv. TH37004 and cv. Lono showed an increase from V3 to V6 and from V6 to V9 stage, while in cv. Notus its expression level decreased from V3 to V6 stages but increased from V6 to V9 stage (Figure 3.11B). At V3 stage, the first internode of cv. Notus showed significantly higher expression levels of *GmGA3ox2* (2.3-fold) and *GmGA3ox3* (2.8-fold) than cv. TH37004 while the first internodes of cv. TH37004 and cv. Lono at V3 stage showed similar levels of expression. There was no difference in the expression level of *GmGA3ox2* and *GmGA3ox3* in the first nodes of the three cultivars at V6 and V9 stages.

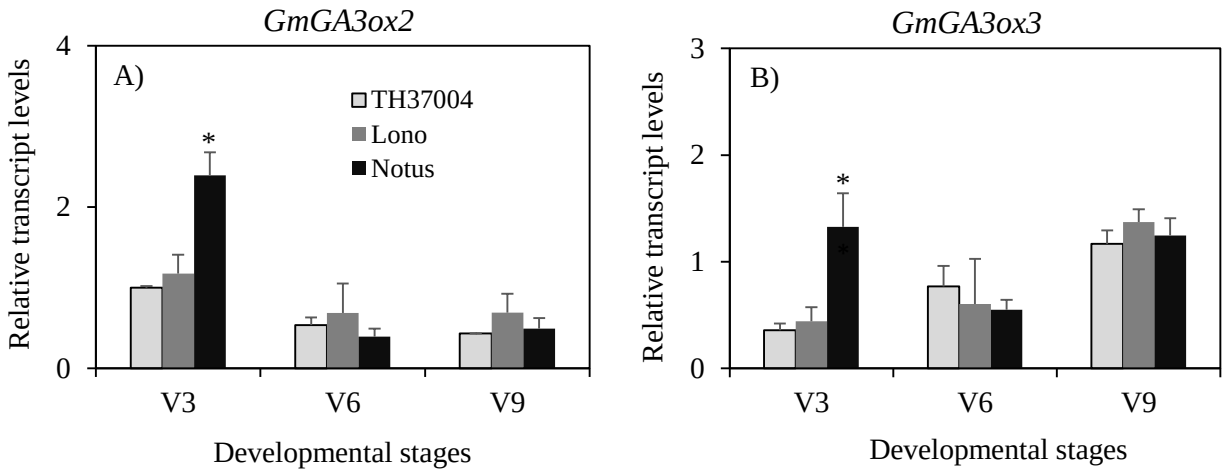

Figure 3.11. Relative expression levels of *GmGA3ox2* (A), *GmGA3ox3* (B) genes in the first internodes of cvs. TH37004, Lono and Notus at three different developmental stages (V3, V6, and V9). The transcript level of each gene was determined relative to the transcript level of *GA3ox2* in cv. TH37004 at V3 stage which was set to 1. The data are means of 2-3 biological replicates ± SE. Asterisks represent statistically significant difference in expression levels relative to that found in cv. TH37004 within a specific developmental stage.

3.3.9. Expression of gibberellin catabolism *GA2ox* genes

The expression level of GA catabolic genes *GmGA2ox1*, *GmGA2ox2-1*, *GmGA2ox2-2, GmGA2ox4 and GmGA2ox8* were analysed in the cotyledonary and first internodes of the three cultivars at V3, V6 and V9 stages of soybean development.

3.3.9.1. Expression of *GmGA2ox* in the cotyledonary internode

The expression level of *GmGA2ox1* in the cotyledonary internodes exhibited an increase from V3 to V6 and from V6 to V9 stages in each cultivar (Figure 3.12A). However, the there was no significant difference in its expression levels among the cultivars at any of the stages.

There was no difference in the expression level of *GmGA2ox2-1* between the cotyledonary internodes of cvs. TH37004 and Lono at V3 and V6 stages (Figure 3.12B). The expression level

of this gene in the cotyledonary internodes of cvs. Lono and Notus increased from V6 to V9 stages, but there was no marked change in cv. TH37004. Higher expression level *GmGA2ox2-1* (2-fold) was observed in the cotyledonary internode of cv. Notus at V6 stage than cvs. TH37004 and cv. Lono, which showed similar expression levels. At V9 stage, its expression level in the cotyledonary internodes of cvs. Lono and Notus was markedly higher (>4-fold) than cv. TH37004.

An increase in the expression level of *GmGA2ox2-2* in the cotyledonary internode was found from V3 to V6 stage in cv. TH37004 but its expression level did not change between V6 and V9 stages (Figure 3.12C). The expression level of the same gene in the cotyledonary internodes of cv. Lono did not show any change between V3, V6 and V9 stages but showed slight decreases from V3 to V6 and V6 to V9 stages cv. Notus. Comparison of the expression level of *GmGA2ox2-2* among the cotyledonary internodes of the three cultivars showed higher level of expression (2.7-fold) in cv. Notus than the other two cultivars at V3 stage. There was no difference in the expression level of the same gene between cvs. TH37004 and Lono. At V6 stage, the expresion levels of *GmGA2ox2-2* in the cotyledonary internodes of cvs. Lono were lower (2.5-fold) than cv. TH37004. At V9 stage, the internodes of the three cultivars had similar expression level of *GmGA2ox2-2*.

The expression level of *GmGA2ox4* in the cotyledonary internode decreased from V3 to V6 but increased from V6 to V9, and its expression level between V3 and V9 stages was similar in cv. TH37004 (Figure 3.12D). In the other two cultivars, its expression level remained at similar level between V3 and V6 but showed an increase from V6 to V9. This genes showed similar expression level among the cotyledonary internodes of the three cultivars at V6 and V9 stages but it had higher level of expression (2.7-fold) than the other two cultivars at V3 stage.

The expression level of *GmGA2ox8* in the cotyledonary internode of cv. Lono showed an increase from V3 to V9 stage (Figure 3.12E). Whereas its expression level in cv. TH37004 was found to be similar between the three stages. In case of cv. Notus, the expression levels of this gene was found to increase from V3 to V6 but decreased from V6 to V9 stage. No difference in its expression level was observed among the internodes of the three cultivars at the V3 stage. However, the expression level of *GmGA2ox8* in the cotyleodnary internode of cv. Notus at V6 and V9 stages was 3.4 and 1.9-fold higher than cv. TH37004, respectively.

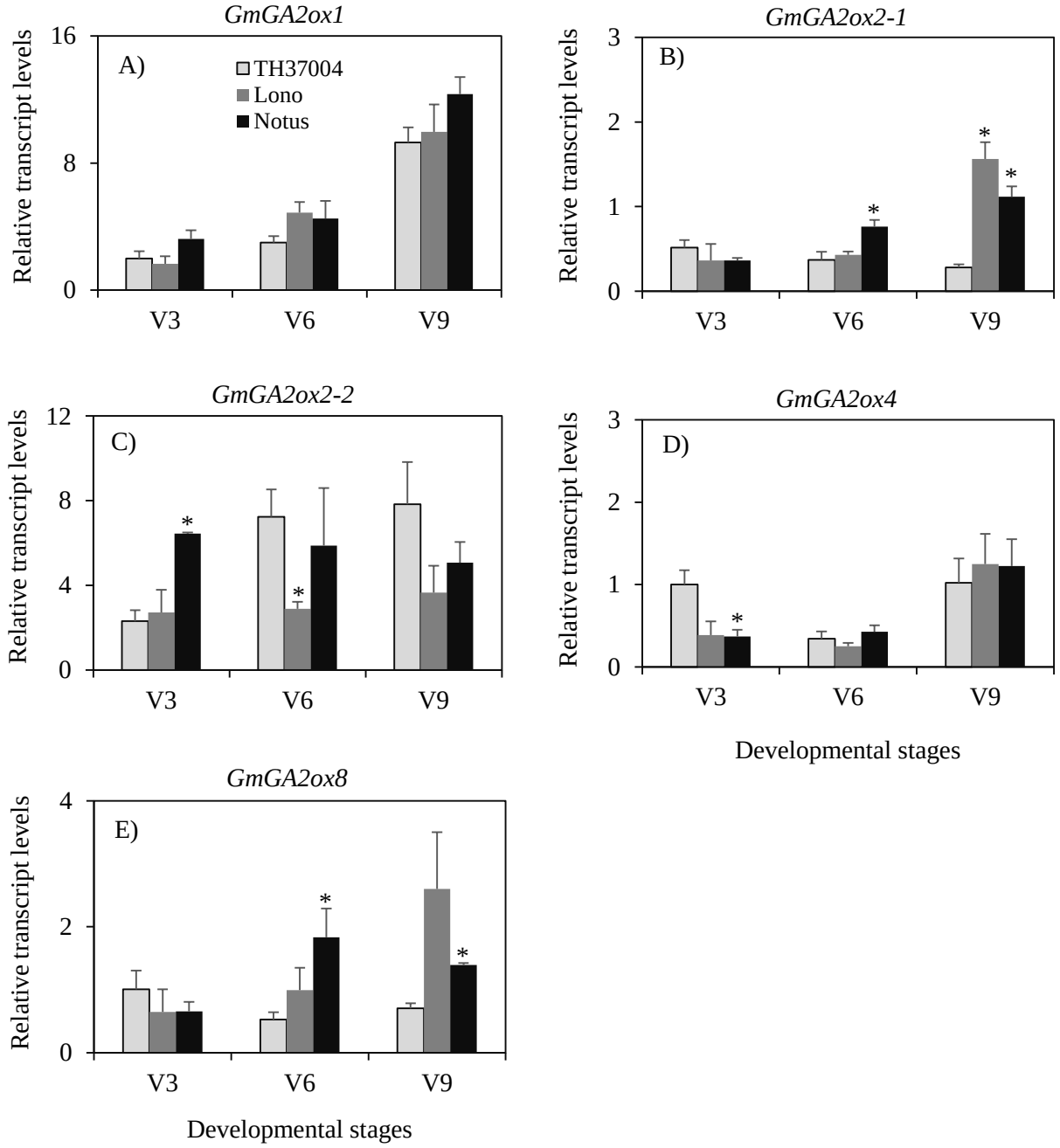

Figure 3. 12. Relative expression levels of *GmGA2ox1* (A), *GmGA2ox2-1* (B), *GmGA2ox2-2* (C), *GmGA2ox4* (D), *GmGA2ox8* (E) genes in the cotyledonary internodes of cvs. TH37004, Lono and Notus at three different developmental stages (V3, V6, and V9). The transcript level of each gene was determined relative to the transcript level of *GA2ox4* in cv. TH37004 at V3 stage which was set to 1. The data are means of 2-3 biological replicates ± SE. Asterisks represent statistically significant difference in expression levels relative to that found in cv. TH37004 within a specific developmental stage.

3.3.9.2. Expression of *GmGA2ox* in the first internode

Expression level of *GmGA2ox1* was found to increase from V3 to V6 and V6 to V9 stage in the first internode of cv. TH37004, however, similar expression level of the gene was found in cv. Lono at V3 and V9 stages and slightly higher at V6 stage (Figure 3.13A). Its expression level in the first internode of cv. Notus decreased from V3 to V6 stage and showed a slight increase from V6 to V9 stage. At V3, the first internode of cv. Notus showed higher (1.8-fold) expression level of the same gene than cv. TH37004. Whereas, no differential expression of the *GmGA2ox1* was shown among the first internodes of the three cultivars at V6 stage. In stage V9, the expression level of *GmGA2ox1* in the first internode of cv. TH37004 was higher (~2-fold) than that found in the first internodes of cvs. Lono and Notus, which showed similar level of expression.

The expression level of *GmGA2ox2-1* in the first internode of cv. TH37004 increased from V3 to V6 and remained at similar level from V6 to V9 (Figure 3.13B). Whereas, the expression level of the same gene showed a decrease from V3 to V9 in the first internode of cv. Notus. In cv. Lono, the expression level of *GmGA2ox2-1* in the first internode showed a slight decrease from V3 to V6 but an increase from V6 to V9. Comparison between the three cultivars showed no difference in the expression level of the same gene in the internodes at V3 stage. At V6 stage, *GmGA2ox2-1* showed higher level of expression (>2-fold) in the first internode of cv. TH37004 than the other two cultivars, Lono and Notus, which showed almost similar level of expression. By stage V9, the first internode of cv. TH37004 also showed higher expression level (3.9-fold) of *GmGA2ox2-1* than cv. Notus. Similar level of expression was found between cv. TH37004 and cv. Lono.

The expression level of *GmGA2ox2-2* in the first internode of all three cultivars showed an increase from V3 to V6, and its expression level also increased from V6 to V9 in cv. Lono but

showed a decrease in cvs. TH37004 and Notus (Figure 3.13C). There was no significant difference in the expression level of *GmGA2ox2-2* in the first internode between the three cultivars at V3 and V6 stages. At V9 stage, the first internode of cv. Notus had similar expression level of this gene with cv. Lono but showed higher expression level (1.6-fold) as compared to the first internode of cv. TH37004.

While the expression level of *GmGA2ox4* in the first internode was found to be almost constant throughout all the developmental stages of cvs. TH37004 and Lono, and it showed a decrease from V3 to V6 and from V6 to V9 in cv. Notus (Figure 3.13D). No difference in the expression level of *GmGA2ox4* was found in the first internodes of the three cultivars at V3 and V6 stages but at V9 stage cv. Notus had lower expression level than the other two cultivars. Similar expression level of *GmGA2ox4* was found in the first internodes of cv. TH37004 and Lono at V9 stage.

Increases in the expression levels of *GmGA2ox8* were exhibited by the first internodes of cvs. TH37004 and Lono from V3 to V6 and from V6 to V9 (Figure 3.13E). The expression level of the same gene in the first internode of cv. Notus showed a slight decrease from V3 to V6 stage and remained constant from V6 to V9 stage. When the cultivars are compared, the expression level of *GmGA2ox8* in the first internode at V6 and V9 stages was >2-fold lower in cv. Notus, respectively, than that observed in cvs. TH37004 and Lono, which showed similar level of expression. At V3 stage, no difference in the expression level of *GmGA2ox8* was found in the first internodes of the three cultivars.

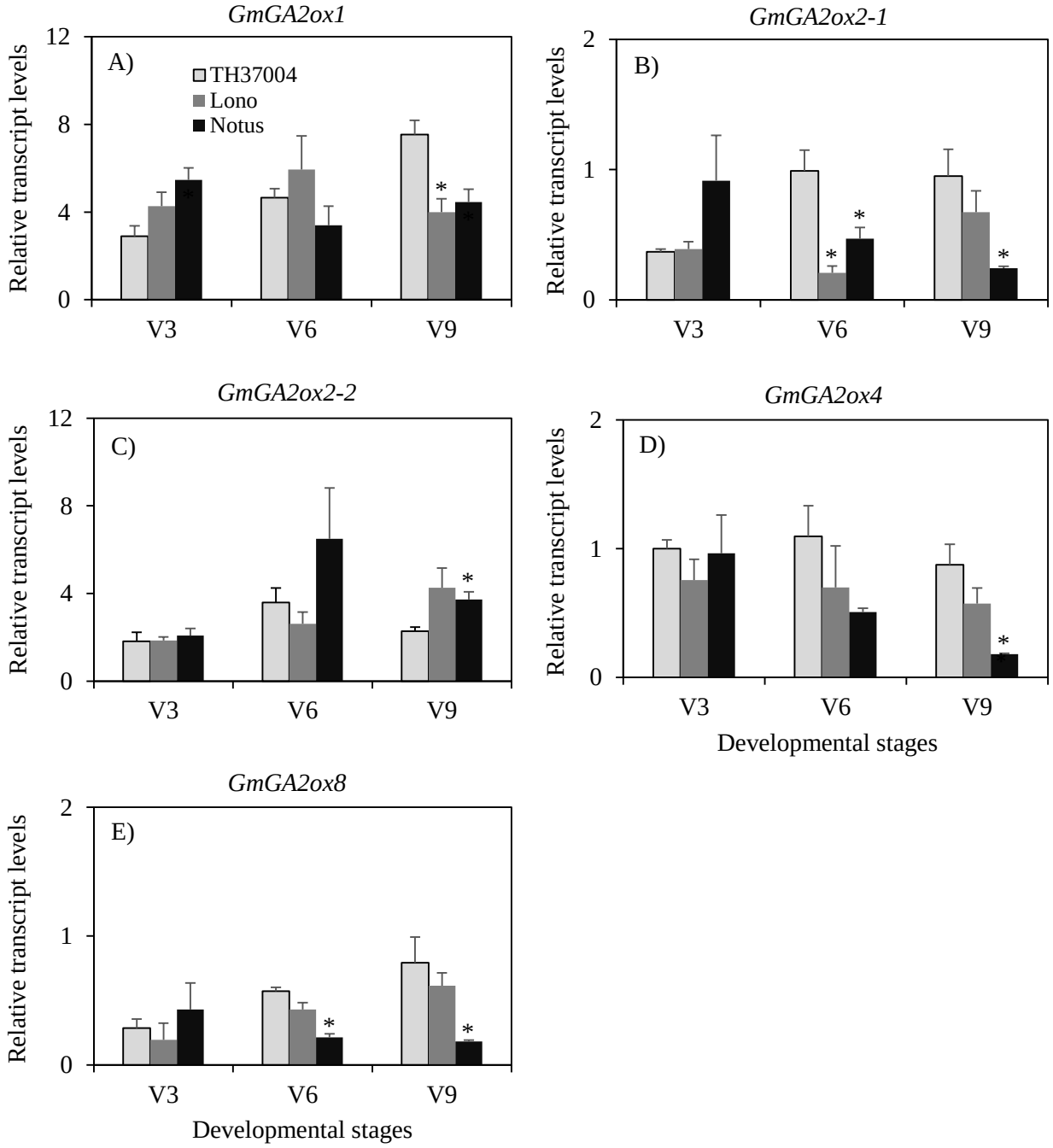

Figure 3.13. Relative expression levels of *GmGA2ox4* (A), *GmGA2ox2-1* (B), *GmGA2ox8* (C), *GmGA2ox2-2* (D), *GmGA2ox1* (E) genes in the first internodes of three cultivars- TH37004, Lono and Notus at three tested stages (V3, V6, and V9) of soybean development. The transcript level of each gene was determined relative to the transcript level of *GA2ox4* in cv. TH37004 at V3 stage which was set to 1. The data are means of 2-3 biological replicates ± SE. Asterisks represent the statistically significant transcript levels and are measured relative to the transcript level of cv. TH37004 at that specific stage.

3.4 Discussion

This thesis project studied three soybean cultivars, namely TH37004, Lono and Notus, to investigate the role of different plant growth regulators on lowest pod height, one of the impediments in soybean production. To this effect, the three cultivars were grown under controlled environment condition, and cv. TH37004 was found to have the shortest cotyledonary and first nodes, and therefore lowest pod heights followed by cvs. Lono and Notus. The lowest pods originate from either the cotyledonary or first nodes. These results are in agreement with the results of a previous study that was conducted under filed conditions (personal communication, Ms. Kristen Podolosky, Agronomist-in-Residence of Soybean and Pulse Crop Agronomy in the Department of Plant Science). These results imply that soybean cultivars are under genetic control and maintain their variation in lowest pod height despite differences in growth conditions (Bakal *et al*., 2017; Beikufner *et al*., 2019).

The effects of GA and/or cytokinin based PGRs on cotyledonary, first node and lowest pod heights were investigated using the cultivar with shortest pod height, cv. TH37004. It is well established that GA plays an important role in promoting stem elongation through enhancing the mechanical extensibility and loosening of cell wall in the stem cells, which is regulated by the actions of xyloglucan endotransglycosylase/hydrolases, pectin methylesterases, expansins and pectin lyases (Behringer *et al*., 1990; Yang *et al*., 1996; Park *et al*., 2017; Gazara *et al*., 2019). Seed treatment with different concentrations of GA resulted in increased cotyledonary and first node heights of the cv. TH37004, indicating that GA has a role in increasing the elongation of the cotyledonary and first internodes in soybeans. The increase in the length of these internodes might be due to the increased cell elongation that is considered as the main factor responsible for stem elongation in response to GA_3 (Khatun *et al*., 2016). Similar to the results of this study, seedlings

grown from soybean and cotton seeds treated with GA have been reported to exhibit increased seedling height (Bradford and Ewing 1958, Bird and Ergle 1960). In addition, application of GA has been shown to cause dramatic increases in the length of oat internodes (Kaufman and Dayanandan, 1983), lettuce hypocotyls (Jones and Moll, 1983) and tomato internodes (Rai *et al*., 2006), and the formation of longer shoots in soybeans (Travaglia *et al*., 2009). The results of this study showed that an increase in the concentration of GA leads to an increase in the cotyledonary and first node, and lowest pod heights. However, no significant difference was observed between the effects of 50 µM and 100 µM concentrations applied to the seed, indicating that the use of 50 µM of GA will be economically feasible for its practical application. These results are in agreement with previous finding which indicated that hypocotyl growth in soybean is directly related to the amount of GA applied (Bensen *et al*., 1990). Moreover, GA has been shown to increase lowest pod height in soybean such that the pods were set high enough to be accessible to combine harvesting (Mislevy *et al*., 1989; Leite *et al*., 2003; Campos *et al*., 2010). It has been suggested previously that yield losses due to inaccessibility of lowest pods to combine cutter could be avoided if the lowest pods are set at height of at least 12 cm from the base of the plant (Ramteke *et al*., 2012). The findings of this study showed that seed treatment with GA containing PGRs can increase lowest pod height to up to16 cm above the soil, indicating the potential significance of treatment with GA containing PGRs in combating stubble losses in soybean. Although increasing the lowest pod height is required to avoid stubble losses, the ratio between lowest pod height and total plant height should be maintained minimum to obtain higher seed yield (Leite *et al*., 2003; Ghodrati *et al*., 2013). The first pod height to total plant height (FPH/TPH) ratio in this study varied from 0.09 to 0.14 which is too low to have significant effect on seed yield (Table 3.3); there was no significant correlation between FPH and seed yield. This result is not in agreement with

the findings of previous study that showed negative correlation between FPH and seed yield (Oz *et al*., 2009; Pirdashti *et al*., 2010; Ramteke *et al*., 2012; Kang *et al*., 2017).

In contrast to the previous reports discussed above, treatment of soybean seeds with the GA containing PGR did not have significant effect on the length of internodes beyond the first internode and total plant height. Moreover, no effect of the treatment was evident on different yield parameters including number of pods/plant, number of seeds/plant and seed weight per plant. The effect of GA applied to the seeds appears to be limited to developmental processes that occur during the early phases of plant development, suggesting sufficient amounts are not available to be translocated to affect developmental/physiological processes occurring during later stages of growth (Leite *et al*., 2003). We found GA treatment of soybean seeds did not affect the elongation of internodes above the first two internodes, total height of the plant at maturity and total seed yield. In addition, soaking seeds with GA solution and subsequent spraying of GA to the plants at vegetative and flowering stages was not shown to affect germination, plant height and yield-related parameters (Domingo, 1981; Mislevy *et al*., 1989; Zhang *et al*., 1997). To the contrary, soybean seeds primed with GA (100 mg/l) has also been found to have a positive impact on yield parameters, particularly number of pods/plant, number of seeds/pod and seed weight (Agawane and Parhe, 2015). Application of GA_3 at 20 mg/l at bud initiation stage, led to an increased seed yield and harvest index in soybean (Upadhyay and Ranjan, 2015). The studies therefore suggest specific response of seed yield to different concentrations of GA_3 when applied at different growth stages.

Lodging has been reported to contribute to approximately 11 to 32% yield loss in soybeans (Woods and Swearingin, 1977), and lodging resistance is mainly determined by stem strength and plant height (Kashiwagi and Ishimaru, 2004; Ma, 2009). This study also examined the effect of

treatment with the PGR containing GA on the thickness and strengths of the cotyledonary and first internodes. Using bending strength, which measures the maximum force the stem could bear before breakage, as an estimate of stem strength, the results of this study showed the absence of significant effect of treatment with the GA containing PGR on the thickness and bending strength of cotyledonary node and first internodes. Previous studies conducted on tamarind and corn also demonstrated that application of GA_3 at V6 stage, does not have significant effect on stem diameter (Dantas *et al*., 2012; Keshavarzi *et al*., 2014).

In addition to the GA containing PGR, the effects of seed treatment with a PGR containing cytokinin in the form of BA, and another PGR containing a mixture of GA and cytokinin on cotyledonary and first node, and lowest pod heights were tested using cv. TH37004. Although previous reports have shown that cytokinins such as BA promote cell division (Hartmann *et al*., 2001) and thereby result in shoot formation (Leclerc *et al*., 2006), treatment with cytokinin containing PGR rather delayed growth, leading to the observation of decreased cotyledonary and first node heights and overall height of plants of cv. TH37004; the plants did not grow to their full potential. This result suggests the negative effect of cytokinin applied in the form seed treatment on the vegetative growth of soybean plants. Similar results were reported by Riedell *et al* (1985), where application of BA as seed treatment caused delayed emergence, decline in growth of internodes and reduction in the size and number of leaf cells in soybean. However, another study that used a different cultivar for its study reported the absence of any effect of cytokinins (30 mg/l) on any of the physiological parameters of soybean when applied at vegetative stages (Leite *et al*., 2003) while cytokinin was shown to be able to reduce pod abortion and thereby increase the number of pods, and increase seed yield when applied at R3 stage and 7 days after anthesis in soybean cvs. BRGSO luziania and IX93-100, respectively (Passos *et al*., 2011; Nonokawa *et al*.,

2012). Therefore, it is likely that responses to cytokinins by soybean plants vary with cultivars, concentrations applied, and the timing of its application as reported previously (Leite *et al*., 2003; Asil *et al*., 2011).

Given that GAs promote stem elongation through cell enlargement and cytokinin is known to enhance cell division, treatment with a combination of GA and cytokinin are expected to promote uniform and controlled growth of plants, making it grow longer and fuller at the same time (Whipker, 2019). However, PGR containing a mixture of GA and cytokinin inhibited not only the elongation of cotyledonary and first internodes, which led to the observation of shorter cotyledonary and first node heights, and overall growth of the plant in this study, suggesting the antagonistic effect of cytokinin on GA action in soybean when these two are applied together (Leite *et al*., 2003). Similar results were found in bean (*Phaseolus vulgaris*) plants where GA and cytokinin applied together at seedling stage caused a negative effect on the stem elongation (Valio & Schwabe, 1978), and these two hormones were also shown to exhibit antagonistic action in tomato (Fleishon *et al*., 2011). A recent study showed that the antagonistic activity of cytokinin with respect to GA in soybeans is attributed to upregulation of the negative regulators of cytokinin signalling, type A- response regulators (Gazara *et al*., 2019).

As discussed above, the results of this study showed that cv. TH37004 exhibits the shortest cotyledonary and first internodes and lowest pod height as compared to cvs. Lono and Notus, and exogenous GA was able to increase the cotyledonary and first internode lengths and lowest pod height. In order to gain insights into the molecular aspects of GA metabolism that are responsible for the variation in the parameters studied among the three cultivars, the expression patterns of GA biosynthetic and catabolism genes were studied in the cotyledonary and first internodes at three developmental stages (V3, V6, V9). At the V3 developmental stage, this study observed higher

expression levels of *GA20ox* and/or *GA3ox* genes in cotyledonary (*GA20ox3*) and first internode (*GA20ox2*, *GA20ox3*, *GA3ox2* and *GA3ox3*) tissues of cultivars that produce longer internodes/higher cotyledonary and first node heights than the one that produces the shortest internodes/lower cotyledonary and first node heights, cv. TH37004. Since *GA20ox* and *GA3ox* are GA biosynthetic genes, our result suggests increased production of biologically active GA, which is known to induce stem elongation (Reinecke *et al*., 2013), in the internodes of cultivars that produce longer cotyledonary and first internodes. In addition, the GA catabolic gene *GA2ox4* was found to have higher level of expression in the cotyledonary internode of cv. TH37004 that produce the shortest cotyledonary internodes/lowest cotyledonary nodes, suggesting the contribution of GA catabolism in reducing the level of bioactive GA and resulting in shorter internodes phenotype (Rieu *et al*., 2008). Overall, it appears from the results of this study that both GA biosynthesis and catabolism contribute in regulating GA levels and therefore variation in cotyledonary and first internode lengths among soybean cultivars at V3 stage.

In the later V6 and/or V9 developmental stages, lowest expression levels of *GA20ox* (*GA20ox2* and *GA20ox3)* and *GA3ox* (*GA3ox2* and *GA3ox3*) genes were evident in the cotyledonary internodes of the cultivar that exhibited the shortest internode/lowest cotyledonary node, cv. TH37004, suggesting reduced production of bioactive GA might be the reason for the observation of shorter cotyledonary internode length/lowest cotyledonary node height in cv. TH37004 as compared to the other two cultivars included in this study. Higher expression levels of *GA2ox* genes (*GA2ox2-1* and *GA2ox8*) were also observed in the cotyledonary internodes of the cultivars that produce longer cotyledonary internode/higher cotyledonary node height and exhibit higher expression levels of *GA20ox* and *GA3ox* at V6 and V9 stages (cv. Notus), implying positive feedforward regulation of the *GA2ox* genes by the elevated level of GA produced in the tissue.

Previous studies have also reported that *GA2ox* genes are upregulated by GA (Ayele *et al*., 2014), demonstrating the role of such feedforward regulation of GA catabolic genes in maintaining GA homeostasis (Phillips *et al*., 1995, Hedden *et al*., 2012). However, at V6 and/or V9 stages, *GA2ox* genes (*GA2ox1*, *GA2ox2-1*, *GA2ox4* and *GA2ox8*) exhibited higher expression levels in the first internodes of the cultivar that exhibited the shortest first internode length/lowest first node height (cv. TH37004) as compared to the cultivars that displayed longer first internode length, suggesting the role of GA catabolism in regulating bioactive GA levels and therefore variation in first internode length/first node height among the soybean cultivars. The observation of lower expression level of the GA biosynthetic gene *GA20ox1* in the first internode of the cultivar that exhibited the highest first node/longest first internode at the V6 developmental stage might be a result of its feedback regulation by the high GA production in the same tissue. This is in agreement with previous reports in which some GA biosynthetic genes are under feedback control which includes a decrease in their expression by GA (Phillips *et al*., 1995; Mitchum *et al*., 2006; Hedden, 2012; Du *et al*., 2015).

4.0. GENERAL DISCUSSION AND CONCLUSION

Soybean is among the most important leguminous crops used for various purposes such as human consumption, livestock feed and industrial products. Cultivation of soybean is increasing in Canada, however, factors such as occurrence of low pod height resulting in stubble losses during harvest cause substantial economic reductions to producers. Stubble losses in soybean include the pods that stay attached to the stubble when the combine cutters cut higher than the lowest pod height and ultimately the bottom pods remain unharvested. Breeding cultivars with higher lowest pod height is time-consuming and agronomic practices such as altering planting date, plant populations and row spacing do not have a significant impact on this trait. Therefore, the use of plant growth regulators to increase the height of the first two internodes such that the plant bears the lowest pods at a higher height can serve to be an alternative solution to mitigate this problem. Plant growth regulators (PGRs) are organic compounds that affect plant growth and development by promoting, delaying, or stimulating certain hormonally directed plant developmental processes. Therefore, this study investigated the effect of PGRs on enhancing the lowest pod height in soybean.

Gibberellins are the major plant hormones that play important roles in inducing stem elongation by promoting cell elongation. Consequently, the current study involved the use of GA-related PGR in enhancing the internode length using a specific soybean cultivar, cv. TH37004, which exhibits shortest cotyledonary node height, first node height and lowest pod height. This study involved a seed treatment approach and seed treatment with GA and cytokinin related PGRs individually or in combination, and the study found that a specific GA related PGR induces increases in cotyledonary and first node heights., The study found that out of the different concentrations tested the 50 µM of the GA related PGR was effective in enhancing the height of

the node bearing the lowest pods. The study also indicated that the same treatment resulted in increases only in the cotyledonary and first internodes and did not have significant impact on the total plant height and total seed yield per plant. In addition, the treatment was found to have no effect on the thickness and bending strength of the cotyledonary and first internode, suggesting that application of the GA related PGR did not make the plant more vulnerable to lodging. Since the current study was carried out under controlled conditions, performing the study under field conditions is necessary. In addition, it is important to test the effect of other PGRs and methods/stages of application on the parameters considered.

In order to assess the factors responsible for variation in lowest pod height among soybean cultivars, the study performed expression analysis of GA biosynthetic and catabolic genes in the cotyledonary and first internodes at three stages of soybean development (V3, V6 and V9) using three different cultivars that exhibit varied lowest pod heights, namely cvs. TH37004, Lono and Notus, which relatively exhibit lowest, intermediate and highest pod heights, respectively. It is well established that balance between GA biosynthesis and catabolism is responsible for maintaining the level of bioactive GAs in higher plants. This study found higher expression levels of *GA20ox3* in the cotyledonary internode, and that of *GA20ox2, GA20ox3, GA3ox2* and *GA3ox3* in the first internode at V3 stage, in the cultivar with highest pod heights. Since functional deficiency in the GA biosynthetic genes (*GA20ox* and *GA3ox*) resulted in plants with dwarf phenotypes, the findings of this thesis project suggest the importance of these genes in GA level and internode elongation. In addition, increased expression of *GA2ox4* in cotyledonary internode of the cultivar with lowest pod height implies that *GA2ox* has a role in decreasing GA level and internode length as functional deficiency in GA catabolic (*GA2ox*) genes have been shown to result in longer stems. During the later stages of soybean development (V6 and V9), higher expression

levels of *GA20ox3*, *GA20ox2*, *GA3ox2* and *GA3ox3* were observed in the cotyledonary internodes of the cultivar with lowest pod height while lower expression level of *GA20ox1* was evident in the first internode of the cultivar highest first pod height. This result may suggest negative feedback regulation of the GA biosynthetic genes by GA levels in the cotyledonary and first internodes of the cultivar. On the other hand, increased expression of *GA2ox* (*GA2ox2-1* and *GA2ox8*) was detected in the cotyledonary internodes of cultivars with highest first pod height. This may suggest that *GA2ox2-1* and *GA2ox8* expression at later stages of soybean development is subjected to feed-forward regulation by the amount of GA since this is associated with elevated expression levels of *GA20ox* and *GA3ox*. On the contrary, elevated expression levels of *GA2ox* was observed in the first internode of the cultivar with lowest first pod height at V6 and V9 stages, suggesting again the role of catabolic genes in lowering the level of bioactive GAs. However, investigating variations in GA levels among the different cultivars is necessary.

This study also investigated the effect of seed treatment with cytokinin related PGR and a PGR containing a mixture of both GA and cytokinin on lowest pod height. Treatment with of these PGRs resulted in delayed emergence, decreased internode lengths and had an overall adverse impact on growth of the plant. Therefore, of the three PGRs tested, the GA-related PGR when applied alone had the potential of enhancing internode lengths and the lowest pod height.

In conclusion, these findings indicate that the GA related PGRs enhance cotyledonary and first node height, and therefore lowest pod height in soybean without having any negative impact on other developmental and yield related parameters considered in this study. This indicates the potential of using the PGR in mitigating the problem of stubble losses in soybean. The expression study indicates the importance of GA biosynthetic and catabolic genes in regulating GA level, and cotyledonary and first internode lengths, and lowest pod height.

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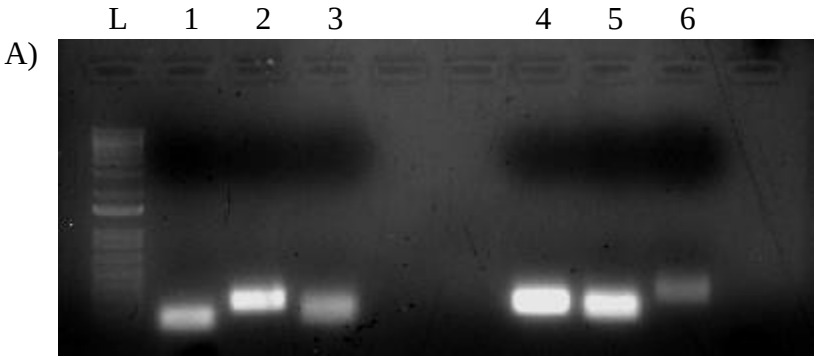
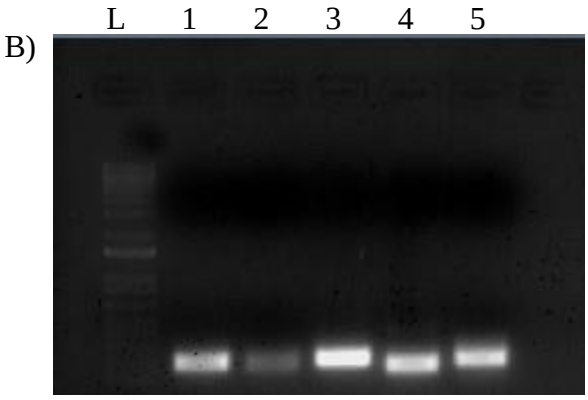
APPENDIX

Appendix 1. Gene specificity of the primers used in qPCR assay. Amplified bands are as follows: A: *GmGA20ox1* (Lane 1), *GmGA20ox2* (Lane 2), *GmGA20ox3* (Lane 3), *GmGA3ox2* (Lane 4), *GmGA3ox3* (Lane 5) and *GmGA3ox4* (Lane 6); B: *GmGA2ox1* (Lane 1), *GmGA2ox2-1* (Lane 2), *GmGA2ox2-2* (Lane 3), *GmGA2ox4* (Lane 4) and *GmGA2ox8* (Lane 5). Lanes marked as L refer to 1 kb DNA ladder.





Appendix 2. Comparison of cotyledonary node height among cvs. TH37004, Lono and Notus (P<0.05; Student's t-test).

Cultivars	Average[a] (cm)	SE	t-test (TH37004 vs.)	t-test (Lono vs.)
TH37004	3.21	0.13		
Lono	4.25	0.35	0.01	
Notus	4.95	0.27	0.00	0.12

[a]Data are means of 15 plants, SE = Standard Error.

Appendix 3. Comparison of first node height among cvs. TH37004, Lono and Notus ($P<0.05$; Student's t-test).

Cultivars	Average [a] (cm)	SE	t-test (TH37004 vs.)	t-test (Lono vs.)
TH37004	7.11	0.23		
Lono	9.92	0.42	0.00	
Notus	11.55	0.35	0.00	0.00

[a]Data are means of 15 plants, SE = Standard Error.

Appendix 4. Comparison of the effects of seed treatment with different concentrations of PGR #1 on cotyledonary node height of cv. TH37004 ($P<0.05$; Student's t-test).

Concentrations (µM)	Average[a] (cm)	SE	t-test (0 µM vs.)	t-test (10 µM vs.)	t-test (50 µM vs.)
0	4.84	0.21			
10	5.82	0.35	0.02		
50	6.54	0.46	0.02	0.68	
100	6.41	0.47	0.00	0.32	0.60

[a]Data are means of 20 plants, SE = Standard Error.

Appendix 5. Comparison of the effects of seed treatment with different concentrations of PGR #1 on first node height of cv. TH37004 ($P<0.05$; Student's t-test).

Concentrations (µM)	Average [a] (cm)	SE	t-test (0 µM vs.)	t-test (10 µM vs.)	t-test (50 µM vs.)
0	12.00	0.32			
10	14.33	0.45	0.00		
50	16.62	0.72	0.00	0.01	
100	17.59	0.97	0.00	0.00	0.42

[a]Data are means of 20 plants, SE = Standard Error.

Appendix 6. Comparison of the effects of seed treatment with different concentrations of PGR #2 on cotyledonary node height of cv. TH37004 ($P<0.05$; Student's t-test).

Concentrations (µM)	Average [a] (cm)	SE	t-test (0 µM vs.)	t-test (10 µM vs.)	t-test (50 µM vs.)
0	5.03	0.37			
10	3.10	0.16	0.00		
50	1.97	0.13	0.00	0.00	
100	1.57	0.21	0.00	0.00	1.10

[a]Data are means of 20 plants, SE = Standard Error.

Appendix 7. Comparison of the effects of seed treatment with different concentrations of PGR #2 on first node height of cv. TH37004 ($P<0.05$; Student's t-test).

Concentrations (µM)	Average [a] (cm)	SE	t-test (0 µM vs.)	t-test (10 µM vs.)	t-test (50 µM vs.)
0	11.01	0.81			
10	7.61	0.57	0.00		
50	6.19	0.43	0.00	0.05	
100	4.93	0.51	0.00	0.00	0.09

[a]Data are means of 20 plants, SE = Standard Error.

Appendix 8. Comparison of the effects of seed treatment with different concentrations of PGR #3 on cotyledonary node height of cv. TH37004 ($P<0.05$; Student's t-test).

Concentrations (µM)	Average [a] (cm)	SE	t-test (0 µM vs.)	t-test (10 µM vs.)	t-test (50 µM vs.)
0	4.95	0.23			
10	3.92	0.23	0.00		
50	3.36	0.15	0.00	0.04	
100	2.94	0.32	0.00	0.02	0.19

[a]Data are means of 20 plants, SE = Standard Error.

Appendix 9. Comparison of the effects of seed treatment with different concentrations of PGR #3 on first node height of cv. TH37004 ($P<0.05$; Student's t-test).

Concentrations (µM)	Average [a] (cm)	SE	t-test (0 µM vs.)	t-test (10 µM vs.)	t-test (50 µM vs.)
0	10.29	0.47			
10	8.11	0.71	0.00		
50	5.53	0.28	0.00	0.00	
100	4.26	0.35	0.00	0.00	0.01

[a]Data are means of 20 plants, SE = Standard Error.

Appendix 10. Comparison of the effect of seed treatment with PGR #1 on the lowest pod height of cv. TH37004 ($P<0.05$; Student's t-test). Pod height determined by measuring the height from the base to the tip of lowest pod.

Concentrations (µM)	Average[a] (cm)	SE	t-test (0 µM vs.)	t-test (10 µM vs.)	t-test (50 µM vs.)
0	10.71	0.78			
10	11.30	0.99	0.64		
50	13.83	0.71	0.01	0.05	
100	13.07	1.07	0.08	0.24	0.55

[a]Data are means of 20 plants, SE = Standard Error.

Appendix 11. Comparison of the effect of seed treatment with PGR #1 on the lowest pod height of cv. TH37004 ($P<0.05$; Student's t-test). Pod height determined by measuring the height from the base to the node bearing the lowest pod.

Concentrations (µM)	Average [a] (cm)	SE	t-test (0 µM vs.)	t-test (10 µM vs.)	t-test (50 µM vs.)
0	13.54	0.76			
10	14.30	0.85	0.52		
50	16.18	0.64	0.02	0.09	
100	15.20	0.95	0.19	0.49	0.41

[a]Data are means of 20 plants, SE = Standard Error.

Appendix **12**. Comparison of effect of seed treatment with different concentrations of PGR #1 on the lengths of second, third and fourth internodes of cv. TH37004 ($P<0.05$; Student's t-test).

Concentrations (µM)	Average[a] (cm)	SE	t-test (0 µM Vs.)	t-test (10 µM Vs.)	t-test (50 µM Vs.)
Effect on second internode length					
0	3.47	0.19			
10	3.21	0.22	0.38		
50	3.22	0.24	0.42	0.99	
100	3.34	0.29	0.72	0.73	0.74
Effect on third internode length					
0	3.47	0.25			
10	3.26	0.20	0.52		
50	3.20	0.21	0.42	0.84	
100	3.37	0.31	0.79	0.77	0.66
Effect on fourth internode length					
0	3.91	0.16			
10	4.14	0.25	0.45		
50	3.91	0.23	1.00	0.51	
100	4.25	0.21	0.20	0.75	0.31

[a]Data are means of 20 plants, SE = Standard Error.

Appendix 13. Comparison of the effect of seed treatment with different concentrations of PGR #1 on total plant height of cv. TH37004 ($P<0.05$; Student's t-test).

Concentrations (µM)	Average[a] (cm)	SE	t-test (0 µM vs.)	t-test (10 µM vs.)	t-test (50 µM vs.)
0	115.13	3.67			
10	121.65	7.23	0.46		
50	115.15	5.22	1.00	0.47	
100	126.27	4.80	0.08	0.62	0.14

[a]Data are means of 20 plants, SE = Standard Error.

Appendix 14. Comparison of the effect of seed treatment with different concentrations of PGR #1 on yield parameters of cv. TH37004 ($P<0.05$; Student's t-test).

Concentrations (µM)	Average[a]	SE	t-test (0 µM Vs.)	t-test (10 µM Vs.)	t-test (50 µM Vs.)
Effect on number of pods/plant					
0	55.65	1.99			
10	53.56	1.29	0.18		
50	57.89	3.31	0.68	0.18	
100	63.19	2.77	0.06	0.00	0.21
Effect on number of seeds/plant					
0	118.13	3.78			
10	112.17	3.46	0.24		
50	119.65	6.07	0.84	0.29	
100	121.40	4.51	0.58	0.11	0.82
Effect on seed weight/plant					
0	21.14	1.03			
10	20.48	0.82	0.62		
50	22.07	1.30	0.58	0.31	
100	23.89	0.94	0.06	0.01	0.27

[a]Data are means of 20 plants, SE = Standard Error.

ABBREVIATIONS

2,4-D	2,4-dichlorophenoxyacetic acid
2ODDs	2-oxoglutarate-dependent dioxygenases
4-CI-IAA	4-chloroindole-3-acetic acid
ABA	abscisic acid
ACC	1-aminocyclopropane-1-carboxylate
AVG	aminoethoxy vinyl glycine
BA	benzyladenine
BLAST	basic local alignment search tool
CDKs	cyclin dependent kinases
cDNA	complementary DNA
CKX	cytokinin oxidase or dehydrogenase
CPS	*ent*-copalyl diphosphate synthase
CYP375A	cytochrome P450 mono-oxygenase family 375 subfamily A
cZ	cis-zeatin
DEPC	diethyl pyrocarbonate
DMAPP	dimethyl allyl diphosphate
DMAPP	dimethylallyl diphosphate
dZ	dihydrozeatin
EUI	*ELONGATED UPPERMOST INTERNODE*
FPH	first pod height
GA	gibberellins
GA13ox	GA 13-oxidase

GA20ox	GA 20-oxidase
GA2ox	GA 2-oxidase
GA3ox	GA 3-oxidase
GAI	GA insensitive
GAMTs	GA methyltransferase
GGDP	geranylgeranyl diphosphate
ha	hectares
IAA	indol-3-acetic acid
IBA	indole-3-butyric acid
IDP	isopentenyl diphosphate
iP	isopenteyl adenine
iP	isopentenyl
IPA	indole-3-pyruvate
IPT	isopenenyltranferase
KAO	*ent*-kaurenoic oxidase
KO	*ent*-kaurene oxidase
KS	*ent*-kaurene synthases
LOG	lonely guy
LPH	lowest pod height
MEP	methylerythritol 4-phosphate
mT	meta-topolin
MVA	mevalonic acid
NAA	1-naphthaleneacetic acid

oT	ortho-topolin
P450s	cytochrome P450 monooxygenases
PAA	phenylacetic acid
PAMI	prairie agricultural machinery institute
PGRs	plant growth regulators
PME	pectin methylesterases
Rn	reproductive stage of soybean development
RT	reverse transcriptase
SPY	spindly
TAA1	tryptophan aminotransferase
TPH	total plant height
TPS's	terpene synthases
tRNA-IPT	t-RNA-isopentenyltransferase;
Trp	tryptophan
tZ	trans-zeatin
USA	united states of america
VC	cotyledon stage of soybean development
VE	cotyledon emergence stage of soybean development
Vn	vegetative stage of soybean development
XETs	xyloglucantransglycolases
XTH	xyloglucan endotransglycosylase/hydrolases