The Effect of Auxins on Seed Yield Parameters in Wheat, Pea and Canola Grown Under Controlled Environment and Western Canadian Field Conditions.

by

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Abstract

The use of plant growth regulators to aid in efficient crop production is a popular research area in agriculture. Among the classes of plant growth regulators, auxins show some promising characteristics in enhancing the vegetative growth and yielding ability of specific crops. Experiments in this thesis test the hypothesis that foliar application of the auxins 4-chloroindole-3-acetic acid (4-Cl-IAA) and 4-methylindole-3-acetic acid (4-Me-IAA) at an early reproductive development stage of wheat (*Triticum aestivum* L.) canola (*Brassica napus* L.) and field pea (*Pisum sativum* L.), will increase the seed yield under normal and heat stress environments, when the stress occurs during the reproductive stage. For wheat, the cultivar CDC Go (contains the *Rht-B1b* mutant allele imparting a semi-dwarf habit) was the most responsive to auxin treatment in both controlled environment and western Canadian field conditions. Relative to control, foliar application of 4-Cl-IAA (1×10^{-6} M) at BBCH 43-45 growth stages increased grain yield per plant by 20% under controlled temperature conditions and 33% when plants were exposed to high temperature (35^{o}C for 6 h per day for 6 days) during initial flowering in controlled environment experiments. The 4-Cl-IAA (1×10^{-6} M) induced increase in grain yield was associated with an increased number of grains per plant under the heat stress conditions. 4-Cl-IAA (1×10^{-6} M) application also increased grain yield per plot of cv. CDC Go in the 2012 (8%) and 2013 (6%) field seasons at the site with higher mid-season temperatures and lower moisture conditions (Saskatoon). In 2013, applications of 4-Cl-IAA resulted in an increase in grain yield at the Saskatoon site that was also associated with an increase in number of grains per plant. Controlled environment experiments using wheat lines isogenic for the wild-type and mutant alleles of Rht-B1 and Rht-D1 (mutations affecting the responsiveness to the plant

hormone gibberellin which interacts with auxins in plant signaling pathways) suggest that the presence of the dwarfing genes *Rht-B1b* (present in CDC Go) or *Rht-D1b* alone do not directly affect the effectiveness of 4-Cl-IAA to induce increases in grain yield.

In canola controlled environment experiments, 4-Cl-IAA (1×10^{-6} M) at BBCH 51 growth stage increased seed weight per plant in the open pollinated cultivar Peace by 46% under controlled temperature conditions and by 51% when plants were exposed to high temperatures (33^{o}C for 6 h per day for 6 days) during initial flowering. Under western Canadian field conditions, 4-Me-IAA (1×10^{-4} M) increased seed yield per plot of the hybrid cultivar SY4114 by 36% at a field site with fluctuating field temperatures and lower moisture condition during the growing season (Saskatoon in 2013). The 4-Me-IAA (1×10^{-4} M) induced increase in plot seed yield in cultivar SY4114 at the Saskatoon site was associated with increased seed size.

For field pea, under controlled environmental conditions, 4-Cl-IAA (1×10^{-4} M) increased the seed weight per plant of the cultivar Carneval by 38% under controlled temperature conditions, and by 38 to 56% (4-Cl-IAA application at 1×10^{-4} to 1×10^{-6} M) when plants were exposed to high temperatures (35^{o}C for 6 h per day for 4 days) during initial flowering. The 4-Cl-IAA-induced increase in seed weight per plant was a reflection of increased number of seeds per plant. 4-Me-IAA application increased the seed weight per plant (at 1×10^{-4} to 1×10^{-7} M) by 61 to 79% under controlled temperature conditions, but had no effect on seed yield when plants were exposed to high temperatures during initial flowering. An earlier 4-Me-IAA application timing (when the floral buds were tightly clustered inside the stipule leaves at the stem apex) was more effective in increasing seed yield per plant (14% higher) and seed size (4.6% higher) than hormone application when the first flowering node of the main stem was near or at anthesis.

Overall, these data suggest that one application of the auxins 4-Cl-IAA or 4-Me-IAA prior to or at flowering has the ability to increase seed yield and/or seed yield components of wheat, canola and field pea crops. Further testing of the effects of 4-Cl-IAA and 4-Me-IAA on these crop species are required to broaden our knowledge of the conditions and genotypes, necessary to obtain optimal auxin response for increased seed yield under a variety of environmental conditions.

Preface

All the experiments in this thesis were designed by the Ozga/Reinecke lab, Plant BioSystems Group, Department of Agricultural, Food, and Nutritional Science, University of Alberta. The auxins (4-Cl-IAA and 4-Me-IAA) used in the experiments covered in this thesis were prepared by Dr. Dennis M. Reinecke (University of Alberta). The field experiments described in Chapters 2, 3, and 4 were a collaborative effort involving the field teams of Syngenta (Western Canadian Group) and the Ozga/Reinecke lab. The St. Albert field trial described in Appendix A of Chapter 2 was a collaborative effort involving the field teams of Dr. Dean Spaner (University of Alberta) and the Ozga/Reinecke lab. As part of the Ozga/Reinecke lab team, I aided with data collection, weed control, and decision making in all field experiments. I organized the field data for statistical analysis and performed all the statistical analyses.

The wheat cv. Harvest greenhouse experiments were designed by the Ozga/Reinecke lab and I aided in data collection and completed the statistical analyses on these data. The wheat experiment using cvs. Pavon and Nesser were designed by the Ozga/Reinecke lab, and I carried out the experiment, collected the data, and performed the statistical analyses on these data. The canola cv. Peace greenhouse experiment described in Chapter 3 was designed and carried out by the Ozga/Reinecke lab and I aided in data collection and completed the statistical analyses. The controlled environment experiments for pea presented in Chapter 4 were designed and carried out by the Ozga/Reinecke lab and I completed the statistical analyses of these data for comparison to those obtain in the field**.**

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2,4D	2,4- dichlorophenoxyacetic acid
4-Cl-IAA	4-chloroindole-3-acetic acid
4-Me-IAA	4-methylindole-3-acetic acid
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
BBCH	Biologische **B**undesanstalt, **B**undessortenamt and **CH**emische Industrie (a scale used to identify the phenological development stages of a plant)
Cf.	Cited from
CRD	Complete randomized design
Cv.	Cultivar
IAA	Indole-3-acetic acid
NAA	1-naphthaleneacetic acid
NIR	Near infrared
RCBD	Randomized complete block design
Rht	Reduced height
SMCS	Soil moisture storage capacity
TKW	Thousand kernel weight

Chapter 1 – Literature review

1.1 Introduction

Canola, wheat and field pea play a major role in global agriculture as they are key staples of the human diet and also produce by-products that comprise animal feed. With population growth and our further understanding of the nutritive qualities and the caloretic value that these crops can provide, there is an increasing trend of worldwide demand for these crops (USDA 2014). However, yields of these crops are limited by changes in environmental conditions. Apart from morphological, physiological and other environmental characters like weed density, fertility level and abiotic stresses (including temperature and drought stress) occurring at flowering and early fruit development have a large negative effect on crop seed yield as they often lead to flower and seed abortion (Saini and Aspinall 1982; Saini and others 1983; Ferris and others 1998). Application of plant growth regulators has shown some promise in reducing the effect of abiotic stress on crop yield when the stress occurs during vegetative and reproductive development (Sakata and others 2010; Sairam 1994; Arfan and others 2007). This introduction, briefly describes the crops used within this thesis. Accordingly, the following literature review summarizes scientific knowledge associated with the application of plant growth regulators (auxin) to crops and their effect on enhancing the vegetative and reproductive parameters with the end goal of increasing crop seed production.

1.2 Introduction to wheat, pea and canola crops

1.2.1 Bread Wheat (*Triticum aestivum* L.)

Wheat is one of the most economically important cereal crops of the poaceae family, which is widely grown around the world in diverse environments. It is one of the first domesticated crops, originating from the Middle Eastern center (includes interior of Asia Minor, all of Transcaucasia, Iran, and the highlands of Turkmenistan) of Vavilov's centers of origin of crops plants and agriculture (Simpson and Conner-Ogorzaly 1986). Bread wheat feeds about 40% of the world's human population and provides 20% of the caloric and protein nutritional

requirements (Gupta and others 2005). It is a major food staple in many countries including China, USA, Canada, Australia, India, Pakistan, France, the Russian Federation, Turkey and Germany (Statista 2014). In the 2013/2014 field season (2013 June to 2014 May), the world wheat production was 650138 million kg (USDA 2014), with Canada (the world's sixth largest wheat producer) producing 34013 million kg (Statistic Canada 2013). In 2012, the total wheat production for western Canada was estimated at 22675 million kg, with 16689 million kg of total spring wheat production and 4199 million kg of durum wheat production (Canadian Grain Commission 2012). The provinces of Alberta, Manitoba and Saskatchewan are the major wheat growing areas of Canada (Canadian Grain Commission 2012).

Climate is an important factor determining the sustainability of wheat crop production. The differential response of cultivars to environmental changes (genotype x environment interaction) will determine the type of cultivars grown in a specific climatic region. The optimum temperature for wheat during its growth cycle is generally between 17 to 23^{o}C (Porter and Garwith 1999). The time and temperature relationship required for plant growth and development is known as thermal time and it is measured using heat units. The total heat unit requirement from germination to wheat crop maturation is calculated in Saskatoon by using 15^{o}C as the average daily temperature and 103 days (spring wheat; 1550 heat units) and 147 days (winter wheat; 2200 heat units) for crop maturation (U of S 2014). The daily rate of water use by wheat is dependent upon various factors like climatic conditions, the density of crop vegetation, and crop growth stage (Ashley and others 2001). Wheat can be grown in a wide range of soils. But, soils with a clay loam or loam texture with good structure and moderate water holding capacity is preferred (U of S 2014). The soil pH for wheat production should range from 6 to 8 (FAO 2013). The crop is moderately tolerant to soil salinity, but the salinity should not exceed 4 mmhos/cm in the upper soil layer during germination (FAO 2013).

Based on its end-use, wheat is categorized into several classes. With respect to seed hardness, wheat is categorized as hard, medium or soft. Wheat is categorized as white, amber or red based on bran color. There are two categories of wheat based on the growing seasons, spring and winter. In this thesis research, we used eight hard red spring wheat cultivars; AC Lilian, AC Harvest, CDC Go, AC Unity, 5604 HR CL, WR859 CL, Nesser and Pavon.

Cv. AC Harvest: Plants have an average height of 84 cm with awn-less tapering-shaped spikes. Grains are small to medium-sized with a short length and medium width, and a broad elliptical shape (Fox and others 2010). Thousand kernel weight (TKW) is about 37g (Fox and others 2010). AC Harvest has good resistance to the leaf rust, excellent resistance to stem rust, moderate susceptibility to common bunt, moderate resistance to loose smut, and poor resistance to Fusarium head blight. This cultivar has good lodging and shattering resistance (Fox and others 2010).

Cv. AC Lillian: This cultivar is hard red spring wheat that has weak to medium anthocyanin content in the coleoptiles (Canadian Food Inspection Agency 2015). Plants have an average height of 92 cm with apically awnleted spikes that are fusiform to oblong, mid-dense, mid-long, erect to slightly nodding, with glumes that are mid-wide to wide (Depauw and others 2004). Grains are medium-sized, mid-wide, mid-long, and elliptical to ovate with rounded to angular cheeks. The TKW is approximately 36 g. AC Lillian has poor Fusarium head blight resistance. This solid stem variety with fair lodging resistance and resistance to seed shelling due to wind is well adapted to Canadian Prairies. AC Lillian has elevated grain protein concentration combined with high grain yield and yielded significantly more grain and protein than cv. Harvest based on data from the 2002 Saskatchewan Advisory Council on Grain Crops (Depauw and others 2004).

Cv. CDC Go: This cultivar was developed by the Crop Development Center at the University of Saskatchewan (Personal communication with Dr. Pierre J. Hucl, University of Saskatchewan). It has an average plant height of 85 cm with awnleted spikes that are nearly 8.6 cm long excluding the awns (Canadian Food Inspection Agency 2015). The plants have strong stems and a semi-dwarf growth habit (Personal communication with Dr. Pierre J. Hucl, University of Saskatchewan) that yielded 3% higher than AC Barrie in the Western Bread Wheat Cooperative Test from 2000 to 2002 conducted at Swift Current, Central Saskatchewan and Parkland areas. TKW is approximately 42 g. CDC Go is resistant to bunt, moderately resistant to leaf rust, and stem rust, and moderately susceptible to loose smut in the wheat growing areas of Western Canada. This cultivar has good lodging and shattering resistance (Alberta Agriculture and Rural Development 2014).

Cv. 5604 HR CL: This cultivar has the Clearfield (CL) herbicide tolerance gene modification, making it tolerant to specific broadleaf herbicides (Alberta Agriculture and Rural Development 2014). Plants have an average height of 87 cm. This cultivar produces awnleted spikes that are tapering, medium density, and white in color with erect attitude at maturity (Canadian Food Inspection Agency 2015). Kernels are dark to medium red, medium size, medium length and width, broad elliptical shape and have rounded cheek (Canadian Food Inspection Agency 2015). The TKW is about 33 g. 5604 HR CL has strong stems with high yield potential, yielding 105% of AC Barrie in 2008/2009 test experiments (Alberta Agriculture and Rural Development 2014). This cultivar is resistant to bunt, loose smut, and stem and leaf rust, and moderately resistant to Fusarium head blight. 5604 HR CL has good lodging resistance (Alberta Agriculture and Rural Development 2014).

Cv. AC Unity: Plants have a semi-erect growth habit with an average height of 90 cm. Spikes are awnleted oblong in shape, medium density, erect and white at maturity, with medium-length awns in relation to the spike length (Canadian Food Inspection Agency 2015). Grains are oblong in shape, small to medium-sized with short to medium length and medium width (Fox and others 2009). There is weak to medium intensity of anthocyanin coloration of the stems of AC Unity at maturity. This cultivar is high yielding; with grain yields 116% of AC Barrie (in Central Bread Wheat Cooperative experiments at Manitoba and Saskatchewan in 2004-2006) (Fox and others 2009). The TKW is about 36g. According to the test results of Cereal Research Center, Manitoba, AC Unity has resistance to the prevalent races of leaf rust, stem rust and common bunt, moderate resistance to loose smut, and it is susceptible to Fusarium head blight (Fox and others 2009). Unity had greater lodging resistance than the check varieties AC Barrie and Superb.

Cv. WR859 CL: This cultivar has the clear field herbicide tolerance gene modification, making it tolerant to specific broadleaf herbicides. Plants have an average height of 81 cm (Alberta Agriculture and Rural Development 2014) with awnleted spikes that are tapering shape, medium density, incline attitude and white at maturity (Canadian Food Inspection Agency 2015). Kernels are hard red type, dark red color, medium sized, short to mid-long, narrow to mid-wide and broad elliptical to elliptical shape (Canadian Food Inspection Agency 2015). The TKW is about 35 g. WR859 CL has high yielding ability, which yields 11% higher yield than AC Barrie

(Manitoba and Saskatchewan seed variety experiments 2011). This cultivar is resistant to leaf rust, common bunt and loose smut, and moderately resistant to Fusarium head blight (Alberta Agriculture and Rural Development 2014).

Pavon and Nesser cultivars: We used hard red spring wheat cultivars that were isogenic for the mutant alleles Rht-D1 (cvs. Pavon tall, *Rht-D1a* and Pavon semi-dwarf, *Rht-D1b*) and Rht-B1 (cvs. Nesser tall, *Rht-B1a* and Nesser semi-dwarf, *Rht-B1b*). Reducing plant height is a dynamic or gradual process in crop domestication and modern breeding. Introduction of dwarfing genes into wheat cultivars in 1960s, tremendously increased the worldwide wheat production and played a major role in green revolution (Davies 2003; Khush 1999). There are many genes associated with a semi-dwarf growth habit in wheat (Ellis and others 2002). They are known as *reduced height* (*Rht*) genes and Norin 10 derived genes; *Rht-B1b* and *Rht-D1b* (previously known as *Rht1* and *Rht2* respectively) were found to prevent the action of gibberellins (Gale and others 1981). Cultivars with those genes are well known to be tolerant to lodging and have an increased harvest index compared with tall cultivars (Gale and others 1981). Fischer and Stockman (1986) suggested that the increased kernel number in semi-dwarf compared with tall wheat cultivars may be due to greater photosynthate partitioning into seeds/ reproductive organs than into vegetative parts of the plant.

Mathews and others (2006) working on a drought environment trial (DET) in northwestern Mexico, tested a number of wheat cultivars (including Pavon and Nesser) for drought resistance. These researchers reported *Rht-B1b* and *Rht-D1b* outperformed the tall alleles (*Rht-B1a* and *Rht-D1a*, respectively) in both drought and irrigated environments. Moreover, they reported the average increase in yield of semi-dwarf lines compared with tall lines over all environments in the DET trial and an international adaptation trial (IAT: 81 experiments around the world) ranged from 7% to 21%. Sing and others (2001) also reported 10 to 30% higher yield in semi-dwarf cultivars (including Nesser and Pavon) in intermediate and high yielding environments compared with their tall isoline. Butler and others (2005) reported that *Rht-B1b* out yielded *Rht-D1b* under fully irrigated conditions.

Brisson and others (2001) compared five wheat cultivars (four bread wheat cultivars including Nesser semi-dwarf and one durum wheat cultivar) in terms of their vegetative growth, water use, phenological development, biomass accumulation and grain yield, under irrigated and

water-stressed (during vegetative growth) conditions in the north-west of Buenos Aires province of Argentina in 1995. According to Brisson and others (2001), leaf area duration (integration of leaf area index and time) was higher (400 m^2m^{-2} days) in Nesser under irrigated condition, but moderate (100 m^2m^{-2} days) under drought stress condition compared with other tested cultivars. Nesser required 950 growing degree days (GDD) to reach to the flag leaf stage under irrigated condition and harvesting occurred 300 GDD earlier in drought condition than under irrigated condition. Nesser produced a vigorous root system under irrigated and drought-stress conditions, and obtained a higher harvest index compared with the durum wheat cultivar (cv. Aconchi) However, Nesser yielded uniformly under irrigated and water stressed (during vegetative growth) condition.

Boogaard and others (1996) compared three bread wheat *(Triticum aestivum* L.) cvs Katya, Nesser, and Mexipak under control and drought stress conditions in a growth chamber experiment. They found that the cultivars with higher root growth rates and higher proportions of root biomass (Nesser and Katya) also had higher leaf growth rates, higher proportions of their biomass as leaves and higher leaf area ratios. These same cultivars had lower rates of transpiration per unit leaf area or unit root weight and higher biomass production per unit water use.

Onyibe (2005) compared two bread wheat cultivars developed from Norin 10 (Siete ceros [maturing in about 110-115 days] and Pavon semi-dwarf [maturing in about 90 - 102 days]) for growth and yield differences under different irrigation regimes (60, 75 and 90% available soil moisture) under field conditions for three years (1995-1997) at the Institute for Agricultural Research, Kadawa, Nigeria (11^o 39'N, 08^o 27'E, 500m asl). In this study, Onyibe (2005) found Pavon had a higher harvest index, more tillers and spikes/m^2 (three year average: 547 tillers/m^2 and 446.6 spikes/m^2) and a larger grain size (three year average: 40.85 g TKW), but had shorter plants, lower grain number/spike and matured earlier (3 year average: 88 days to mature) than Siete ceros under all irrigation levels.

In summary, semi-dwarf wheat cultivars outperformed the tall varieties and show more adaptations to perform well under stressed and non-stressed environments.

1.2.2 Canola (*Brassica napus* L.)

Canola is an annual oil seed crop belonging to the brassicaceae family (Edwards 2008). It is a genetically improved version of rapeseed developed by plant breeders in Saskatchewan and Manitoba, Canada, during the 1960s and 1970s (Bell 1982). To obtain a food grade quality oil, it was produced with less than 2% of erucic acid and lower glucosinolates compared to rapeseed oil (Raymer 2002). Demand for canola oil continues to grow due to its many uses including food grade oil that has lower saturated fatty acid content and other advantages for human health, use as a livestock feed, and also as a feedstock for bio-fuels (Raymer 2002; Dupont and others 1989). Winter canola is planted in parts of China, Ukraine and Russia while spring canola is popular in parts of China, India, Canada and the United States (USDA 2012). Canada is ranked second in canola production, and in 2013 Canada produced 16289720 kg on 8007 ha (Canola Council of Canada 2014).

Canola plants have a tap root system and are able to extract water from deeper soil layers of around 114 to 165 cm (Downey and others 1974). According to Nielsen (1997), canola can extract water from a soil depth of about 165 cm, but 92 to 95% of total seasonal water use comes from growing season precipitation and water extracted from soil depths above 119 cm. Canola does not tolerate water-logged conditions as roots require a good mix of both air and water. Water logging for three days or more during flowering reduces the number of pods per branch as well as seeds per pod (Canola council of Canada 2014). Based on growth chamber experiments conducted for two years, optimum day time temperature during flowering of *B. napus* is around 20^{o}C (Angadi and others 2000). Temperature differences during vegetative and reproductive stages can cause extensive yield losses. In the data presented in my thesis, the following four canola cultivars were used:

Cv. Peace: This cultivar is an open-pollinated early maturing *B. napus* cultivar developed by the canola breeding program at University of Alberta (released as a registered line in 2001). Cv. Peace has slightly lower oil content and higher protein content than the check varieties Maverick, Parkland and Reward. Plant height was taller (>7 cm) than the check varieties and, in cooperative experiments Peace yielded 44% higher than the checks (Alberta Agriculture and Rural Development 2014). It is moderately resistant to the blackleg disease (Alberta Agriculture and Rural Development 2014).

Cv. Pioneer hybrid 45H21: Dupont Pioneer assigned numbers to identify their cultivars and in cultivar 45H21, 4 denote that it is a canola cultivar; 5 denote that it has a medium maturity; H denotes it is a hybrid *napus*, and 2 and 1 are random numbers (Dupont Pioneer 2014). It is a Roundup resistant hybrid canola cultivar which facilitates easy weed control. In a variety comparison trial conducted at Roseau, Minnesota in 2004, cv. 45H21 yielded 2293 kg/ha and the seed oil percentage was 43.5 (Canola Council of Canada 2014). It is a blackleg resistant variety. It has excellent yield potential, higher oil content and very good standability (Dupont Pioneer 2014).

Cv. Canterra 1852 H: This is a Roundup ready early maturity hybrid canola variety with higher yield potential; it yielded 108% of the check cultivars 46A65 and Q2 in 2004 and 2005 performance experiments at 25 or more locations in Canadian Prairie region (Canola performance experiments and grower directory 2011). It is resistant to blackleg and fusarium wilt.

Cv. SY 4114: This cultivar is a new Roundup ready mid-season (95-115 frost free days) hybrid canola variety selected specially for Western Canada developed by Syngenta. According to canola performance experiments at 20 different locations around the Canadian Prairies in 2013, in the long-season zone (more than 115 frost free days) the average yield was 5634 kg/ha, in the mid-season zone (95-115 frost free days) the average yield was 4015 kg/ha, and in the short-season zone the average yield was 4194 kg/ha. In the long-season zone the plant height was 122 cm, in the mid-season zone 111 cm and in the short-season zone 113 cm (Canola performance experiments 2013).

1.2.3 Field pea (*Pisum sativum* L.)

Field pea (*Pisum sativum* L.) is a commercial crop that belongs to the fabaceae family. Field pea is a cool season pulse crop based primarily on its growing pattern of emergence in cool soil conditions and tolerance to early season frost. It is an annual crop grown in temperate zones and at high elevations in the tropics throughout the world including Russia, China, Canada, USA, Australia and Europe (Schatz and Endres 2009). In the 2012 August to 2013 July crop year, field pea was seeded into 1509 kha producing 3341 kt of seed in Canada (Agriculture and Agri-Food Canada 2014). The provinces of Saskatchewan, Alberta, Manitoba and British Columbia are

recognized as the major Canadian field pea growing areas where Saskatchewan accounts for nearly two thirds, and Alberta nearly one third of the dry pea growing area (Agriculture and Agri-Food Canada 2014).

Rhizobium bacteria form a symbiotic relationship with crop legumes, living in root nodules and providing fixed nitrogen to the plant (changes inert N_2 to biologically useful NH_3) (Lindemann and Glover 2003). Specific species of *Rhizobium* can form effective symbiotic relationships with specific crops, such as *R. leguminosarum* with *Pisum*, *Vicia*, *Lathyrus* and *Lens*, and *R. phaseoli* with *Phaseolus* (Beynon and others 1980). Pea plants can fix 17-77 kg N/ ha through symbiotic nitrogen fixation depending on management practices, soil conditions, genetic factors and other environment factors like moisture and temperature (Townley-Smith 1993). Pea, being a high nitrogen producing pulse crop, has many agricultural uses including use as a seed crop, a green manure crop (Townley-Smith 1993), a cover crop (Akemo and others 2000), a rotational crop (Stevenson and Kessel 1996) and an intercrop (Hauggaard-Nielsen and others 2001). Field pea seeds contain high protein levels (averaging 20 to 27 g per 100 gdwt) that are abundant in lysine and tryptophan amino acids (required in the human diet) (Wang and Dawn 2004). Pea seeds also contain high carbohydrate levels (averaging 42 to 49 g per 100 gdwt), and 86 to 87% total digestible nutrients, which makes them an excellent food source for both human and livestock diets (Wang and Daun 2004; Schatz and Endres 2009).

On the western Canadian prairie, daytime temperature for best growth and development of pea ranges from 13 to 23^{o}C (Cutforth and others 2007). A temperature of 27^{o}C will often decrease flower number and flowering duration for field pea (Cutforth and others 2007). Thermal time for full flowering of pea is about 1180^{o}C days or 90-112 calendar days (Iannucci and others 2008). Field pea is well adapted to moist dark brown and black soils in Canada and can be grown in wide range of other soil types ranging from light sandy to heavy clay. Pea is moderately tolerant to water-logged or saline soils (Schatz and Endres, 2009). In this study we used the field pea cultivar Carneval.

Cv. Carneval: 'Carneval' is a semi-dwarf (*le-1*; single base-pair mutation in *PsGA3ox1)* and semi-leafless (*af*; *afila*, leaflets are replaced by tendrils of normal anatomy) field pea cultivar (Reinecke and others 2013) which is adapted to all field pea growing areas of western Canada (Alberta Agriculture and Rural Development 2014). This cultivar has white flowers and yellow

cotyledons at maturity. The TKW is about 222 g and plant field height is about 73 cm. 'Carneval' is resistant to seed coat dimpling, and fairly resistant to mycosphaerella blight, powdery mildew and seed coat breakage. It is susceptible to fusarium wilt (Alberta Agriculture and Rural Development 2014).

1.3 Abiotic stress (drought and heat)

Drought and heat stress are major environmental factors (or abiotic stresses) that affect plant growth and development (Zinn and others 2010; Kalapos and others 1996; Mohommadi and others 2012). Long or short term abiotic stress particularly during sensitive crop growth stages, can result in drastic yield and quality reductions (Zinn and others 2010; Prasad and others 2008). Heat stress affects vegetative and reproductive growth and development in crop plants (Sato and others 2000; Ahmed and others 1992). According to current speculations about global warming and climate change, most agricultural regions will experience more extreme environmental fluctuations (Solomon and others 2007). An inter-governmental panel on Climatic Change (IPCC) reported that the global mean temperature will rise by 0.3°C every decade (Jones and others 1999). The annual mean temperature has increased by about 1.4^{o}C in Canada as a country, and western and northwestern eco-zones showed the strongest warming trends from 1950 to 2007 (Zhang and others 2011). A trend of increasing precipitation and growing degree days was detected along with an increase in annual mean temperature in Canada during this period (Zhang and others 2011). However, an increase in the rate of evapo-transpiration of crop plants due to elevated temperatures would reduce the available moisture content for field crops (Nayirfa and Harron 2001). Rising temperatures can create heat stress conditions and, thus, have a dramatic effect on crop production. Heat stress during the reproductive growth stage of crop plants can cause flower, fruit, and/or seed abortion, leading to lower seed number per pod and/or pod number per plant, and ultimately reduce seed yield (Angadi and others 2000; Farooq and others 2011; Guilioni and others 1997; Faraji and others 2009). Drought stress can directly affect photosynthesis, and reduce assimilate partitioning into reproductive organs (Prasad and others 2008), thereby reducing TKW, spike or pod weight, and final seed yield. Reproductive organs in pea (*Pisum sativum* L.), wheat (*Triticum aestivum* L.) and canola (*Brassica napus* L.) are highly

sensitive to heat stress conditions during development, even a single hot day (>25^{o}C) can reduce the reproductive success of these crop plants.

1.3.1 Effect of heat stress on wheat during the reproductive growth stage

An optimum temperature for wheat during its growth cycle is between 17 to 23^{o}C (Porter and Garwith 1999). Although temperature fluctuations can damage many growth and development stages, wheat is less sensitive to heat stress during vegetative growth than reproductive growth (Porter and Garwith 1999). Balla and others (2009) worked on heat stress at the shooting stage (8th week after planting) and adult stage (12 days after heading) of wheat and found the shooting stage was more sensitive to heat-induced reduction in grain number and grain yield (at the shooting stage grain yield decreased by 49–55%, grain number by 46–63% while at the adult stage grain yield decreased by 32–49% and grain number by 6–17%). A short period of high temperature stress (40^{o}C for 6 h given for 5 days) imposed on wheat plants during the grain filling period (15 days after anthesis (DAA) to 30 DAA) resulted in heat stress-induced reductions in grain yield and mature individual kernel weight (Stone and Nicolas 1995). In wheat, heat stress can affect grain yield and other yield parameters including TKW, grain number per spike, spike weight, and spike number per plant. Temperatures above 20^{o}C (up to 30^{o}C) between ear initiation and anthesis substantially reduce the wheat grain number per spike due to reduction in grain set (Saini and Aspinall 1982). The number of spikelets per spike or number of grains per spike can also be affected by high temperatures during early spike development in winter wheat (Johnson and Kanemasu 1983). Other studies also reported grain number, grain weight and grain yield reductions due to heat stress during reproductive development (Spiertz and others 2006; Rahman and others 2009; Balla and others 2009). Pollen and ovule sterility, changes in normal functions of somatic tissues or inhibition of early cell divisions in the fertilized embryo-sac, thus preventing its transition to a seed, were identified as reasons behind the grain set reduction in heat stress-affected wheat plants during anthesis (Saini and Aspinall 1982). Rijven (1986) also found that high temperature (30-35^{o}C range) reduced the conversion of sucrose to starch by reductions in starch synthase activity (in both *in vivo* and *in vitro* experiments) in the wheat endosperm, and this was correlated with reductions in starch accumulation in wheat grains. Rahman and others (2009) worked with 10 spring-type wheat

cultivars and found cultivar variation with respect to grain yield under heat stress conditions. Dupont and others (2006) found that moderate heat (24/17^{o}C day/night) with post-anthesis mineral application (N:P:K 20:20:20; 0.1g per pot per day) increased the grain and flour protein content and grain weight compared with no mineral application at post anthesis. There was no effect of fertilizer application on final yield under a high heat regime (37/28^{o}C day/night), as high temperature compressed development without disrupting coordinate synthesis of gliadins and glutenin subunits.

1.3.2 Effect of heat stress on canola during the reproductive growth stage

Growth chamber and field experiments have shown that seed production is reduced in *Brassica napus* L*., B. juncea* L. and *B. rapa* L., when the plants were exposed to high temperature stress conditions (Morrison and Stewart 2002). This effect has been attributed to three different phenomena occurring during the reproductive growth stage of these canola species; 1) reduction in flower number before anthesis; 2) reduction in flower fertility due to pollen sterility or damage to the ovary or stigmatic surface; and 3) reduction in the plant capacity to produce pods and seeds after fertilization (Morrison and Stewart 2002). *B. napus, B. juncea* and *B. rapa* plants subjected to 35/15°C light/dark temperatures during early flowering for 7 days had a reduced rate of seed production (Angadi and others 2000). *B. napus* plants grown at 27/ 17°C light/dark temperatures throughout their life were found to be almost totally sterile (Morrison 1993). Polowick and Sawhney (1988) found both male and female sterility in plants (*B. napus* cv. Westar) which were grown at 32^{o}C/ 26^{o}C day night temperatures from their 1st leaf stage to maturity. Heat stress decreased seed yield of canola (*B napus* L. cvs. Monty, Oscar, and Range) compared with non-heat stressed plants (Aksouh and others 2001). The effect of heat stress on seed yield (when applied to canola plants 29 days after opening of first flower) was more severe under short-term heat shock conditions (exposure to 40°C for 4 h in the middle of each day for 5 days from an ambient temperature of 21^{o}C) than when the temperature was gradually increased over 5 days (from 21^{o}C to 40°C over 5 days by adding 5^{o}C for daytime temperature each day) (Aksouh and others 2001). Young and others (2004) reported that short-term heat stress during the reproductive growth stage (one week or two weeks of high temperature stress [8/16 h dark/light, 18°C night, ramped at 2°C h±1, over 6 h, to 35°C for 4 h,

ramped at 2°C h±1 back to 23°C for 6 h] given at 50% flowering stage) can induce the production of lateral inflorescences, and thereby result in increased seed and silique production of canola *B. napus* (cv. DH12075). In general, high temperatures at flowering are more delirious to final seed yield than high temperatures at early pod development (Angadi and others 2000). Under high heat stress (35/18^{o}C day/night), 15% seed weight loss was observed when the stress occurred at the bud formation stage, 58% during the flowering stage, and 77% during the pod development/seed filling stage (Gan and others 2004). Abiotic stress during the seed filling stage (14 to 35 weeks after pollination; Fowler and Downey 1970) can change the canola (*B. napus* cv. Nugget) seed oil quality. Aksouh and others (2001) found that the oil content and composition changed in canola seed (*B napus* cvs. Monty, Oscar, and Range) when heat stress occurred after 29 days from the opening of first flower. In their experiment under heat shock conditions (as described above), the quantity (oil content, protein content) and composition (fatty acid composition and oleic content) of the seed was reduced compared with a gradual increase in temperature (Aksouh and others 2001). Moderately high temperature (28^{o}C/23^{o}C day/night) given continuously for 10 days from 20 to 30 days after flowering increased the seed oil/protein ratio in three different canola (*B. napus* 'Insignia, Emblem', 'Surpass400') cultivars (Aksouh-Harradj and others 2006). Overall, the literature suggests that high temperature changes usually reduce the quality and quantity of canola seeds.

1.3.3 Effect of heat stress on pea during the reproductive growth stage

Seed and fruit abortion due to heat and drought stress at the time of flowering is a particular constraint to legume crop production in Canada and worldwide. Abiotic stress occurring at the flowering and early fruit development stage of crop development has the greatest negative effect on seed yield (up to 40% yield reduction in soybean; Muchow and Sinclair 1986). The heat stress effect on seed yield strongly depends on the magnitude and the duration of the heat stress received. Severe heat stress interrupted pea fruit development regardless of the age or position of the fruit on the plant resulting in fruit abortion (Guilioni and others 1997; Guilioni and others 2003), while moderate heat stress mainly affected the development of reproductive organs in the apical phytomeres (Guilioni and others 1997).

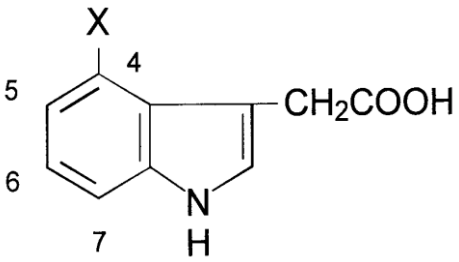
1.4 Use of plant growth regulators to ameliorate abiotic stress effects in plants

Plant hormones are signal molecules that occur naturally within the plant and regulate many processes in vegetative and reproductive growth and development. The use of plant growth regulators to ameliorate abiotic stress effects on seed yield and vegetative growth in agronomic crops is currently of great interest to the plant research community. There are reports that brassinosteroid application can be used to overcome abiotic stresses like drought and salinity effects on wheat (Sairam 1994; Bajguz and Hayat 2009). Salicylic acid is also a plant growth regulator that has been reported to ameliorate salinity stress effects on crops (Arfan and others 2007; Hussain and others 2007). Gibberellin biosynthesis inhibitors are also used to overcome stress effects in field crops usually by reducing vegetative growth, while preserving or enhancing the yielding ability (Cf. Kurepin and others 2013). Abscisic acid is another plant growth regulator which uses to ameliorate the water stress symptoms of plants (it can help with plant survival, but does not directly help with seed yield) (Hiron and Wright 1973; Sharp and others 1994). Auxins have shown some promise in reducing the effects of abiotic stress on plant growth and reproductive development.

1.4.1 Auxins

Auxins are a class of plant growth regulators that are defined by their activity, not by their chemical structure. Four naturally occurring auxins were identified in different plant species. The principal naturally occurring auxin in all higher plants is indole-3-acetic acid (IAA) (Bentley 1958). IAA is synthesized from either the tryptophan or tryptophan-independent pathway in plants (Bartel 1997; Zhao 2010). Indole-3-butyric acid (IBA) is also an endogenous auxin present in many plants including pea (Schneider and others 1985; Simon and Petrasek 2011). IBA can be converted back to IAA and this conversion suggests that IBA may function as a storage form for IAA (Simon and Petrasek 2011). 4-chloroindole-3-acetic acid (4-Cl-IAA) is another endogenous auxin present in some plants and first identified in developing pea (*Pisum sativum* L.) seeds (Marumo and other 1968). 4-Cl-IAA occurs naturally in some species of the Vicieae tribe of the Fabaceae family, and one species outside the Vicieae tribe, *Pinus sylvestris* (Reinecke 1999). Phenylacetic acid (PAA) is the weakest auxin among the four endogenous

auxins (Simon and Petrasek 2011). PAA has been shown to occur in various plants like *Pisum sativum* (Schneider and other, 1985), *Helianthus annuus*, *Lycopersicon esculentum*, *Nicotiana tobacum* and *Hordeum vulgare* (Wightman and Lighty 1982). There are also synthetic compounds designed to mimic the properties of naturally occurring auxins, and the synthetic auxins including 1-naphthaleneacetic acid (NAA) and 2, 4-dichlorophenoxy acetic acid (2,4-D), are widely used in horticultural and field crop industries as growth regulators (NAA) and herbicides (2,4-D). 4-methylindole-3-acetic acid (4-Me-IAA) is a 4-substituted IAA that stimulates pea pericarp growth in a manner similar to the naturally occurring auxin 4-Cl-IAA (Reinecke and others 1999).



X = H: IAA

X = Cl: 4-Cl-IAA

X = CH_3: 4-Me-IAA

Figure 1.1 Structural formulae of IAA, 4-Cl-IAA and 4-Me-IAA auxins

(Source: Modified from Reinecke and others 1999)

As an important plant hormone, auxin modulates diverse plant processes including stem/cell elongation (Thimann 1938; Masuda 1990), apical dominance (Leyser 2005; Blake and others 1983), light and gravity tropism (Went 1942; Estelle 1996), root initiation and development (Thimann 1938), and xylem regeneration/vascular tissue development (Jacob 1952; Mattsson and others 2003). Auxins also have been implicated in the modulation of photoassimilate partitioning into reproductive organs (Bangerth and others 1985; Cole and Patrick 1998), a potentially advantageous function of auxin when considering methods to increase seed yield. IAA application (three concentrations 25, 50 and 100 mg/L) to cowpea (*Vigna sinensis* L.) ten days before and after flowering was reported to increase the number and

weight of pods and number and weight of seeds with the lowest IAA concentration tested (25, 50 mg/L; El-Saeid and others 2010). Shi and others (2014) reported that endogenous and exogenous auxin (IAA) affects the expression of some abiotic stress controlled genes in *Arabidopsis* seedlings, and this response was correlated with improved drought stress resistance in IAA-treated seedlings. Sakata and others (2010) reported that YUCCA (a gene involve in tryptophan dependent auxin biosynthesis) auxin biosynthesis gene expression was reduced under high temperature conditions, and this was correlated with data indicating reduction in anther auxin levels and pollen abortion in barley (*Hordeum vulgare*) and *Arabidopsis*. Multiple applications of auxin (indole-3-acetic acid [IAA], 1-naphthaleneacetic acid [NAA], or 2,4-dichlorophenoxyacetic acid [2,4-D]) at 1×10^{-7}, 1×10^{-6}, 1×10^{-5} and 1×10^{-4} M at 18, 19, 21, and 23 days after sowing in barley and single application of IAA and NAA at 10^{-7} or 10^{-6} M just before increasing the temperatures to 31°C were able to reverse high temperature-induced pollen abortion (Sakata and others 2010). Sarkar and others (2002) sprayed soybean cv. BS-3 with IAA at three different times (two single applications: at 20 days after sowing (DAS) and 42 DAS, and one double application: at 20 DAS and 42 DAS) using two different concentrations (100 and 200 ppm). IAA application increased the number of pods per plant, number of seeds per plant and seed yield in soybean (Sarkar and others 2002). Shahid and others (2013) applied NAA at 0, 50, 100 and 200 ppm to okra (*Abelmoschus esculentus*) cv. Sabz Pari when plants were at the 2-true leaf stage; they observed higher seed yield and thousand seed weight at all concentrations compared with the control.

4-Cl-IAA is generally 1.3 to 50 time more active than IAA in standard biological assays (Reinecke 1999). 4-Cl-IAA showed optimum auxin activity at a lower concentration (10^{-6} M) than the other auxins (IAA, 2,4-D, NAA, and 5-Cl-IAA) used in an Avena coleoptile straight growth bioassay (Böttger and others 1978). In both pea stem and wheat coleoptile section assays, 4-Cl-IAA reached maximum response at a 10-fold lower concentration than IAA, suggesting 4-Cl-IAA is more active than IAA in these assays (Katekar and Geissler 1983). 4-CI-IAA stimulated growth of deseeded fruit (pericarp) of pea *in planta*, but, IAA did not (Reinecke and others 1995). 4-Me-IAA was found to be the second most effective auxin in stimulating deseeded pea pericarp growth (Reinecke and others 1999). It is hypothesized that 4-Cl-IAA-stimulation of the pericarp gibberellin biosynthesis pathway is a major factor in the auxin-

stimulated growth response (Ozga and others 2009). In summary, literature suggests that auxins have the potential to be effectively used in horticultural and field crop research to enhance or sustain crop growth and yielding ability under stress or non-stress conditions.

1.5 Objectives

As auxins have been implicated in the modulation of photoassimilate partitioning into reproductive organs, the main objective of my thesis was to test the working hypothesis that application of auxins, specifically 4-Cl-IAA and 4-Me-IAA, to plants prior to or at initial flowering can increase the seed yield of canola, field pea, and wheat under non-stress and heat stress controlled environmental conditions and under western Canadian field conditions.

The specific objectives of this study were to:

- Determine if 4-Cl-IAA or 4-Me-IAA can increase seed yield of wheat, pea and canola
- Determine the optimum concentration(s) of these auxins for increasing seed yield in these crops.
- Determine which yield component parameters are modified if increased seed yield is achieved (parameters include pod/spike number; number of seeds per pod/spike; increased seed size).

Chapter 2 – The effect of auxins on grain yield parameters on wheat grown under controlled environment and western Canadian field conditions

2.1 Introduction

The world population reached 7.2 billion in 2014 and is expected to increase by more than 2 billion by 2050 (UN 2014). The need to feed an increasing world population, and our further understanding of food nutritive qualities, has led to an increasing demand for wheat and other global foodstuffs worldwide (USDA 2014). However, grain yield in crops including wheat is limited by variability in environmental conditions and associated biotic and abiotic stresses. Abiotic stresses (including high temperature and drought stress) occurring at flowering and early seed development have the greatest negative effect on crop seed/grain yield, as it often leads to flower and seed abortion which collectively lowers grain yield (kg/ha) and grain quality (Angadi and others 2000; Farooq and others 2011; Guilioni and others 1997; Faraji and others 2009). Heat stress during reproductive development can cause anther abortion, pollen sterility, ovule sterility, inhibition of pollen tube growth, fruit abortion, and reduction in photoassimilate partitioning to reproductive organs (Saini and Aspinall 1982; Saini and others 1983; Ferris and others 1998). Application of synthetic or natural auxins has shown some promise in reducing the effect of abiotic stress on plant growth and seed yield. Sakata and others (2010) reported that YUCCA auxin biosynthesis gene expression was reduced under high temperature conditions, and this was correlated with data indicating reduction in anther auxin levels and pollen abortion in barley (*Hordeum vulgare*) and *Arabidopsis*. Multiple applications of auxin (indole-3-acetic acid [IAA], 1-naphthaleneacetic acid [NAA], or 2,4-dichlorophenoxyacetic acid [2,4-D]) at 1 x 10^{-7}, 1 x 10^{-6}, 1 x 10^{-5} and 1 x 10^{-4} M on 18, 19, 21, and 23 days after sowing in barley and single applications of IAA and NAA at 10^{-7} or 10^{-6} M just before increasing the temperatures to 31°C were able to reverse high temperature-induced pollen abortion (Sakata and others 2010). Shi and others (2014) investigated the effect of IAA on ameliorating drought stress symptoms in *Arabidopsis* seedlings. Enhanced drought resistance was observed in wild-type (WT) *Arabidopsis* seedlings treated with 1 mM IAA daily for 7 days and in iaaM-OX transgenic seedlings (which have higher endogenous IAA levels) compared with WT seedlings. Seedlings

of the yuc1yuc2yuc6 IAA biosynthesis triple mutant with (have lower endogenous IAA levels) showed decreased stress resistance in comparison with non-treated WT seedlings (Shi and others 2014). Furthermore, data from gene expression analysis suggested that auxin might positively regulate drought stress resistance in *Arabidopsis* seedlings by regulating stress responsive genes and reactive oxygen species metabolism (Shi and others 2014).

Auxins also modulate photoassimilate partitioning into reproductive organs. Bangerth and others (1985) found a positive correlation between grain dry matter accumulation and estimated IAA grain concentration in the spring wheat (*Triticum aestivum* L.) cultivars Solo and Kolibi during the grain filling period. Furthermore, an agar gel of IAA (1×10^{-7} M) injected into the flag internode lacuna of durum wheat (*Triticum turgidum* cv. Franshawi) promoted the transport of ^{14}C-labelled photoassimilate from the flag leaf to the grain, and grain dry matter accumulation throughout the grain filling phase (Cole and Patrick 1998). These data suggest that endogenous auxins may play an important role in regulating photoassimilate import into developing grains. Three sources of photoassimilate contribute to grain growth, flag leaf and ear photosynthesis, and re-mobilization of non-structural carbohydrate from stem storage (Schnyder 1993). Cole and Patrick (1998) found that the net rates of photosynthesis by the flag leaf and ear were unaffected by IAA treatment that stimulated photoassimilate import into the grain. Studies also suggested that IAA does not affect photoassimilate export from the flag leaf (Cole and Patrick 1998; Bauermeister and others 1980) or re-mobilization of stem reserves (Cole and Patrick 1998). However, IAA did increase sucrose concentration and osmolality of the endosperm cavity sap, the sucrose concentration of the bulk filial sap, and [^{14}C] sucrose accumulation rate of the endosperm cavity (Cole and Patrick 1998). The auxin-enhanced [^{14}C] sucrose accumulation into the endosperm cavity was abolished by membrane transport inhibitors (Cole and Patrick 1998). Taken together, Cole and Patrick (1998) suggested that IAA stimulates photoassimilate flow to and within the developing wheat grain by acting directly on plasma membrane transport processes mediating photoassimilate exchange to and from the endosperm cavity.

Using excised tissue biological assays, it has been demonstrated that 4-chloroindole-3-acetic acid (4-Cl-IAA) is a highly active auxin (Katekar and Geissler 1983; Bottger and others 1978; cited from Reinecke 1999). 4-Cl-IAA showed optimum auxin activity at relatively lower

concentration (1 μM) than the other auxins assayed (IAA, 2,4-D, NAA, and 5-Cl-IAA) using the *Avena* coleoptile straight growth bioassay (Böttger and others 1978). In both pea stem section and wheat coleoptile section assays, 4-Cl-IAA reached its maximum response (elongation) at a 10-fold lower concentration than IAA (Katekar and Geissler 1983). Furthermore, 4-CI-IAA stimulated growth of deseeded fruit (pericarp) of pea *in planta* (Reinecke and others 1995). Applications of 4-CI-IAA promoted pea pericarp growth, with increasing response from 1 to 100 μM, but IAA was ineffective in stimulating growth (from 0.1 to 100 μM; Reinecke and others 1995). 4-Me-IAA was found to be the second most effective auxin in stimulating deseeded pea pericarp growth (Reinecke and others 1999).

Given the high auxin activity of 4-Cl-IAA in a number of excised tissue assays (including wheat tissue section assays), as well as the ability of 4-Cl-IAA and 4-Me-IAA to stimulate pea pericarp growth in *planta*, we investigated the ability of these auxins to increase grain yield in wheat when applied one time to the whole plant near or at the beginning of flowering. The effects of auxin were assessed on wheat plants exposed to non-stress or heat-stress conditions following auxin treatment in environmentally controlled conditions, or under field conditions.

2.2 Materials and methods

2.2.1 Experiments under controlled environmental conditions

2.2.1.1 Planting protocol

For all greenhouse experiments, grains were planted at an approximate depth of 1.5 cm in 12.7 cm square plastic pots (15.2 cm pot depth; 2 to 3 grains per pot) in 1:4 Sunshine #4 potting mix (Sun Gro Horticulture, Vancouver, Canada) and sand. The seedlings were thinned to one seedling per pot approximately 2 weeks after seeding.

2.2.1.2 Greenhouse experiment -1- Wheat cv. Harvest

Grains of the hard red spring wheat (*Triticum aestivum* L.) cv. Harvest were sown on February 27, 2012 and maintained in a climate-controlled greenhouse at the University of Alberta (Edmonton, Alberta) with an average temperature of 21^{o}C day/19^{o}C night from February

27 to June 21, 2012. The plants also received supplemental lighting daily (average photon flux density of 250 µmol $m^{-2}s^{-2}$) for 16 h per day (from 6:00 to 22:00 h). Aqueous solutions of 4-Me-IAA at $1x10^{-6}$ and $1x10^{-7}$ M in 0.25% (v/v) Adigor or Agnique, or 0.1% (v/v) Tween 80, or adjuvants only solutions (0.25% [v/v] Adigor or Agnique; 0.1% [v/v] Tween 80) were applied to plants (sprayed to cover) at three growth stages (BBCH 24, four tiller stage; BBCH 45, late-boot stage, flag leaf sheath swollen with inflorescence; BBCH 59, end of heading, inflorescence fully emerged). The experiment was arranged in a completely randomized design (CRD) with six replications (six pots; one plant per pot) per treatment. At the BBCH stage 24, 4-Me-IAA in Adigor or Agnique solutions was applied to the plants and the response to the application was tested under non-heat stress conditions. At the BBCH stages 45 and 59, 4-Me-IAA in Adigor, Agnique, or Tween 80 solutions was applied to the plants and the response to the application was tested under non-heat stress and heat stress conditions. The heat stress treatment was imposed by moving plants to receive the heat stress to a growth chamber with the following conditions for six days within 12 h after hormone application. In the heat stress chamber, the light cycle began at 7:00 h at a 24°C air temperature. The heat treatment began at 11:00 h (33°C air temperature) and was maintained for 6 h (until 17:00 h). Following the heat treatment, the remainder of the light cycle was maintained at a 24°C air temperature. The dark cycle (began at 23:00 h) was maintained at 20°C. The photoperiod was 16 h light/8 h dark at an average photon flux density of 492 µmol $m^{-2}s^{-2}$ using 54 W/835/HO high fluorescent bulbs (Phillips, Holland). After six days, the heat stress-treated plants were returned to the original greenhouse and maintained at non-heat stress conditions to develop to maturity. The plant potting medium was maintained moist in all treatments throughout the experiment. Plants were fertilized with 175 ppm water soluble 20: 20: 20 (N: P: K) every three to four days.

2.2.1.3 Greenhouse experiment - 2 - Wheat cvs. with Rht genes

The effect of 4-Cl-IAA and 4-Me-IAA application on grain yield was tested on greenhouse grown hard red spring wheat lines that were isogenic for the mutant alleles Rht-D1 (cv. Pavon tall, *Rht-D1a* and Pavon semi-dwarf, *Rht-D1b*) and Rht-B1 (cv. Nesser tall, *Rht-B1a* and Nesser semi-dwarf, *Rht-B1b*), as well as the semi-dwarf cultivar CDC Go (*Rht-B1b*). 'Nesser' (tall and semi-dwarf lines) seeds were planted on May 16, 2014, Pavon (tall and semi-

dwarf lines) on May 26, 2014, and CDC Go on June 5, 2014. Pots were maintained in a greenhouse at the University of Alberta (Edmonton, Alberta) with an average maximum day temperature of 30^{o}C and average minimum night temperature of 17^{o}C prior to, and during flowering. The plants also received supplemental lighting daily (average photon flux density of 250 μmol $m^{-2}s^{-2}$) for 16 h per day (from 6 am to 10 pm).

Aqueous solutions of 4-Me-IAA and 4-Cl-IAA at $1x10^{-6}$ M in 0.1% (v/v) Tween 80 or adjuvant only solution (0.1% [v/v] Tween 80) were applied to plants (sprayed to cover) when the majority of the plants were at mid-boot to late-boot stage (BBCH 43-45). The experiment was arranged as a CRD with 10 replications (10 pots; one plant per pot) per treatment. Auxin responses were tested under two temperature levels after application. One set of plants remained in the greenhouse the entire length of the experiment (temperature control). Another set of plants were exposed to 35^{o}C for 6 h per day for six days within 12 h after hormone application by moving plants from the greenhouse to a growth chamber set as described above for cv. Harvest, except the heat treatment temperature was 35^{o}C. After six days, the heat stress-treated plants were returned to the greenhouse and maintained there to maturity. The plant potting medium was maintained moist in all treatments throughout the experiment, and the plants were fertilized weekly with 10: 52: 10 (N: P: K, early in the week) and 12: 2: 14 (later in the week) at 50 ppm.

2.2.2 Field experiments

2.2.2.1 Wheat cv. 5604 HR CL dose response experiments

4-Cl-IAA and 4-Me-IAA dose-response field experiments were conducted in four locations in the prairies eco-zone in western Canada. There were two locations in Alberta (Calgary [Latitude: 50.72/ Longitude: -113.32] and Red Deer [Latitude: 52.24/ Longitude: -113.86]) and two in Saskatchewan (Regina [Latitude: 50.44 / Longitude: -104.37] and Saskatoon [Latitude: 52.42/ Longitude: -106.38]) in 2012 and 2013. The Calgary and Red Deer sites were dominated with black chernozemic soils (Soil group map of Alberta 2002), where as the Saskatoon sites had dark brown regosolic soil, and the Regina sites had dark brown vertisolic soil (Saskatchewan Ministry of Agriculture 2009; Anderson 2010).

Grains of the wheat cultivar 5604 HR CL (treated with CruiserMaxx® at 325 ml/100 kg grain) were sown to a depth of 3.8 to 5 cm using a calibrated cone seeder at a rate of 100 kg/ha. Plots were arranged in a Randomized Complete Block Design (RCBD) consisting of 10 treatments per replicate (4-Cl-IAA and 4-Me-IAA at 1×10^{-7} M, 1×10^{-6} M, 1×10^{-5} M and 1×10^{-4} M in aqueous 0.25% Adigor, 0.25% aqueous Adigor only control, and a no treatment control), with four (in 2012) or six (in 2013) replicates per treatment. Treatments were randomly applied to plots within each replicate. Individual treatment plot size was 6 m ×1.5 m in 2012 and 7 m x 1.5 m in 2013. Inter-plot spacing was 0.3 m, with a 2 m buffer between replicates. Each treatment plot consisted of seven rows with an inter-row spacing of 18 cm from the middle of one row to the middle of the next row. Guard plots bordered the outermost treatment plots of the replicate row (Appendix A; Figure A.1; Plot illustration: 4-Me-IAA and 4-Cl-IAA dose-response field experiments for wheat, canola and pea).

All the auxins and adjuvant solutions used were made by Dr. Dennis M. Reinecke, University of Alberta, and stored in freezers in dark bottles and delivered to field locations under frozen and dark conditions. Frozen hormone and adjuvant control solutions were completely thawed in the dark, at ambient temperature, and mixed thoroughly, just prior to field application. In almost all sites, the solutions were applied at BBCH 43-45 (mid-boot stage to late-boot stage) except in Calgary 2013 where hormones were applied at BBCH 58 (80% of inflorescence emerged) growth stage. Hormones were applied to cover the plot plant canopy using a hand boom having 3 nozzles (110-02 low drift air induction nozzle with 275 kpa pressure) with a 90^{o} angle to the crop at a rate of 200 L/ha spray volume. Application of herbicides, fungicides or any other chemical treatment for pest and or disease control was not done seven days before or after the treatment application. Phenoxy or auxinic based products, Pyrasulfotole or other HPPD-inhibitors or bleachers were not used for any crop management purposes throughout the growing season. All other crop maintenance practices were completed following normal commercial wheat cultivation in western Canada (Alberta Agriculture and Rural development 2012).

Plot and yield component data were collected throughout the field seasons as follows. In 2013 to estimate plot plant density, the number of plants per meter was counted before the tillering stage, after the final plot trimming was completed (1 m in each row in a diagonal pattern across the plot, using the middle five rows). In the 2012 field season, the number of spikes per

metre at BBCH 83 were counted (as described above) to estimate plot plant density because the number of plants per meter assessment was considered inaccurate due to difficulty in determining individual plants due to tillering. Plant height per plot was estimated by measuring the height (from the ground level to the top of the major spike excluding awns) of randomly selected plants (10-20) at the BBCH 83 (early dough) growth stage.

Ten plants per plot were randomly selected from the inner rows of each plot to estimate if the treatments affected plant dry weight, spike dry weight, number of spikes per plant, or number of grains per spike or plant. For plant dry weight, the entire plant (excluding roots) was dried at 45^{o}C for two days prior to weighing. Individual plots were harvested using a Wintersteiger Classic plot combine harvester with a 150 cm wide header. Plot grain yield as measured from the combine harvester was normalized for 14.5% moisture and dockage weight. Weight of dockage per plot was estimated by calculating the percent dockage in 500 g grain subsamples from each plot (using a Clipper seed cleaner model 400: Seedburo, IL, USA). To calculate thousand kernel weight (TKW), two lots of 200 grains were weighed, and the average weight of 200 grains was multiplied by five. A SpectraStar RTW Near-Infrared (NIR) food analyzer (Unity Scientific, CT, USA) was used to measure the protein content in the grain samples. The calibration curve used for NIR protein analysis was developed at the Field Crop Development Centre in Lacombe, Alberta, using wheat grain samples collected throughout Canada and wet chemistry protein analysis using the Kjeldahl method from the Cereal Quality Lab in Winnipeg, Manitoba. For construction of the protein calibration curve, the complete wavelength region from 400 – 2500 nm and standard multivariate statistics with scatter correction and outlier elimination were used.

Weather data were collected using an on-site weather station in Calgary, Regina and Saskatoon fields while, Red Deer weather data were collected from the Red Deer, Alberta, weather station WMO ID 71878. Soil moisture (vol%) was measured using a Watermark Soil Moisture Sensor (Spectrum Technologies, IL, USA) placed at a depth of 10 cm in Saskatoon and Regina. Soil moisture storage capacity (SMSC) was measured at Calgary site using a Watchdog data logger with a Watermark Soil Moisture Sensor (Spectrum Technologies, IL, USA) placed at a depth of 20 cm.

2.2.2.2 Cultivar response field experiments

Field experiments were conducted in 2012 and 2013 on the wheat cultivars AC Lilian, AC Harvest, CDC Go, AC Unity, 5604 HR CL and WR859 CL (in 2012 only) at two sites, Alberta (Red Deer [Latitude: 52.24/ Longitude: -113.86]) and Saskatchewan (Saskatoon [Latitude: 52.42/ Longitude: -106.38]). The Red Deer site was predominantly composed of black chernozemic soils (Soil group map of Alberta 2002), where as the Saskatoon site had dark brown regosolic soil (Saskatchewan Ministry of Agriculture 2009; Anderson 2010). Seed treatments, seeding procedure, and seeding rate were completed as described for the dose-response experiment. Plots were arranged in a RCBD consisting of 18 plots per replicate in 2012 (6 cultivars × 3 treatments [4-Cl-IAA and 4-Me-IAA at 1×10^{-6} M in aqueous 0.25% Adigor and 0.25% aqueous Adigor only control]) and 20 plots in 2013 (5 cultivars × 4 treatments [4-Cl-IAA and 4-Me-IAA at 1×10^{-6} M in aqueous 0.25% Adigor, 0.25% aqueous Adigor only control, and a no treatment control]),with four (in 2012) or six (in 2013) replicates per treatment (Appendix A; Figure A.2; Plot illustration- wheat cultivar response field trial). Treatments were randomly applied to plots within each replicate. Plot dimensions, number of rows per plot, inter-row spacing, inter-plot spacing, buffer zones and guard plots were as described for the field dose-response experiment above. The plot management practices, hormone application, data collection, harvesting, and sample processing were also as described for the field dose-response experiment above.

2.2.3 Statistical analysis

2.2.3.1 Greenhouse experiments

Analyses were performed using the PROC MIXED procedure of SAS 9.3 software (SAS Institute Inc. Cary, NC, USA, 2010). For greenhouse experiment 1, statistical significance of the data was determined at the four-tiller stage (BBCH 24) by using a 2×3 factorial analysis of variance (adjuvant type×4-Me-IAA concentration), at the late-boot stage (BBCH 45) by using a 3×2×3 factorial analysis of variance (adjuvant type×temperature×4-Me-IAA concentration), and at the end of heading, 1st inflorescence fully emerged stage (BBCH 59) by using a 2×2×3 factorial analysis of variance (adjuvant type×temperature×4-Me-IAA concentration). Mean separation (main effects, treatments, and interactions) was determined using the Least Significant

Difference (LSD) test. For greenhouse experiment 2, statistical significance of the data was determined for the cultivar CDC Go by using a 3×2 factorial analysis of variance (hormone type × temperature). Statistical significance of the data was determined for the cultivars Nesser (tall and semi-dwarf) and Pavon (tall and semi-dwarf) by using a 2×3×2 factorial analysis of variance (height × hormone type× temperature). Mean separation (main effects, treatments, and interactions) was determined using the LSD test. Statistical significance was declared at $P \leq 0.05$.

2.2.3.2 Field experiments

For the cv. 5604 HR CL dose-response trial, a one-way analysis of variance (ANOVA) or analysis of covariance (ANCOVA) (for grain yield only) were performed on field data within each location and year using the PROC MIXED procedure of SAS 9.3 software (SAS Institute Inc. Cary, NC, USA, 2010). A one-way ANCOVA was carried out to analyze plot grain yield data with treatment as the main effect and the number of spikes per meter in 2012 (the number of plants per meter data was not accurate as it was counted after the tillering stage) and number of plants per meter in 2013 as covariates to account for the variation in plot plant density. The one-way ANOVA analyses were carried out for yield component parameters (here treatment level was taken as the main effect and analyses were carried out for 4-Me-IAA treatments and controls as well as for 4-Cl-IAA treatments and controls separately). Preplanned single degree of freedom contrast analysis was performed between the adjuvant only control (0.25% Adigor) and 4-Me-IAA treatments, the adjuvant only control (0.25% Adigor) and 4-Cl-IAA treatments, the no treatment control and 4-Me-IAA treatments, and the no treatment control and 4-Cl-IAA treatments, within each location and year. These contrasts were preplanned to examine specific treatment effects that would be of agronomic or biological importance. However, only yield parameters that showed similar mean trends for both no treatment control and the adjuvant control with respect to the hormone treatments were discussed. Statistical significance was declared at $P \leq 0.05$.

For the wheat cultivar response trials, one-way ANOVA or ANCOVA (for grain yield only) were performed on field data within each location, year and cultivar using the PROC MIXED procedure of SAS 9.3 software (SAS Institute Inc. Cary, NC, USA, 2010). The one-way ANOVA analyses were carried out for yield component parameters (here treatment level was

taken as the main effect and analyses were carried out for 4-Me-IAA treatments and controls as well as for 4-Cl-IAA treatments and controls separately). Similarly, the one-way ANCOVA was carried out to analyze plot grain yield data with treatment as the main effect and the number of spikes per meter in 2012 (the number of plants per meter data were not accurate as measurements were taken after the tillering stage) and number of plants per meter in 2013 as covariates to account for the variation in plot plant density. Preplanned single degree of freedom contrast analysis was performed between the adjuvant only control (0.25% Adigor), 4-Me-IAA 1×10^{-6} M, and 4-Cl-IAA 1×10^{-6} M treatments within each location, year and cultivar, as a no treatment control was not available for the 2012 field season. Statistical significance was declared at $P\leq0.05$.

2.3 Results

2.3.1 Experiments under controlled environmental conditions

2.3.1.1 Greenhouse experiment-1- Wheat cv. Harvest

To examine the effects of 4-Me-IAA application timing, and type of adjuvant, on grain yield parameters of wheat cv. Harvest, 4-Me-IAA was applied at three growth stages using 2 to 3 different adjuvant types. At the four tiller stage (BBCH 24), 4-Me-IAA application at 1×10^{-7} or 1×10^{-6} M had no effect on grain number and number of spikes per plant (Table 2.1). A small decrease in spike length was observed in plants treated with 4-Me-IAA in the adjuvant Adigor applied at the four tiller stage (Table 2.1). At the late-boot stage (BBCH 45, when the boot (flag leaf sheath) was swollen with the inflorescence) the effect of heat stress and 4-Me-IAA application on grain yield parameters was studied. Heat stress imposed at the late-boot stage decreased the number of grains and spikes per plant compared with the control temperature treatment (compare heat treatment main effect means within adjuvant type, Table 2.2). The effect of 4-Me-IAA on the number of grains and spikes per plant varied with the adjuvant used when applied at BBCH 45. 4-Me-IAA at 1×10^{-7} and 1×10^{-6} M in Adigor increased the number of grains per plant under non-heat stress (18.6% and 25.8%, respectively), but not under heat stress conditions (Table 2.2). 4-Me-IAA at 1×10^{-6} M in Agnique increased number of grains (by 21%)

and spikes per plant (by 28%) only under heat stress conditions. 4-Me-IAA in Tween 80 did not affect the number of grains or spikes per plant under either temperature treatment (Table 2.2). When applied at the end of heading (BBCH 59), 4-Me-IAA in Agnique increased the number of grains per plant at 1×10^{-7} M (by 22%) and 1×10^{-6} M (by 19%), and the number of spikes per plant at 1×10^{-7} M compared with the Agnique only control treatment regardless of the temperature treatment (see Agnique mean, Table 2.3); however, there was no difference in the number of grains per plant when 4-Me-IAA was applied with Adigor (Table 2.3).

2.3.1.2 Greenhouse experiment- 2 - Wheat cvs. with Rht genes

To determine if the mutations in the gibberellin response genes, Rht-B1 and Rht-D1, affect the efficacy of the auxin treatments with respect to grain yield, we applied 4-Me-IAA and 4-Cl-IAA to hard red spring wheat lines that were isogenic for the mutant alleles Rht-D1 (cv. Pavon tall, *Rht-D1a* and Pavon semi-dwarf, *Rht-D1b*) and Rht-B1 (cv. Nesser tall, *Rht-B1a* and Nesser semi-dwarf, *Rht-B1b*), and to the semi-dwarf cultivar CDC Go (*Rht-B1b*). The average maximum daily temperature in the greenhouse during this experiment (without added heat stress) was 30^{o}C, enabling assessment of auxin response under higher temperatures throughout their life-cycle. Plants of the semi-dwarf cultivar CDC Go (*Rht-B1b*) exposed to 35^{o}C for 6 h per day for 6 days at the early flowering stage of development produced fewer grains, tillers, and spikes per plant, resulting in lower grain weight per plant compared with the plants that did not receive this heat treatment (see temperature mean; Table 2.4). The 4-Cl-IAA (1×10^{-6} M) treatment increased grain weight per plant regardless of the temperature treatment at flowering (20% higher under greenhouse conditions and 33% higher under the additional heat condition compared with the Tween 80 control; Table 2.4). The 4-Cl-IAA treatment increased the number of grains per plant compared with the Tween 80 control (compare number of grains per plant 4-Cl-IAA mean to Tween 80 mean; Table 2.4), but had no effect on grain size (Table 2.4). The auxin treatments did not affect the plant height of cv. CDC Go (Table 2.4).

The plants of Nesser and Pavon with the semi-dwarf growth habit produced more grains and higher grain weight per plant than their corresponding isogenic lines with a tall growth habit (compare growth habit mean; Tables 2.5 and 2.6). Nesser semi-dwarf plants that were exposed to 35^{o}C for 6 h per day for 6 days at the early flowering stage of development produced lower grain

weight per plant and were shorter in height than plants not exposed to the 35°C treatment (Table 2.5; compare semi-dwarf control mean to semi-dwarf 35°C mean). The auxin treatment did not affect grain, tiller, spike or height parameters for tall or semi-dwarf lines of Nesser or Pavon (Tables 2.5 and 2.6).

2.3.2 Experiments under field conditions

2.3.2.1 Wheat cv. 5604 HR CL dose response experiment

Among the four sites over the 2012 and 2013 field seasons, only the 2012 Saskatoon field site exhibited both moderate heat stress (total of 15 days above 25°C daily maximum) and lower soil moisture conditions 14 days before and after hormone treatment at BBCH 43-45 (mid-boot to late-boot stage; see Appendix table A.2). Application of 4-Cl-IAA at 1×10^{-6} M or 1×10^{-7} M increased grain yield (by 7-11%) per plot compared with the no treatment control ($P\leq0.05$ for single degree of freedom contrasts; Table 2.7) at the 2012 Saskatoon site. Yield component analysis did not associate a particular measured parameter with the increase in yield observed at the 2012 Saskatoon site [Tables 2.8 (Thousand Kernel Weight), 2.9 (Protein content), 2.10 (Individual spike weight), A.3 (Total spike weight), A.4 (Plant height), A.5 (Plant dry weight), A.6 (Number of grains per plant), and A.7 (Number of grains per spike)]. The site with the greatest number of days above 25°C before and after hormone treatment at BBCH 43-45 (total of 17 days), but adequate soil moisture throughout this period, was the Regina site in 2012 (see Appendix table A.2). Although no increase in grain yield per plot was observed at the Regina site in 2012 (Table 2.7), application of 4-Cl-IAA at 1×10^{-5} or 1×10^{-6} M increased individual spike weight (Table 2.10) and spike weight per plant (Table A.3), as well as plant dry weight at 1×10^{-6} and 1×10^{-7} M (Table A.5) compared with the no treatment control ($P\leq0.05$) for single degree of freedom contrasts).

Application of 4-Cl-IAA or 4-Me-IAA did not affect grain protein content compared with the control (no treatment) at the 2012 Saskatoon or Regina sites (Table 2.9).

2.3.2.2 Cultivar response experiments

Among the cultivars tested, CDC Go showed the most consistent response to auxin treatment with respect to increasing grain yield parameters. At the Saskatoon site, in 2012 application of 4-Cl-IAA and 4-Me-IAA at 1×10^{-6} M, and in 2013 4-Cl-IAA at 1×10^{-6} M, increased the grain yield per plot of cv. CDC Go compared with the adjuvant control (0.25% Adigor) using single degree of freedom contrast analysis ($P \leq 0.05$) by 8%, 5.5%, and 6%, respectively (Table 2.12). The 4-Cl-IAA-induced increase in grain yield per plot for cv. CDC Go (Table 2.12) in 2013 was reflected in greater weight per spike (8% higher spike weight) (Table 2.13) and number of grains per plant (16% higher grain number) (Table 2.14). The 4-Cl-IAA-induced increase in grain yield per plot for cv. CDC Go also did not affect grain protein content (Table 2.13).

The auxin treatments did not increase grain yield per plot in the cvs. AC Lillian, AC Unity, Harvest, 5604 HR CL and WR859 CL in 2012 or 2013 (Table 2.12). However, 4-Cl-IAA application increased grain size (TKW; Table 2.12) in 2012 and spike weight per plant in 2013 (Table A.8) for cv. 5604 HR CL in Saskatoon. For AC Lillian in Saskatoon in 2013, 4-Cl-IAA application increased spike weight per plant (Table A.8) and number of grains per plant (Table 2.14). 4-Me-IAA application increased spike weight per plant for 5604 HR CL in Red Deer in 2013, and AC Lillian in Saskatoon in 2013 (Table A.8).

2.4 Discussion

Heat stress treatment (33-35^{o}C for 6 h per day for 6 days) imposed on hard red spring wheat plants at the mid-boot to late-boot stage reduced grain number (19%) and number of spikes (18%) per plant in cv. Harvest (Table 2.2), and grain weight (28%), grain number (31%), and number of spikes (18%) per plant in cv. CDC Go (Table 2.4). These data are similar to those reported by other researchers. Rahman and others (2009) reported high temperatures (compared day/night temperatures of 20/15^{o}C, 25/20^{o}C and 30/25^{o}C with control day/night temperatures which were maintained 5^{o}C lower) imposed on 10 spring wheat genotypes at three different times (from sowing to 60 days after sowing (DAS), 61 DAS to 80 DAS, or 81 DAS to maturity) reduced the number of productive tillers, grains per spike and grain yield per plant. The extent of

grain yield reduction induced by heat stress has also been reported to be genotype dependent (Rahman and others 2009; Balla and others 2009; Ferris and others 1998; Stone and Nicolas 1994; Gibson and Paulsen 1999; Spiertz and others 2006).

The effect of auxin application on wheat grain yield parameters varied with type of auxin, genotype, adjuvant, temperature at flowering, and environment. Under controlled environment conditions, the effect of 4-Me-IAA on grain number per plant in cv. Harvest (when treatments were applied at the late-boot stage) varied with adjuvant and temperature at flowering. 4-Me-IAA increased grain yield per plant of cv. Harvest under heat stress with adjuvant Agnique (alcohol ethoxylated surfactant), and under controlled conditions with adjuvant Adigor (petroleum hydrocarbon and surfactant blend; Table 2.2). No effect on grain number per plant was observed when 4-Me-IAA was applied with adjuvant Tween 80 (polyoxyethylenesorbitan monooleate surfactant) under these conditions (Table 2.2). Under field conditions, 4-Me-IAA (1×10^{-6} M in Adigor) did not increase grain yield per plot of cv. Harvest in 2012 or 2013 (Table 2.12). 4-Cl-IAA (1×10^{-6} M) also did not increase grain yield per plot of cv. Harvest in 2012 or 2013, but did increase grain size (TKW) at the Saskatoon site in 2013 (Table 2.12).

In the cultivar CDC Go, under controlled environment conditions, 4-Cl-IAA (1×10^{-6} M) increased grain yield per plant (Table 2.4) under additional heat stress (during initial flowering) and control conditions (33% higher grain weight per plant under heat stress and 20% higher yield under controlled treatment conditions). The 4-Cl-IAA-induced increase in grain yield per plant of CDC Go was a reflection of increased number of grains per plant under the additional heat stress conditions (Table 2.4). 4-Cl-IAA (1×10^{-6} M) also increased plot grain yield in the 2012 (8%) and 2013 (6%) field seasons at the site with higher mid-season temperatures and lower moisture conditions (Saskatoon). In 2013, the 4-Cl-IAA-induced increase in the grain yield at the Saskatoon site was also associated with an increase in number of grains per plant (Table 2.14). 4-Me-IAA (1×10^{-6} M) increased (5.5%) plot grain yield at the Saskatoon site in 2012.

Even though the auxin treatments did not increase grain yield per plot in the cvs. AC Lillian and 5604 HR CL in 2012 or 2013 in Saskatoon (Table 2.12), 4-Cl-IAA application increased grain size (TKW) in 2012 and spike weight per plant in 2013 for cv. 5604 HR CL (Tables 2.12 and 2.13). For AC Lillian, 4-Cl-IAA application increased spike weight per plant and number of grains per plant, and 4-Me-IAA application increased spike weight per plant in

2013 (Tables 2.13 and 2.14). These data suggest that both auxins can be effective in increasing grain yield parameters in hard red spring wheat, with 4-Cl-IAA (at 1×10^{-6} M) being more effective than 4-Me-IAA at the same concentration.

With respect to auxin concentration, the application of 4-Cl-IAA at 1×10^{-6} M or 1×10^{-7} M increased grain yield per plot (by 7-11%) compared with the no treatment control in cv. 5604 HR CL at the 2012 Saskatoon site (Table 2.7). Yield component analysis did not associate a particular measured parameter with the increase in grain yield per plot observed at the 2012 Saskatoon site (Tables 2.7, 2.9, 2.10 and 2.11). However, grain size (TKW) increased with auxin (4-Cl-IAA and 4-Me-IAA) treatment in cv.5604 HR CL in 2012 and 2013 at the Saskatoon site (Table 2.8). Data from the Regina site also indicates that 4-Cl-IAA (at 1×10^{-5} and 1×10^{-6} M) was effective in increasing specific grain yield parameters in cv. 5604 HR CL (increases in individual spike weight, total spike weight and plant dry weight; Tables 2.10, A.3 and A.5).

Semi-dwarf hard-red spring wheat cultivars are widely grown as they are high yielding under conventional agricultural management systems and they are more resistant to lodging than their taller counterparts (Butler and others 2005; Mathews and others 2006). The main dwarfing genes used in most commercial wheat lines are mutant alleles of the Rht-B1 and Rht-D1 genes. The wild-type (non-mutant) alleles of Rht-B1 and Rht-D1 (designated *Rht-B1a* and *Rht-D1a*, respectively) were isolated by Peng and others (1999) and shown to encode DELLA proteins, which are components of the gibberellic acid (GA) signal transduction pathway. It is also known that 4-Cl-IAA can stimulate GA biosynthesis in pea fruit, and data suggests that this auxin-induced GA biosynthesis promotes pea fruit set and growth (Ozga and others 2009). Therefore, to determine if the mutations in Rht-B1 and Rht-D1(designated *Rht-B1b* and *Rht-D1b*, respectively) that reduce GA sensitivity and cause the semi-dwarf habit affect the efficacy of the auxin treatments with respect to grain yield, we applied 4-Me-IAA and 4-Cl-IAA to hard red spring wheat lines that were isogenic for the mutant alleles Rht-D1 (cv. Pavon tall, *Rht-D1a* and Pavon semi-dwarf, *Rht-D1b*) and Rht-B1 (cv. Nesser tall, *Rht-B1a* and Nesser semi-dwarf, *Rht-B1b*). Additionally, the cultivar CDC Go has the *Rht-B1b* gene for semi-dwarf habit (Dr. Spaner, University of Alberta, personal communication).

We found that the semi-dwarf lines produced more grains and higher grain weight per plant than their corresponding isogenic tall lines (compare growth habit means; Nesser *Rht-B1b*,

23.7% higher grain weight and 99% higher number of grains per plant [Table 2.5], and Pavon *Rht-D1b* 40% higher grain weight and 37% higher number of grains per plant [Table 2.6]). Our data are consistent with those reported in the literature. Mathews and others (2006) reported that the semi-dwarf Nesser *Rht-B1b* and Pavon *Rht-D1b* lines out yielded their corresponding tall alleles (*Rht-B1a* and *Rht-D1a,* respectively) in both drought and irrigated environments by approximately 7% to 21%. Sing and others (2001) also reported a 10 to 30% higher grain yield in semi-dwarf cultivars (including Nesser and Pavon) in intermediate and high yielding environments compared with their tall isolines. Butler and others (2005) reported that *Rht-B1b* out yielded *Rht-D1b* under fully irrigated conditions. In our study, seed yield and seed number per plant means were higher for Nesser semi-dwarf (*Rht-B1b*) than that of Pavon semi-dwarf (*Rht-D1b*) under normal and heat stress conditions (Tables 2.5 and 2.6); however, due to our experimental design, the yield of these two cultivars cannot be directly compared. Fischer and Stockman (1986) hypothesized that increased grain number in the semi-dwarf cultivars may be the result of greater resource partitioning into spikes due to decreased competition for the nutrient resources from the shorter stems when compared with the longer stems in the taller counterparts.

Neither wild-type or mutant lines of Rht-B1 and Rht-D1 in Nesser or Pavon backgrounds were responsive to one application of 4-Cl-IAA or 4-Me-IAA (at 1×10^{-6} M) with respect to increasing grain yield in these experiments. However, CDC Go (*Rht-B1b*) was very responsive to 4-Cl-IAA-induced grain yield increases. These data suggest that the presence of the *Rht-B1b* or *Rht-D1b* alone does not directly affect the effectiveness of 4-Cl-IAA to induce increases in grain yield.

Sakata and others (2010) found that multiple applications of auxins (IAA, NAA, or 2,4-D) functioned to ameliorate the effects of high temperature on male sterility/anther abortion and seed setting rate in barley and *Arabidopsis*. Here we show that a single application of auxin (4-Cl-IAA or 4-Me-IAA) to the whole wheat plant has the ability to increase grain yield parameters under both normal and heat stress conditions, and under field conditions. Our data also suggest that, in general, 4-Cl-IAA was more effective than 4-Me-IAA in increasing grain yield in hard red spring wheat cultivars tested. Reinecke and others (1999) also found that 4-Cl-IAA was more effective in stimulating pea pericarp growth than 4-Me-IAA. Furthermore, our field data suggest

that the genotype is a factor that affects auxin-induced enhancement of grain yield in hard-red spring wheat. Further testing of the effects of 4-Cl-IAA and 4-Me-IAA on the ability to increase wheat grain yield under both normal and heat stress conditions, and under field conditions, would broaden our knowledge of the conditions and genotypes to use to obtain optimal response.

Table 2.1 Effect of 4-Me-IAA and type of adjuvant on grain number, number of spikes per plant and spike length of greenhouse-grown wheat (cv. Harvest) plants when applied at the four tiller stage.

Adjuvant	4-Me-IAA concentration[z]	Number of grains per plant	Number of spikes per plant	Spike length (cm)
Adigor	1×10^{-6} M	382 a[w]	14.0 a	6.7a
Adigor	1×10^{-7} M	385 a	14.0 a	6.8 a
Adigor	0	361 a	14.0 a	7.3 b
Mean		376 m	14.0 m	6.9 m
Agnique	1×10^{-6} M	373 a	15.2 a	6.8 a
Agnique	1×10^{-7} M	291 a	12.8 a	6.6 a
Agnique	0	366 a	13.8 a	6.9 a
Mean		343 m	13.9 m	6.8 m
Mean	1×10^{-6} M	378 r	14.6 r	6.8 r
Mean	1×10^{-7} M	338 r	13.4 r	6.7 r
Mean	0	364 r	13.9 r	7.1 s

[z] 4-Me-IAA concentration: aqueous solutions of 4-Me-IAA ($1x10^{-7}$ and $1x10^{-6}$ M) in 0.25% Adigor or 0.25% Agnique; Control adjuvant solutions: aqueous 0.25% Adigor or 0.25% Agnique only; one application sprayed on plants to cover when the plants were at the BBCH 24 developmental stage (four tiller stage).

[w] The means of parameters with significantly different factorial treatment effects and/or significant interaction effects (at ($P\leq 0.05$) were separated using the LSD test. Means followed by a different letter are significantly different within sets (a, b: within adjuvant), (m, n), and (r, s) by the LSD test, $P<0.05$.

Table 2.2 Effect of 4-Me-IAA, type of adjuvant and heat stress or non-heat stress condition on grain number, number of spikes per plant and spike length of greenhouse-grown wheat (cv. Harvest) plants when applied at the late-boot stage.

Adjuvant	4-Me-IAA concentration[z]	Heat trt[y]	Number of grains per plant	Number of spikes per plant	Spike length (cm)
Adigor	1×10^{-6} M	-	317 a[w]	13.0 a	6.8 a
Adigor	1×10^{-7} M	-	299 a	12.8 a	7.0 a
Adigor	0	-	252 b	11.8 a	7.0 a
Mean		-	289 m	12.6 m	6.9 m
Adigor	1×10^{-6} M	+	213 a	9.2 a	7.1 a
Adigor	1×10^{-7} M	+	235 ab	10.5 ab	7.0 a
Adigor	0	+	261 b	11.8 b	7.0 a
Mean		+	236 n	10.5 n	7.0 m
Agnique	1×10^{-6} M	-	298 a	13.3 a	6.9 a
Agnique	1×10^{-7} M	-	311 a	12.8 a	7.1 a
Agnique	0	-	302 a	12.8 a	7.4 a
Mean		-	304 m	13.0 m	7.1 m
Agnique	1×10^{-6} M	+	271 a	12.2 a	7.1 a
Agnique	1×10^{-7} M	+	241 ab	10.8 ab	7.1 a
Agnique	0	+	224 b	9.5 b	7.1 a
Mean		+	245 n	10.8 n	7.1 m
Tween 80	1×10^{-6} M	-	341 a	13.5 ab	6.9 a
Tween 80	1×10^{-7} M	-	359 a	15.2 a	7.0 a
Tween 80	0	-	348 a	13.0 b	7.3 a
Mean		-	349 m	13.9 m	7.1 m
Tween 80	1×10^{-6} M	+	295 a	11.7 a	7.1 a
Tween 80	1×10^{-7} M	+	280 a	11.2 ab	6.8 a
Tween 80	0	+	255 a	10.5 b	7.2 a
Mean		+	277 n	11.1 n	7.0 m

[z] 4-Me-IAA concentration : aqueous solutions of 4-Me-IAA ($1x10^{-7}$ and $1x10^{-6}$M) in 0.25% Adigor or 0.25% Agnique or 0.1% Tween 80; Control adjuvant solutions: aqueous 0.25% Adigor or 0.25% Agnique or 0.1% Tween 80 only; one application sprayed on plants to cover when the majority of the plants were at the BBCH 45 developmental stage (late-boot stage).

[y] Heat stress: The heat stress treatment was imposed by moving plants to receive the heat stress to a growth chamber (heat stress chamber) for 6 days, within 12 h after the hormone application. In the heat stress chamber, the light cycle began at 7:00 h at a 24°C air temperature. The heat treatment began at 11:00 h (33°C air temperature) and was maintained for 6 h (until 17:00 h). Following the heat treatment, the remainder of the light cycle was maintained at a 24°C air temperature. The dark cycle (began at 23:00 h) was maintained at 20°C; photoperiod was 16 h light/8 h dark. After 6 days, the heat stress-treated plants were returned to the greenhouse maintained at non-heat stress conditions to develop to maturity.

[w] The means of parameters with significantly different factorial treatment effects ($P \leq 0.05$) were separated using the LSD test. Means followed by a different letter are significantly different within sets (a, b within adjuvant and heat treatment) and (m, n) by the LSD test, $P \leq 0.05$.

Table 2.3 Effect of 4-Me-IAA, type of adjuvant and heat stress or non-heat stress condition on grain number, number of spikes per plant and spike length of greenhouse-grown wheat (cv. Harvest) plants when applied at the end of heading.

Adjuvant	4-Me-IAA concentration[z]	Heat trt[y]	Number of grains per plant	Number of spikes per plant	Spike length (cm)
Adigor	1×10^{-6} M	-	304 a[w]	13.0 a	7.3 a
Adigor	1×10^{-7} M	-	272 a	12.0 a	7.3 a
Adigor	0	-	297 a	12.3 a	7.3 a
Adigor	1×10^{-6} M	+	309 a	13.7 a	6.9 a
Adigor	1×10^{-7} M	+	274 a	12.2 a	6.9 a
Adigor	0	+	309 a	12.5 a	7 .0 a
Adigor mean	1×10^{-6} M		307 m	13.3 m	7.1 m
Adigor mean	1×10^{-7} M		273 m	12.1 m	7.1 m
Adigor mean	0		303 m	12.4 m	7.1 m
Agnique	1×10^{-6} M	-	342 ab	13.5 a	7.0 a
Agnique	1×10^{-7} M	-	357 b	14.3 a	7.0 a
Agnique	0	-	290 a	12.5 a	7.0 a
Agnique	1×10^{-6} M	+	312 a	12.3 a	7.0 a
Agnique	1×10^{-7} M	+	311 a	12.7 a	7.2 a
Agnique	0	+	258 a	10.8 a	7.0 a
Agnique mean	1×10^{-6} M		327 m	12.9 mn	6.9 m
Agnique mean	1×10^{-7} M		334 m	13.5 n	7.1 m
Agnique mean	0		274 n	11.7 m	7.0 m

[z] 4-Me-IAA concentration: aqueous solutions of 4-Me-IAA ($1x10^{-7}$ and $1x10^{-6}$ M) in 0.25% Adigor or 0.25% Agnique; Control adjuvant solutions: aqueous 0.25% Adigor or 0.25% Agnique only; one application sprayed on plants to cover when the majority of the plants were at the BBCH 59 developmental stage (end of heading).

[y] Heat stress: The heat stress treatment was imposed by moving plants to receive the heat stress to a growth chamber (heat stress chamber) for 6 days, within 12 h after the hormone application. In the heat stress chamber, the light cycle began at 7:00 h at a 24°C air temperature. The heat treatment began at 11:00 h (33°C air temperature) and was maintained for 6 h (until 17:00 h). Following the heat treatment, the remainder of the light cycle was maintained at a 24°C air temperature. The dark cycle (began at 23:00 h) was maintained at 20°C; photoperiod was 16 h light/8 h dark. After 6 days, the heat stress-treated plants were returned to the greenhouse maintained at non-heat stress conditions to develop to maturity.

[w] The means of parameters with significantly different factorial treatment effects and/or significant interaction effects ($P\leq0.05$) were separated using the LSD test. Means followed by a different letter are significantly different within sets (a, b: within adjuvant) and (m, n) by the LSD test, $P\leq0.05$.

Table 2.4 Effect of 4-Cl-IAA and 4-Me-IAA when applied at the mid to late-boot stage on grain yield parameters of greenhouse grown wheat cv. CDC Go plants that were exposed or not exposed to 35°C for 6 h per day for 6 days at the early flowering stage of development[z].

Hormone treatment[y]	Heat trt[x]	Grain weight per plant (g)	Grain size (mg)	Number of grains per plant	Number of tillers per plant before hormone application	Number of tillers per plant	Number of spikes per plant	Plant height (cm)
4-Cl-IAA	-	2.877 a[w]	32 a	99.6 a	5.6 a	12.8 a	11.8 a	60.6 a
4-Me-IAA	-	2.180 b	27 a	79.5 b	5.2 a	10.6 a	9.3 b	62.0 a
Tween 80 (0.1%)	-	2.399 b	26 a	93.8 ab	5.6 a	11.2 a	10.6 ab	64.1 a
Temperature mean		2.485 m	28 m	91.0 m	5.5 m	11.5 m	10.6 m	62.2 m
4-Cl-IAA	+	2.103 a	28 a	76.3 a	5.4 a	10.8 a	9.2 a	59.0 a
4-Me-IAA	+	1.711 ab	29 a	60.2 ab	5.7 a	8.4 b	7.9 a	63.6 a
Tween 80	+	1.580 b	31 a	53.0 b	6.0 a	9.5 ab	8.9 a	62.9 a
Temperature mean		1.798 n	29 m	63.2 n	5.7 m	9.6 n	8.7 n	61.8 m
4-Cl-IAA mean		2.490 r	30 r	88.0 r	5.5 r	11.8 r	10.5 r	59.8 r
4-Me-IAA mean		1.946 s	28 r	69.9 s	5.5 r	9.5 s	8.6 s	62.8 r
Tween 80 mean		1.990 s	28 r	73.4 s	5.8 r	10.4 rs	9.8 rs	63.5 r
Treatment x heat stress		NS[v]	NS	NS	NS	NS	NS	NS

[z] Data were taken at harvest maturity unless otherwise noted.

[y] 4-Cl-IAA or 4-Me-IAA at $1X10^{-6}$ M in 0.1 % Tween 80, or 0.1 % Tween 80 alone was applied to plants as a spray mid to late-boot stage.

[x]The heat stress treatment was imposed by moving plants to receive the heat stress to a growth chamber (heat stress chamber) for 6 days, after the hormone application. In the heat stress chamber, the light cycle began at 7:00 h at a 24°C air temperature. The heat treatment began at 11:00 h (35°C air temperature) and was maintained for 6 h (until 17:00 h). Following the heat treatment, the remainder of the light cycle was maintained at a 24°C air temperature. The dark cycle (began at 23:00 h) was maintained at 20°C; photoperiod was 16 h light/8 h dark. After 6 days, the heat stress-treated plants were returned to the greenhouse maintained to develop to maturity. The average maximum daily temperature in the greenhouse during this period was 30°C.

[w] The means of parameters with significantly different ANOVA treatment effects were separated using the LSD test. Means followed by a different letter are significantly different from one another within sets (a, b within temperature treatment), (m, n), and (r, s), by the LSD test, $P < 0.05$.

[v] NS = non significant, $P > 0.05$.

Table 2.5 Effect of 4-Cl-IAA and 4-Me-IAA when applied at the mid to late-boot stage on grain weight per plant, grain size, number of grains per plant, number of tillers per plant before hormone application and at harvest, number of spikes per plant, and plant height of greenhouse grown wheat cv. Nesser with two growth habit (tall and semi-dwarf) plants that were exposed or not exposed to 35°C for 6 h per day for 6 days at the early flowering stage of development[z].

Treatment[y]	Growth Habit	35°C Trt[x]	Grain weight per plant (g)	Grain size (g)	Number of grains per plant	Number of tillers per plant before hormone application	Number of tillers per plant at harvesting	Number of spikes per plant	Plant height (cm)
4-Cl-IAA	Tall	-	2.813 a[w]	30 a	99.0 a	5.6 a	12.3 a	9.8 a	70.0 a
4-Me-IAA	Tall	-	2.509 a	29 a	92.2 a	5.9 a	10.5 a	8.9 a	71.9 a
Tween 80 (0.1%)	Tall	-	3.309 a	28 a	121.0 a	5.8 a	11.6 a	10.4 a	73.7 a
Mean			2.877 e	29 e	104.1 e	5.8 e	11.5 e	9.7 e	71.9 e
4-Cl-IAA	Tall	+	2.436 a	28 a	93.7 a	6.1 a	10.9 a	9.8 a	68.5 a
4-Me-IAA	Tall	+	2.918 a	29 a	102.2 a	5.9 a	10.6 a	9.5 a	67.9 a
Tween 80 (0.1%)	Tall	+	2.212 a	29 a	84.9 a	6.1 a	12.1 a	9.6 a	70.3 a
Mean			2.522 e	29 e	93.6 e	6.0 e	11.2 e	9.6 e	68.9 f
4-Cl-IAA	Semi-dwarf	-	3.725 a	19 a	196.4 a	9.0 a	10.6 a	8.2 a	67.3 a
4-Me-IAA	Semi-dwarf	-	4.137 a	20 a	206.8 a	8.3 a	9.3 a	7.7 a	67.7 a
Tween 80 (0.1%)	Semi-dwarf	-	3.928 a	19 a	207.8 a	8.9 a	12.2 a	9.3 a	65.9 a
Mean			3.930 e	19 e	203.7 e	8.7 e	10.7 e	8.4 e	67.0 e
4-Cl-IAA	Semi-dwarf	+	3.071 a	15 a	197.2 a	7.7 a	10.1 a	7.3 a	58.8 a
4-Me-IAA	Semi-dwarf	+	3.224 a	18 a	182.8 a	8.8 a	9.5 a	7.3 a	60.6 a
Tween 80 (0.1%)	Semi-dwarf	+	3.149 a	17 a	188.5 a	8.7 a	11.1 a	8.1 a	61.5 a
Mean			3.148 f	17 e	189.5 e	8.4 e	10.3 e	7.6 e	60.3 f
Growth Habit mean	Tall		2.700 m	29 m	98.8 m	5.9 m	11.4 m	9.7 m	70.4 m
	Semi-dwarf		3.539 n	18 n	196.6 n	8.6 n	10.5 m	8.0 n	63.6 n
4-Cl-IAA mean			3.011 r	23 r	146.6 r	7.1 r	11.0 r	8.8 r	66.1 r
4-Me-IAA mean			3.197 r	24 r	146.0 r	7.2 r	10.0 r	8.4 r	67.0 r
Tween 80 (0.1%) mean			3.150 r	23 r	150.6 r	7.4 r	11.8 r	9.4 r	67.9 r
Growth habit x 35°C treatment			NS[v]	NS	NS	NS	NS	NS	SD

[z] Data were taken at harvest maturity unless otherwise noted.

[y] 4-Cl-IAA or 4-Me-IAA at $1X10^{-6}$ M in 0.1 % Tween 80, or 0.1 % Tween 80 alone was applied to plants as a spray mid to late-boot stage.

[x] The heat stress treatment was imposed by moving plants to receive the heat stress to a growth chamber (heat stress chamber) for 6 days, after the hormone application. In the heat stress chamber, the light cycle began at 7:00 h at a 24°C air temperature. The heat treatment began at 11:00 h (35°C air temperature) and was maintained for 6 h (until 17:00 h). Following the heat treatment, the remainder of the light cycle was maintained at a 24°C air temperature. The dark cycle (began at 23:00 h) was maintained at 20°C; photoperiod was 16 h light/8 h dark. After 6 days, the heat stress-treated plants were returned to the greenhouse maintained to develop to maturity. The average maximum daily temperature in the greenhouse during this period was 30°C.

[w] The means of parameters with significantly different ANOVA treatment effects were separated using the LSD test. Means followed by a different letter are significantly different from one another within sets (e, f) and (m, n) by the LSD test, and means followed by a similar letter are not significantly different from one another within sets (a: within growth habit and temperature treatment) and (r) by the LSD test, $P < 0.05$

[v] NS = non significant, $P > 0.05$

Table 2.6 Effect of 4-Cl-IAA and 4-Me-IAA when applied at the mid to late-boot stage on grain weight per plant, grain size, number of grains per plant, number of tillers per plant before hormone application and at harvest, number of spikes per plant, and plant height of greenhouse grown wheat cv. Pavon with two growth habit (tall and semi-dwarf) plants that were exposed or not exposed to 35°C for 6 h per day for 6 days at the early flowering stage of development[z].

Treatment[y]	Growth Habit	35°C Trt[x]	Grain weight per plant (g)	Grain size (g)	Number of grains per plant	Number of tillers per plant before hormone application	Number of tillers per plant at harvesting	Number of spikes per plant	Plant height (cm)
4-Cl-IAA	Tall	-	1.460 a[w]	23 a	65.2 a	4.4 a	8.4 a	8.0 a	69.3 a
4-Me-IAA	Tall	-	1.500 a	23 a	66.6 a	5.1 a	7.7 a	7.3 a	66.3 a
Tween 80 (0.1%)	Tall	-	1.302 a	23 a	58.2 a	4.4 a	7.7 a	6.9 a	67.9 a
Mean			1.421 e	23 e	63.4 e	4.6 e	7.9 e	7.4 e	67.8 e
4-Cl-IAA	Tall	+	1.913 a	25 a	82.9 a	5.6 a	8.3 a	6.5 a	75.0 a
4-Me-IAA	Tall	+	1.536 a	23 a	69.6 a	5.2 a	8.7 a	7.7 a	69.9 a
Tween 80 (0.1%)	Tall	+	1.562 a	25 a	64.0 a	4.4 a	7.8 a	6.9 a	65.9 a
Mean			1.670 e	24 e	72.1 e	5.1 e	8.3 e	7.0 e	70.3 e
4-Cl-IAA	Semi-dwarf	-	2.381 a	24 a	101.1 a	4.7 a	6.2 a	5.1 a	66.5 a
4-Me-IAA	Semi-dwarf	-	2.531 a	25 a	103.7 a	4.5 a	5.6 a	4.7 a	68.0 a
Tween 80 (0.1%)	Semi-dwarf	-	2.267 a	22 a	107.2 a	4.0 a	6.2 a	5.9 a	67.2 a
Mean			2.393 e	24 e	104.0 e	4.4 e	6.0 e	5.3 e	67.3 e
4-Cl-IAA	Semi-dwarf	+	2.955 a	24 a	127.4 a	4.6 a	5.2 a	4.8 a	65.6 a
4-Me-IAA	Semi-dwarf	+	2.495 a	25 a	102.7 a	4.7 a	5.8 a	4.5 a	65.9 a
Tween 80 (0.1%)	Semi-dwarf	+	2.713 a	30 a	106.4 a	4.7 a	6.4 a	5.0 a	65.6 a
Mean			2.721 e	26 e	112.2 e	4.7 e	5.8 e	4.8 e	65.7 e
Growth Habit mean	Tall		1.546 m	24 m	67.7 m	4.9 m	8.1 m	7.2 m	69.0 m
	Semi-dwarf		2.557 n	25 m	108.1 n	4.5 m	5.9 n	5.0 n	66.5 n
4-Cl-IAA mean			2.177 r	24 r	94.1 r	4.8 r	7.0 r	6.1 r	69.1 r
4-Me-IAA mean			2.015 r	24 r	85.6 r	4.9 r	6.9 r	6.0 r	67.5 r
Tween 80 (0.1%) mean			1.961 r	25 r	84.0 r	4.4 r	7.0 r	6.2 r	66.6 r
Growth habit x heat stress			NS[v]	NS	NS	NS	NS	NS	SD

[z] Data were taken at harvest maturity unless otherwise noted.

[y] 4-Cl-IAA or 4-Me-IAA at $1X10^{-6}$ M in 0.1 % Tween 80, or 0.1 % Tween 80 alone was applied to plants as a spray mid to late-boot stage.

[x] The heat stress treatment was imposed by moving plants to receive the heat stress to a growth chamber (heat stress chamber) for 6 days, after the hormone application. In the heat stress chamber, the light cycle began at 7:00 h at a 24°C air temperature. The heat treatment began at 11:00 h (35°C air temperature) and was maintained for 6 h (until 17:00 h). Following the heat treatment, the remainder of the light cycle was maintained at a 24°C air temperature. The dark cycle (began at 23:00 h) was maintained at 20°C; photoperiod was 16 h light/8 h dark. After 6 days, the heat stress-treated plants were returned to the greenhouse maintained to develop to maturity. The average maximum daily temperature in the greenhouse during this period was 30°C.

[w] The means of parameters with significantly different ANOVA treatment effects were separated using the LSD test. Means followed by a different letter are significantly different from one another within sets (e, f) and (m, n) by the LSD test, and means followed by a similar letter are not significantly different from one another within sets (a: within growth habit and temperature treatment) and (r) by the LSD test, $P < 0.05$.

[v] NS = non significant, $P > 0.05$.

Table 2.7 Effect of 4-Cl-IAA and 4-Me-IAA on grain yield when applied to field-grown wheat (cv. 5604 HR CL) at Calgary, Red Deer, Regina and Saskatoon sites during the 2012 and 2013 field seasons.

		Grain Yield (kg/ha) per plot							
		Calgary		Red Deer		Regina		Saskatoon	
Hormone[a]	Concentration	2012	2013	2012	2013	2012	2013	2012	2013
4-Cl-IAA	1×10^{-4} M	3495	5387	3971	4586	2930	nd[b]	4218	4617
	1×10^{-5} M	3443	5109	3486	4838	2920	nd	4250	4553
	1×10^{-6} M	3570	5095	3840	4655	2878	nd	4429*[c]	4779
	1×10^{-7} M	2857	5320	4169	4539	3022	nd	4600*	4484
	0 (0.25% Adigor)	3667	5174	4292	4721	2940	nd	4246	4515
	0 (no treatment)	3235	5270	4241	4671	2850	nd	4135	4358
4-Me-IAA	1×10^{-4} M	3330	5096	4033	5029	2973	nd	4451	4649
	1×10^{-5} M	3325	5114	4249	4776	2893	nd	4196	4448
	1×10^{-6} M	3130	5156	4053	4599	2901	nd	4444	4403
	1×10^{-7} M	3469	5218	4252	4802	2900	nd	4442	4575
	0 (0.25% Adigor)	3664	5133	4037	4700	2945	nd	4247	4467
	0 (no treatment)	3249	5228	4038	4672	2871	nd	4133	4338

[a] Hormone: aqueous solutions of 4-Me-IAA and 4-Cl-IAA ($1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$ and $1x10^{-4}$ M) in 0.25% Adigor; Control solutions aqueous 0.25% Adigor and control no solution; one application sprayed on plants to cover when the majority of the plants were at the BBCH scale 43-45 developmental stages (mid-boot to late-boot stage).

[b] nd = not determined.

[c] Denotes means compared within hormone that are different from the no treatment control and adjuvant only (0.25% Adigor) control at $P \leq 0.05$ using single degree of freedom contrast analysis.

Table 2.8 Effect of 4-Cl-IAA and 4-Me-IAA on grain size (thousand kernel weight) when applied to field-grown wheat (cv. 5604 HR CL) at Calgary, Red Deer, Regina and Saskatoon sites during the 2012 and 2013 field seasons.

		Thousand kernel weight (g)							
		Calgary		Red Deer		Regina		Saskatoon	
Hormone[a]	Concentration	2012	2013	2012	2013	2012	2013	2012	2013
4-Cl-IAA	1×10^{-4} M	29.97	35.82	28.71	34.69	33.07	nd[b]	31.63*[c]	36.72
	1×10^{-5} M	29.18	35.68	28.37	34.60	33.19	nd	32.10*	36.85
	1×10^{-6} M	30.25	35.87	29.40	34.84	32.87	nd	32.80*	37.35*
	1×10^{-7} M	29.32	35.73	29.12	34.71	33.47	nd	31.97*	37.46*
	0 (0.25% Adigor)	30.55	35.95	29.23	34.95	33.61	nd	32.65*	36.66
	0 (no treatment)	30.04	35.60	29.00	34.59	32.69	nd	29.42	36.27
4-Me-IAA	1×10^{-4} M	30.43	35.31	29.14	34.57	33.08	nd	30.47	37.41*
	1×10^{-5} M	29.70	35.82	29.25	34.47	33.70	nd	31.40*	37.33*
	1×10^{-6} M	30.33	36.28	29.59	34.87	33.33	nd	31.97*	36.92
	1×10^{-7} M	30.06	35.71	28.88	34.83	33.31	nd	31.69*	36.86
	0 (0.25% Adigor)	30.55	35.95	29.23	34.95	33.61	nd	32.65*	36.67
	0 (no treatment)	30.04	35.60	29.00	34.59	32.69	nd	29.42	36.27

[a] Hormone: aqueous solutions of 4-Me-IAA and 4-Cl-IAA ($1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$ and $1x10^{-4}$ M) in 0.25% Adigor; Control solutions aqueous 0.25% Adigor and control no solution; one application sprayed on plants to cover when the majority of the plants were at the BBCH scale 43-45 developmental stages (mid-boot stage to late-boot stage).

[b] nd = not determined

[c] Denotes means compared within hormone that are different from the no treatment control and adjuvant only (0.25% Adigor) control at $P \leq 0.05$ using single degree of freedom contrast analysis.

Table 2.9 Effect of 4-Cl-IAA and 4-Me-IAA on protein content (%) when applied to field-grown wheat (cv. 5604 HR CL) at Calgary, Red Deer, Regina and Saskatoon sites during the 2012 and 2013 field seasons.

		Protein content (%)							
		Calgary		Red Deer		Regina		Saskatoon	
Hormone[a]	Concentration	2012	2013	2012	2013	2012	2013	2012	2013
4-Cl-IAA	1×10^{-4} M	14.55	13.00	13.11	12.44	14.61	nd[b]	13.34	12.31 *[c]
	1×10^{-5} M	14.46	13.05	13.18	12.84	14.47	nd	13.38	11.99
	1×10^{-6} M	14.38	13.14	13.07	12.44	14.43	nd	13.28	11.96
	1×10^{-7} M	14.63	13.05	13.20	12.40*	14.59	nd	13.82	11.89
	0 (0.25% Adigor)	14.89	13.11	13.00	12.67	14.12	nd	13.57	11.92
	0 (no treatment)	14.57	13.30	13.07	12.67	14.25	nd	13.85	11.85
4-Me-IAA	1×10^{-4} M	14.51	13.04	13.33	12.58	14.31	nd	13.26	12.10
	1×10^{-5} M	14.58	13.17	13.23	12.38	14.39	nd	13.10*	12.22
	1×10^{-6} M	14.62	13.09	13.20	12.60	14.46	nd	13.62	12.22
	1×10^{-7} M	14.76	13.05	12.92	12.27*	14.68	nd	13.83	12.06
	0 (0.25% Adigor)	14.89	13.11	13.00	12.67	14.12	nd	13.57	11.92
	0 (no treatment)	14.57	13.30	13.07	12.67	14.25	nd	13.85	11.85

[a] Hormone: aqueous solutions of 4-Me-IAA and 4-Cl-IAA ($1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$ and $1x10^{-4}$ M) in 0.25% Adigor; Control solutions aqueous 0.25% Adigor and control no solution; one application sprayed on plants to cover when the majority of the plants were at the BBCH scale 43-45 developmental stages (mid-boot stage to late-boot stage).

[b] nd = not determined.

[c] Denotes means compared within hormone that are different from the no treatment control and adjuvant only (0.25% Adigor) control at $P \leq 0.05$ using single degree of freedom contrast analysis.

Table 2.10 Effect of 4-Cl-IAA and 4-Me-IAA on spike weight when applied to field-grown wheat (cv. 5604 HR CL) at Calgary, Red Deer, Regina and Saskatoon sites during the 2012 and 2013 field seasons.

		Individual spike weight (g)							
		Calgary		Red Deer		Regina		Saskatoon	
Hormone[a]	Concentration	2012	2013	2012	2013	2012	2013	2012	2013
4-Cl-IAA	1×10^{-4} M	1.13	1.15	0.66	0.46	1.06*[c]	nd[b]	1.05	0.92
	1×10^{-5} M	1.09	1.15	0.57	0.46	1.10*	nd	1.18	0.95
	1×10^{-6} M	1.01	1.17	0.67	0.43	1.08*	nd	1.09	0.88
	1×10^{-7} M	0.95	1.10	0.64	0.44	0.98	nd	1.07	0.95
	0 (0.25% Adigor)	1.13	1.23	0.61	0.43	0.94	nd	1.06	0.91
	0 (no treatment)	1.09	1.15	0.65	0.45	0.89	nd	1.09	0.93
4-Me-IAA	1×10^{-4} M	1.10	1.13	0.60	0.43	1.03*	nd	1.20	0.92
	1×10^{-5} M	1.11	1.16	0.60	0.46	0.96	nd	1.10	0.92
	1×10^{-6} M	1.10	1.17	0.66	0.43	0.96	nd	1.04	1.07
	1×10^{-7} M	1.05	1.16	0.72	0.43	0.92	nd	1.19	1.03
	0 (0.25% Adigor)	1.13	1.23	0.61	0.43	0.94	nd	1.06	0.91
	0 (no treatment)	1.09	1.15	0.65	0.45	0.89	nd	1.09	0.93

[a] Hormone: aqueous solutions of 4-Me-IAA and 4-Cl-IAA ($1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$ and $1x10^{-4}$ M) in 0.25% Adigor; Control solutions aqueous 0.25% Adigor and control no solution; one application sprayed on plants to cover when the majority of the plants were at the BBCH scale 43-45 developmental stages (mid-boot stage to late-boot stage).

[b] nd = not determined.

[c] Denotes means compared within hormone that are different from the no treatment control and adjuvant only (0.25% Adigor) control at $P \leq 0.05$ using single degree of freedom contrast analysis.

Table 2.11 Effect of 4-Cl-IAA and 4-Me-IAA on number of spikes per plant when applied to field-grown wheat (cv. 5604 HR CL) at Calgary, Red Deer, Regina and Saskatoon sites during the 2012 and 2013 field seasons.

		Number of spikes per plant							
		Calgary		Red Deer		Regina		Saskatoon	
Hormone[a]	Concentration	2012	2013	2012	2013	2012	2013	2012	2013
4-Cl-IAA	1×10^{-4} M	3.00	2.80	4.30	3.70	3.00*[c]	nd[b]	3.30	2.50
	1×10^{-5} M	3.00	2.90	4.00*	3.80	3.00*	nd	3.30	2.50
	1×10^{-6} M	3.00	2.70	3.80*	3.60	3.00*	nd	3.00	2.60
	1×10^{-7} M	3.00	2.70	4.00*	3.50	3.00*	nd	3.50	2.50
	0 (0.25% Adigor)	3.00	2.80	4.30	3.60	3.00*	nd	3.30	2.50
	0 (no treatment)	3.00	2.80	4.50	3.60	3.50	nd	3.00	2.60
4-Me-IAA	1×10^{-4} M	3.00	2.90	3.80	3.70	3.00*	nd	3.30	2.50
	1×10^{-5} M	3.00	2.80	4.30	3.80	3.00*	nd	3.30	2.50
	1×10^{-6} M	3.00	3.00	4.80	4.00	3.00*	nd	3.00	2.50
	1×10^{-7} M	3.00	2.90	4.00	4.00	3.00*	nd	3.00	2.70
	0 (0.25% Adigor)	3.00	2.80	4.30	3.60	3.00*	nd	3.00	2.50
	0 (no treatment)	3.00	2.80	4.50	3.60	3.50	nd	3.30	2.60

[a] Hormone: aqueous solutions of 4-Me-IAA and 4-Cl-IAA ($1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$ and $1x10^{-4}$ M) in 0.25% Adigor; Control solutions aqueous 0.25% Adigor and control no solution; one application sprayed on plants to cover when the majority of the plants were at the BBCH scale 43-45 developmental stages (mid-boot stage to late-boot stage).

[b] nd = not determined.

[c] Denotes means compared within hormone that are different from the no treatment control and adjuvant only (0.25% Adigor) control at $P \leq 0.05$ using single degree of freedom contrast analysis.

Table 2.12 Effect of 4-Cl-IAA and 4-Me-IAA on grain yield and grain size (thousand kernel weight) of six field-grown wheat cultivars (cv. 5604 HR CL, AC Lillian, AC Unity, CDC Go, Harvest, and WR859 CL) at Red Deer and Saskatoon sites during the 2012 and 2013 field seasons.

Variety	Hormone treatments[a] (1×10^{-6} M)	Grain Yield per plot (kg/ha)				Thousand kernel weight (g)			
		Red Deer		Saskatoon		Red Deer		Saskatoon	
		2012	2013	2012	2013	2012	2013	2012	2013
5604 HR CL	4-Cl-IAA	5314	4092	4214	4884	34.00	33.53	33.11*[c]	36.93
	4-Me-IAA	5458	4315	3937*	4890	34.68	33.82	32.12	36.90
	0 (0.25% Adigor)	5299	4039	4184	5083	32.37	33.57	31.73	37.02
	0 (no treatment)	nd[b]	4433	nd	5039	nd	33.29	nd	37.33
AC Lillian	4-Cl-IAA	5067	5741	4289	4562	34.28	40.52	37.73	40.42
	4-Me-IAA	4461	5865	4100	4563	33.30	41.51	36.49	40.69
	0 (0.25% Adigor)	5318	5806	4052	4673	33.90	41.01	36.96	40.91
	0 (no treatment)	nd	5722	nd	4628	nd	41.09	nd	40.77
AC Unity	4-Cl-IAA	5197	4365	5078	5407	34.01	37.42	35.83	37.36
	4-Me-IAA	5466	4443	4878	5427	32.47	37.19	35.20	38.40
	0 (0.25% Adigor)	5290	4359	4999	5578	33.09	37.63	35.92	37.29
	0 (no treatment)	nd	4382	nd	5667	nd	36.94	nd	37.96
CDC Go	4-Cl-IAA	5152	5467	3950*	5892*	35.68	43.12	39.02	44.08
	4-Me-IAA	5005	5244	3851*	5715	35.15	42.96	38.53	44.77
	0 (0.25% Adigor)	4845	5349	3650	5565	35.61	43.47	38.27	44.37
	0 (no treatment)	nd	5128	nd	5635	nd	43.43	nd	43.53
Harvest	4-Cl-IAA	4782	5211	5103	5270	33.81	37.79	35.55	38.87*
	4-Me-IAA	5430	5146	4343	5243	33.58	37.85	35.07	38.32
	0 (0.25% Adigor)	5462	5361	4736	5454	33.54	37.77	35.87	37.91

	0 (no treatment)	nd	5243	nd	5133	nd	37.28	nd	38.92
WR859 CL	4-Cl-IAA	5168	nd	4608	nd	33.25	nd	34.24	nd
	4-Me-IAA	5508	nd	4653	nd	33.55	nd	33.85	nd
	0 (0.25% Adigor)	5193	nd	4680	nd	33.82	nd	34.05	nd
	0 (no treatment)	nd	nd	nd	nd	nd	nd	nd	nd

[a] Hormone treatments: aqueous solutions of 4-Me-IAA and 4-Cl-IAA ($1x10^{-6}$ M) in 0.25% Adigor; Control solutions aqueous 0.25% Adigor and control no solution; one application sprayed on plants to cover when the majority of the plants were at the BBCH scale 43-45 developmental stages (mid-boot stage to late-boot stage) in each variety.

[b] nd = not determined.

[C] Denotes means compared within cultivar that are different from the 0.25% Adigor control at $P \leq 0.05$ using single degree of freedom contrast analysis.

Table 2.13 Effect of 4-Cl-IAA and 4-Me-IAA on protein content (%) and spike weight of six field-grown wheat cultivars (cv. 5604 HR CL, AC Lillian, AC Unity, CDC Go, Harvest, and WR859 CL) at Red Deer and Saskatoon sites during the 2012 and 2013 field seasons.

Variety	Hormone treatments[a] (1×10^{-6} M)	Protein content (%)				Individual spike weight (g)			
		Red Deer		Saskatoon		Red Deer		Saskatoon	
		2012	2013	2012	2013	2012	2013	2012	2013
5604 HR CL	4-Cl-IAA	13.33	12.85	13.13	12.78	0.78	0.92	1.11	1.44
	4-Me-IAA	13.16	12.90	12.57*[c]	12.46	0.81	0.96	1.13	1.39
	0 (0.25% Adigor)	13.23	12.94	13.09	12.78	0.81	0.86	1.20	1.47
	0 (no treatment)	nd[b]	12.95	nd	13.14	nd	0.99	nd	1.35
ACLillian	4-Cl-IAA	13.37	13.83	14.03	13.34	0.75	0.73	0.97	1.19
	4-Me-IAA	14.06	13.86	14.03	12.77	0.65	0.75	1.02	1.10
	0 (0.25% Adigor)	13.70	13.45	14.19	13.14	0.69	0.72	0.99	1.07
	0 (no treatment)	nd	13.64	nd	13.69	nd	0.72	nd	1.11
ACunity	4-Cl-IAA	12.71	12.92	12.88	12.05	0.69	0.66	1.04	1.16
	4-Me-IAA	12.94	12.92	12.48	11.87	0.73	0.76	1.07	1.11
	0 (0.25% Adigor)	13.15	12.97	12.45	12.37	0.76	0.68	1.13	1.18
	0 (no treatment)	nd	12.88	nd	12.28	nd	0.79	nd	1.26
CDC Go	4-Cl-IAA	12.93*	12.18	13.11	11.38	0.78	0.92	1.09	1.35*
	4-Me-IAA	13.26	11.80	13.01	11.73	0.71	0.95	1.04	1.27
	0 (0.25% Adigor)	14.00	12.28	13.28	11.89	0.69	0.90	0.96	1.25
	0 (no treatment)	nd	12.17	nd	12.06	nd	0.89	nd	1.25
Harvest	4-Cl-IAA	12.90	11.80	11.84	11.26	0.69	0.82	1.05	1.17
	4-Me-IAA	13.17	11.84	11.32*	10.85	0.77	0.80	1.05	1.21
	0 (0.25% Adigor)	12.94	12.10	11.98	11.16	0.75	0.76	1.01	1.14

	0 (no treatment)	nd	11.70	nd	10.92	nd	0.72	nd	1.18
WR859 CL	4-Cl-IAA	13.47	nd	12.57	nd	0.73	nd	0.97	nd
	4-Me-IAA	12.65	nd	12.36	nd	0.76	nd	0.94	nd
	0 (0.25% Adigor)	13.48	nd	12.14	nd	0.64	nd	1.00	nd
	0 (no treatment)	nd	nd	nd	nd	nd	nd	nd	nd

[a] Hormone treatments: aqueous solutions of 4-Me-IAA and 4-Cl-IAA ($1x10^{-6}$ M) in 0.25% Adigor; Control solutions aqueous 0.25% Adigor and control no solution; one application sprayed on plants to cover when the majority of the plants were at the BBCH scale 43-45 developmental stages (mid-boot stage to late-boot stage) in each variety.

[b]nd = not determind.

[c] Denotes means compared within cultivar that are different from the 0.25% Adigor control at $P \leq 0.05$ using single degree of freedom contrast analysis.

Table 2.14 Effect of 4-Cl-IAA and 4-Me-IAA on number of grains and spikes per plant of six field-grown wheat cultivars (cv. 5604 HR CL, AC Lillian, AC Unity, CDC Go, Harvest, and WR859 CL) at Red Deer and Saskatoon sites during the 2012 and 2013 field seasons.

Variety	Hormone treatment[a] (1×10^{-6} M)	Number of grains per plant				Number of spikes per plant			
		Red Deer		Saskatoon		Red Deer		Saskatoon	
		2012	2013	2012	2013	2012	2013	2012	2013
5604 HR CL	4-Cl-IAA	95	67	90	86	4.00	3.17	3.50	3.00
	4-Me-IAA	96	77	86	74	4.25	3.00	3.00	2.67
	0 (0.25% Adigor)	95	64	89	80	4.00	3.17	3.00	2.83
	0 (no treatment)	nd[b]	66	nd	71	nd	3.00	nd	2.83
AC Lillian	4-Cl-IAA	93	57	79	68 *[c]	4.03	3.33	3.75	3.17
	4-Me-IAA	90	61	77	67	4.10	3.50	3.75	3.17
	0 (0.25% Adigor)	97	52	75	56	4.25	3.00	3.50	3.17
	0 (no treatment)	nd	56	nd	58	nd	3.33	nd	3.00
AC Unity	4-Cl-IAA	91	52	75	77	4.50	3.17	3.00	3.33
	4-Me-IAA	99	63	87	72	4.25	3.50	3.50	3.00
	0 (0.25% Adigor)	96	56	88	79	3.90	3.00	3.50	3.17
	0 (no treatment)	nd	70	nd	73	nd	3.67	nd	2.83
CDC Go	4-Cl-IAA	82	61	79	72 *	3.98	3.00	3.75	3.17
	4-Me-IAA	86	55	74	60	4.05	2.83	3.50	3.17
	0 (0.25% Adigor)	88	55	71	63	4.25	3.00	3.75	3.00
	0 (no treatment)	nd	55	nd	62	nd	2.83	nd	3.00
Harvest	4-Cl-IAA	90	59	84	75	3.75	3.00	3.50	3.33
	4-Me-IAA	93	58	79	74	3.75	3.00	3.00	3.00
	0 (0.25% Adigor)	99	56	83	73	4.25	3.00	3.25	3.17

	0 (no treatment)	nd	52	nd	68	nd	3.00	nd	3.17
WR859 CL	4-Cl-IAA	92	nd	83	nd	4.1	nd	3.50	nd
	4-Me-IAA	97	nd	82	nd	4.25	nd	3.50	nd
	0 (0.25% Adigor)	94	nd	83	nd	4.25	nd	3.75	nd
	0 (no treatment)	nd	nd	nd	nd	nd	nd	nd	nd

[a] Hormone treatments: aqueous solutions of 4-Me-IAA and 4-Cl-IAA ($1x10^{-6}$ M) in 0.25% Adigor; Control solutions aqueous 0.25% Adigor and control no solution; one application sprayed on plants to cover when the majority of the plants were at the BBCH scale 43-45 developmental stages (mid-boot stage to late-boot stage) in each variety.

[b] nd = not determined.

[c] Denotes means compared within cultivar that are different from the 0.25% Adigor control at $P \leq 0.05$ using single degree of freedom contrast analysis.

Chapter 3 – The effect of auxins on seed yield parameters in canola grown under controlled environment and western Canadian field conditions

3.1 Introduction

Canola oil properties including low saturated fatty acid (4%; and high monounsaturated fatty acid (55%) content (Dupont 1989), a good linoleic to linolenic fatty acid ratio, and greater temperature stability than rapeseed oil (Raymer 2002) make this oil the third most widely consumed food grade oil type in the world (Raymer 2002: USDA 2012). *Brassica napus* L. and *Brassica rapa* L. are the most widely grown canola species in the world which contain more than 40% seed oil content (Raymer 2002). The high protein levels (35-40%), less than 2% of erucic acid and lower glucosinolate content in canola seed meal makes it a more popular feedstock for animals than rapeseed meal (Raymer 2002). Canola is a cool season crop (Angadi and others 2000) that can be adversely affected by abiotic stresses including high temperature (Morrison and Stewart 2002; Angadi and others 2000; Polowick and Sawhney 1988) and drought (Nuttal and others 1992; Bérard and others 2001; Gan and others 2004). Pollowick and Sawhney (1988) reported 32^{o}C/26^{o}C day/night temperatures caused male and female sterility in the flowers of *B. napus* (cv.Westar). Yield reduction in canola due to high temperature stress during flowering can be due to a number of factors including anther abortion, pollen sterility, ovule sterility, inhibition of pollen tube growth and fruit abortion (Morrison and Stewart 2002; Young and others 2004).

The use of plant growth regulators to ameliorate the effects of abiotic stress on crops is an emerging area of research in agriculture (Ullah and others 2012; Farooq and others 2009; Kagale and others 2007), where plant growth regulators are used to regulate and adjust the endogenous hormone levels in favor of normal physiological processes and restore the crops yielding ability. Among the classes of plant growth regulators, auxins show some promising characteristics in regulating abiotic stress (heat and drought) effects related to plant growth and yielding ability of specific crops (Sakata and others 2010; Shi and others 2014). Application of auxin can be effective in increasing yield and yield component parameters even under non-stress conditions (El-Saeid and others 2010). Indole-3-acetic acid (IAA) application (at 25, 50 and 100 mg/L) in cowpea (*Vigna sinensis* L.) 10 days before and after flowering were reported to increase the

number and weight of pods and number and weight of seeds with the lowest and moderate IAA concentrations used in this study (El-Saeid and others 2010). Asghar and others (2004) found that inoculation of auxin producing rhizobacteria onto *B. napus* seeds under gnotobiotic conditions increased the plant height, root length, number of racemes per plant, stem diameter, number of pods per plant, thousand kernel weight, yield and oil content compared with the uninoculated control. Auxin production assays with these rhizobacteria showed that *in vitro* auxin production ability was related to the number of racemes per plant and seed oil content (Asghar and others 2004).

It is important to use highly active auxin types to reduce the bulk application and/or concentration of the solution and runoff of the excess solution. Using excised tissue biological assays, it has been demonstrated that 4-chloroindole-3-acetic acid (4-Cl-IAA) is a highly active auxin (Katekar and Geissler 1983; Bottger and others 1978; cited from Reinecke 1999). Furthermore, after 4-Cl-IAA, 4-methylindole-3-acetic acid (4-Me-IAA) was found to be the second most effective auxins in stimulating deseeded pea pericarp growth (Reinecke and others 1999).

This study was undertaken to determine the ability of 4-Cl-IAA and 4-Me-IAA to increase the seed yield and yield component parameters in canola when applied as a single application to cover the whole plant, when the floral buds were at the green bud stage prior to bolting. The auxin effects were assessed on canola plants exposed to non-stress or heat-stress conditions following auxin treatment in environmentally controlled conditions, or under field conditions.

3.2 Materials and methods

3.2.1 Experiments under controlled environmental conditions

3.2.1 1 Greenhouse Experiment -1-Canola cv. Peace greenhouse experiment

This greenhouse experiment was designed and carried out by the Ozga/Reinecke lab. I aided in data collection and completed the statistical analysis presented in this chapter. Canola seeds (*Brassica napus* L.) from the cultivar Peace were sown at an approximate depth of 1 cm in

12.7 cm square plastic pots (15.24 cm pot depth; 4 seeds per pot) in 1:1 Sunshine #4 potting mix (Sun Gro Horticulture, Vancouver, Canada) and sand. The seedlings were thinned to one seedling per pot approximately 2 weeks after seeding. Plants were maintained in a climate-controlled greenhouse at the University of Alberta (Edmonton, Alberta) from November 14, 2011 to March 5, 2012. The average temperature was approximately 18^{o}C day/16^{o}C night from November 14, 2011 to February 8, 2012 and 21^{o}C day/19^{o}C night from February 8 to March 5, 2012. The plants also received supplemental lighting daily (average photon flux density of 250 μmol $m^{-2}s^{-2}$) for 16 h per day (from 6:00 to 22:00).

Aqueous solutions of 4-methylindole-3-acetic acid (4-Me-IAA) and 4-chloroindole-3-acetic acid (4-Cl-IAA) at 1×10^{-6}, 1×10^{-5} and 1×10^{-4} M in 0.1% (v/v) Tween 80 or an adjuvant only control solution (0.1% [v/v] Tween 80) were applied to canola plants (sprayed to cover). All the plants were treated at the green bud stage (BBCH scale 51; prior to bolting when flower buds are visible from above, but they are tightly clustered and have not extended above smallest leaves surrounding the inflorescence) and they were separated into two sets. In the first set, half of the plants were exposed to a heat stress treatment for 6 days by moving plants to a heat stress chamber just after the treatment application. The other half of the plants of the first set remained in the greenhouse. In the second set, all the plants remained in the greenhouse for one week after the first hormone application, then the plants were treated a second time with the same hormone solution (second treatment one week after the first application). Half of the plants in the second set were exposed to a heat stress treatment for 6 days, with the remainder of the plants remaining in the greenhouse. The entire experiment was arranged in a completely randomized design (CRD) with 6 replications (plants) per treatment.

The heat stress treatment was imposed by moving plants to receive the heat stress from the greenhouse into a growth chamber for 6 days. The light cycle began at 7:00 h at a 19^{o}C air temperature. The heat treatment began at 11:00 h (33^{o}C air temperature) and was maintained for 6 h (until 17:00 h). Following the heat treatment, the remainder of the light cycle was maintained at a 22^{o}C air temperature. The dark cycle (began at 23:00 h) was maintained at 17^{o}C. The photoperiod was 16 h light/8 h dark at an average photon flux density of 492 μmol $m^{-2}s^{-2}$ using 54W/835/HO high fluorescent bulbs (Phillips, Holland). After 6 days, the heat stress-treated plants were returned to the original greenhouse and maintained at non-heat stress conditions to

develop to maturity. The plant potting medium was maintained moist in all treatments throughout the experiment and fertilized with 20: 20: 20 (N: P: K) as required.

3.2.1.2 Greenhouse Experiment -2-Canola cv. 45H21greenhouse experiment

Roundup ready Pioneer hybrid canola cultivar 45H21 was seeded on February 27, 2012. Seeds were sown at an approximate depth of 1.5 cm in 3.79 L round plastic pots (3 seeds per pot) filled with 1:1 Sunshine #4 potting mix (Sun Gro Horticulture, Vancouver, Canada) and sand. The seedlings were thinned to one seedling per pot approximately 2 weeks after seeding. Pots were maintained in a climate-controlled greenhouse at the University of Alberta (Edmonton, Alberta) until the maturity, with an average temperature of 21^{o}C day/19^{o}C night from February 27 to June 29, 2012. The plants also received supplemental lighting daily (average photon flux density of 250 μmol $m^{-2}s^{-2}$) for 16 h per day (from 6:00 to 22:00 h). Pots were watered to maintain the plants in a fully hydrated condition.

Aqueous solutions of 4-Me-IAA at 1×10^{-6} M and 1×10^{-7} M in 0.25% (v/v) Adigor, Agnique or 0.1% [v/v] Tween 80, or adjuvants only solutions (0.25% [v/v] Adigor or Agnique; 0.1% [v/v] Tween 80) were sprayed on plants to cover using a hand sprayer when the majority of plants were at BBCH 50 (flower buds present, still enclosed by leaves) to BBCH 52 (green buds free from the level of young leaves). The experiment was arranged in a CRD with 12 replications (plants) per treatment in the treatments using Adigor or Agnique as adjuvants, and 6 replications (plants) per treatment in the treatment using Tween 80 as the adjuvant. Plant responses to 4-Me-IAA in different adjuvant solutions were tested under non-heat stress condition.

3.2.2. Field experiments

3.2.2.1 Canola cvs. Canterra 1852 H and SY 4114 dose-response experiments

4-Cl-IAA and 4-Me-IAA dose-response field experiments were conducted in four locations in the prairies eco-zone in western Canada. There were two locations in Alberta (Calgary [Latitude: 50.7167/ Longitude: -113.3167 and Red Deer [Latitude: 52.24/ Longitude: -113.86]) and two in Saskatchewan (Regina [Latitude: 50.44 / Longitude: -104.37] and Saskatoon [Latitude: 52.42/ Longitude: -106.38]) in 2012 and 2013. The Calgary and Red Deer sites were

dominated with Black Chernozemic soils (Soil group map of Alberta 2014), where as Saskatoon sites had dark brown regosolic soil, and Regina sites had dark brown vertisolic soil (Saskatchewan Ministry of Agriculture 2009; Anderson 2010).

Helix XTra 289FS-treated seeds (at 434 g active ingredient/100kg = 400 g Thiamethoxam/100 kg seed + 24 g Difenoconazole/100kg seed + 7.5 g Metalaxyl-M/100kg seed + 2.5 g Fludioxonil / 100kg seed) of Roundup ready hybrid canola cultivars Canterra 1852 H (in 2012) or SY 4114 (in 2013) were sown into the soil at a 1.25 to 2.5 cm depth, using a calibrated cone seeder at a rate of 6.5 kg/ha. Plots were arranged in a Randomized Complete Block Design (RCBD) consisting of 10 treatments per replicate (4-Cl-IAA and 4-Me-IAA at 1×10^{-7} M, 1×10^{-6} M, 1×10^{-5} M and 1×10^{-4} M in aqueous 0.25% Adigor, 0.25% aqueous Adigor only control, and a no treatment control), with 4 (in 2012) or 6 (in 2013) replicates per treatment. Treatments were randomly applied to plots within each replicate. Individual treatment plot size was 6 m×1.5 m in 2012 and 7 m×1.5 m in 2013. Inter-plot spacing was 0.3 m, with a 2 m buffer between replicates. Each treatment plot consisted of 7 rows with an inter-row spacing of 18 cm from the middle of one row to the middle of next row. Guard plots bordered the outermost treatment plots of the replicate row (Appendix A; Figure A.1. Plot illustration: 4-Me-IAA and 4-Cl-IAA dose response field experiments for wheat, canola and pea).

All the auxins and adjuvant solutions used were made by Dr. Dennis M. Reinecke, University of Alberta and stored in freezers in dark bottles and delivered to field locations under frozen and dark conditions. Frozen hormone and adjuvant control solutions were completely thawed in the dark, at ambient temperature, and mixed thoroughly, just prior to field application. In almost all sites the solutions were applied at BBCH 50-52 (when flower buds are enclosed by leaves to flower buds free from the level of younger leaves) plant growth stages except in Saskatoon 2013 (Saskatoon 2013 canola plots were sprayed a little later at BBCH 53 [flower buds raised above the youngest leaves] to BBCH 57 [individual flower buds visible, but still closed]. Hormones were sprayed to cover the plot plant canopy using a hand boom having 3 nozzles (110-02 low drift air induction nozzle with 275 kpa pressure) with a 90^{o} angle to the crop at a rate of 200 L/ha spray volume. The application of herbicides, fungicides or any other chemical treatment for pest and or disease control was not carried out 7 days before or after the treatment application. Phenoxy or auxinic based products, Pyrasulfotole or other HPPD-

inhibitors or bleachers were not used for any crop management purposes throughout the growing season. All of the other crop maintenance practices were completed following normal commercial canola cultivation in western Canada (Canola Council of Canada 2012).

In 2012, plot plant density was determined by counting the number of plants in each plot after final plot trimming was completed. In 2013, plot plant density was estimated by counting the number of plants per meter (1 m per row in a diagonal pattern across the plot using the middle 5 rows) after the final plot trimming was completed (five plants per plot were randomly selected from the inner rows of each plot for determination of plant dry weight, number of racemes per plant, number of pods per plant and plant height, when plants were at the BBCH 80 growth stage (beginning of ripening). For dry weights, plants (excluding roots) were dried at 45^{o}C for two days prior to weighing. Individual plots were harvested using a Wintersteiger Classic plot combine harvester with a 150 cm wide header. Plot seed yield as measured from the combine harvester was normalized for 8% moisture content and dockage weight. Weight of dockage per plot was estimated by calculating the percent dockage in 500 g seed subsamples from each plot (using a Clipper seed cleaner model 400: Seedburo, IL, USA). To calculate 1000 kernel weight (TKW) per plot, 10 lots of 1000 seeds were counted using a Seedburo 801 count-a-pak seed counter (IL, USA) at sensitivity 33 and speed 47), weighed, then averaged. A Foss NIR system (Denmark) was used to measure the seed oil content, Data were normalized using an ISI Window Near Infrared WinISI II, Version 1.02A. Hormone treatments were compared with an adjuvant only (2.5% Adigor) control and no treated control.

Weather data were collected using an onsite weather station at the Calgary, Regina and Saskatoon field sites, while Red Deer weather data were collected from a Red Deer, Alberta, weather station WMO ID 71878. Soil moisture (vol%) was measured using a Watermark Soil Moisture Sensor (Spectrum Technologies, IL, USA) placed at a depth of 10 cm in Saskatoon and Regina. Soil moisture storage capacity (SMSC) was measured at the Calgary site using a Watchdog data logger with a Watermark Soil Moisture Sensor (Spectrum Technologies, IL, USA) placed at a depth of 20 cm.

3.2.3 Statistical analysis

3.2.3.1 Greenhouse experiments

Analyses were performed using the PROC MIXED procedure of SAS 9.3 software (SAS Institute Inc. Cary, NC, USA, 2010). For greenhouse experiment 1, statistical significance of the data was determined by using a 4×2×2 factorial analysis of variance (4-Cl-IAA concentration×heat stress condition×number of hormone applications). Mean separation (main effects, treatments, and interactions) were determined using the Least Significant Difference (LSD) test. For greenhouse experiment 2, statistical significance of the data was determined by using a 3×3 factorial analysis of variance (adjuvant type×4-Me-IAA concentration). Mean separation (main effects, treatments, and interactions) were determined using the LSD test.

3.2.3.2 Field experiments

For the canola cultivar dose response field trails, an analysis of variance (ANOVA) or analysis of covariance (ANCOVA) (for seed yield) was performed on field data within location and year using the PROC MIXED procedure of SAS 9.3 software (SAS Institute Inc. Cary, NC, USA, 2010). The one-way ANOVA analyses were carried out for yield component parameters (here treatment level was taken as the main effect and analyses were carried out for 4-Me-IAA treatments and controls as well as for 4-Cl-IAA treatments and controls separately). Similarly, the one-way ANCOVA was carried out to analyze plot seed yield data with the treatment as the main effect and the number of plants per meter as the covariate. Preplanned single degree of freedom contrast analysis was performed between the adjuvant only control, 4-Me-IAA, and 4-Cl-IAA treatments, and the no treatment control, 4-Me-IAA, and 4-Cl-IAA treatments at each location and year. These contrasts were preplanned to examine specific treatment effects that would be of agronomic or biological importance. However, only yield parameters that showed significant differences compared with the adjuvant control with respect to the hormone treatments were discussed. Statistical significance was declared at $P \leq 0.05$.

3.3 Results

3.3.1 Experiments under controlled environmental conditions

3.3.1.1 Greenhouse Experiment -1- Canola cv. Peace greenhouse experiment

This experiment was carried out to examine the effects of 4-Cl-IAA and 4-Me-IAA concentrations, number of applications and heat stress effects on seed yield and yield component parameters of the early maturing open-pollinated canola cv. Peace. Different concentrations (1×10^{-4}, 1×10^{-5} and 1×10^{-6} M) of 4-Cl-IAA or 4-Me-IAA were applied on plants at two developmental stages with plants subsequently exposed to heat stress or non-heat stress conditions. One application of 4-Cl-IAA at 1×10^{-6} M increased seed weight per plant when plants were grown under non-heat stress conditions (46% increase) and when the plants were exposed to heat stress conditions following auxin application (51% increase) compared with the adjuvant only control (Table 3.1). Reduction in number of pods per plant was observed in the plants which received two applications of the highest concentration (1×10^{-4} M) of 4-Cl-IAA under control (109% reduction) and heat stress (86% reduction) conditions compared with the adjuvant only control (Table 3.1). Similar results were observed in the plants that received one application of the highest concentration of 4-Cl-IAA under controlled temperature condition (59% reduction in number of pods per plant compared with the adjuvant only control; Table 3.1). 4-Cl-IAA had no effect on number of racemes per plant under either temperature treatments with one auxin application. However, fewer racemes per plant were observed in the plants that received two applications of the highest concentration of 4-Cl-IAA under heat stress conditions (Table 3.1).

Plants which received one or two applications of 4-Me-IAA had no effect on seed weight under either temperature treatment of cv. Peace. One application of 4-Me-IAA (1×10^{-5} M) to plants that were subsequently exposed to 6 days of heat stress increased the number of pods per plant (36%), but the increase in pod number was not correlated with an increase in seed yield compared with the adjuvant only control (Table 3.2). Two applications of the highest concentration (1×10^{-4} M) of 4-Me-IAA reduced the number of pods per plant and number of racemes per plant under non-heat stress conditions (Table 3.2).

The 6-day heat treatment increased the number of pods and racemes per plant in cv. Peace independent of the auxin treatments (Tables 3.1 and 3.2, compare temperature treatment means).

3.3.1.2 Greenhouse experiment -2- Canola cv.45H21greenhouse experiment

To examine the effects of 4-Me-IAA concentration and type of adjuvant on seed yield and yield component parameters of the hybrid canola cv. 45H21, different concentrations (1×10^{-6}, 1×10^{-7}M and adjuvant only controls) were applied at BBCH 50-52 growth stages using 3 different adjuvant types. 4-Me-IAA had no effect on seed weight, number of pods per plant or number of racemes per plant in cv. 45H21 when applied with the adjuvants Adigor, Agnique or Tween 80 under non-heat stress conditions (Table 3.3).

3.3.2. Field experiments

3.3.2.1 Canola cvs. Canterra 1852 H and SY 4114 dose-response experiments

Among the four sites over the 2012 and 2013 field seasons, the Regina site in 2012, and the Saskatoon and Calgary sites in 2013 exhibited moderate heat stress (12 or more days at or above 25^{o}C daily maximum temperature) 14 days before and after hormone treatment (at BBCH 50-52 growth stages of canola), with adequate soil moisture conditions (Appendix, Table B.1). Application of 4-Me-IAA at 1×10^{-4} M increased plot seed yield of hybrid canola cv. SY 4114 by 36% compared with the adjuvant only control ($P\leq0.05$ for single degree of freedom contrasts; Table 3.4) at the Saskatoon site in 2013. This yield increase was reflected in greater seed size (TKW; Table 3.5). 4-Cl-IAA at 1×10^{-5} M also increased seed size (TKW) at the Saskatoon site in 2013 (Table 3.5). Application of 4-Me-IAA at 1×10^{-7} M also increased seed yield per plot in cv. Canterra 1852 H at the Calgary site in 2012 (Table 3.4). Higher seed oil content was observed at the Red Deer site in 2012 with all 4-Me-IAA concentrations, and at the higher concentrations of 4-Cl-IAA (1×10^{-5} and 1×10^{-4} M) in cv. Canterra 1852 H (Table 3.6). Also, higher seed oil content was observed with 4-Me-IAA treatment at 1×10^{-6} M in cv. SY 4114 at the Calgary site in 2013 compared with the adjuvant only control (Table 3.6). The auxins had minimal to no effect on plant height, number of pods per plant and number of racemes per plant in Canterra 1852 H in 2012 or SY 4114 in 2013 (Table 3.7, 3.8, and 3.9).

3. 4 Discussion

The heat stress treatment (33^{o}C for 6 h per day for 6 days) increased the number of pods per plant (20-21%) and number of racemes per plant (19-25%) in the self-pollinating canola cv. Peace (Tables 3.1 and 3.2). Young and others (2004) also reported that short-term heat stress during the reproductive growth stage (one week or two weeks of high temperature stress [8/16 h dark/light, 18 °C night, ramped at 2°C h±1, over 6 h, to 35 °C for 4 h, ramped at 2 °C h±1 back to 23 °C for 6 h] given at 50% flowering stage) induced the production of lateral inflorescences, and thereby resulted in increased seed and silique production of canola *B. napus* (cv. DH12075).

The effect of auxin application on canola seed yield parameters varied with type of auxin, genotype and temperature at flowering. In cv. Peace under controlled environment conditions, 4-Cl-IAA (1×10^{-6} M) increased seed yield per plant (Table 3.1) under heat stress (during initial flowering; 51% higher grain weight per plant) and heat control conditions (46% higher grain weight per plant). 4-Me-IAA had no effect on seed yield in the canola cv. Peace (Table 3.2). In the situations where a higher level of 4-Cl-IAA is expected to be absorbed by the plant such as one application of 1×10^{-4} M under non-heat stress conditions (auxins are liable under high temperature conditions) or two applications of 1×10^{-4} M, reduced the number of pods per plant, but not the seed (Table 3.1). Similarly, two applications of 4-Me-IAA (a less active auxin than 4-Cl-IAA in the pea split-pericarp assay; Reinecke and others 1999) at 1×10^{-4} M under non-heat stress conditions (likely the treatment with the highest available 4-Me-IAA for plant up-take) reduced the number of pods per plant, but not the seed weight per plant (Table 3.2). Katekar and Geissler (1983) found 4-Cl-IAA at 1×10^{-4} M reduced elongation of pea and wheat stem sections in *invitro* bioassays, where lower concentrations (1×10^{-8} and 1×10^{-6} M) increased stem elongation. Using *Avena* coleoptile (cv. Carsten Phoenix) section bioassays, 4-Cl-IAA and IAA dose-response curves also showed that both auxins were inhibitory to growth at 1×10^{-4} M (Böttger and others 1978). These studies show that supra-optimal concentrations of auxins can negatively affect tissue growth. In the present canola experiment, high concentrations of 4-Cl-IAA and 4-Me-IAA were observed to cause flower or pod abortion; however, our data suggest that high auxin levels increased the assimilate partitioning to the first developing pods, increasing final seed size in the remaining pods (and possibly the number of seeds per pod, but

this was not assessed). Consistently, Cole and Patrick (1998) found that an agar gel of IAA (1×10^{-7} M) injected into the flag internode lacuna of durum wheat (*Triticum turgidum* cv. Franshawi) promoted the transport of ^{14}C-labelled photoassimilate from the flag leaf to the grain, and grain dry matter accumulation throughout the grain filling phase. It is possible that the increased assimilate partitioning into the first developing canola pods may have reduced the longevity of the floral meristem leading to a reduced number of pods per plant. Alternately, the auxin affect on floral meristem longevity may be independent of its effect on assimilate partitioning into the pods.

Our data suggest that auxin response for increasing canola seed yield is likely genotype dependent. In cv. 45H21 under normal temperature conditions, lower concentrations of 4-Me-IAA (1×10^{-6} and 1×10^{-7} M) resulted in higher average seed weight per plant and pod number per plant, but these data did not reach the significant level (Table 3.3). The cultivar Peace was responsive to 4-Cl-IAA, but not 4-Me-IAA with respect to increasing seed yield (Tables 3.1 and 3.2).

In the field studies conducted in 2012 and 2013, 4-Me-IAA was effective in increasing plot seed yield in the hybrid canola cultivar 1852 H at the Calgary site in 2012 (1×10^{-7} M) and SY 4114 at the Saskatoon site in 2013 at 1×10^{-4} M (Table 3.4). The increase in plot seed yield for cv. SY 4114 was reflected in greater seed size (TKW; Table 3.5) without affecting the percent seed oil content (Table 3.6). It should be noted that the lower daily temperature following hormone application in 2012 at the Calgary site likely facilitated auxin up-take into the plant, whereas the higher temperature following hormone application at the 2013 Saskatoon site likely reduced auxin up-take into the plant (Appendix Table B.1). This temperature difference following hormone application may partially explain the large difference in the concentration that was effective in increasing seed yield at these two sites. These data suggest that both auxins can be effective in increasing seed yield parameters in canola (*B. napus*) under normal and heat stress conditions. Sakata and others (2010) found that multiple applications of auxins (IAA, NAA, or 2,4-D) functioned to ameliorate the effects of high temperature on male sterility/anther abortion and seed setting rate in *Arabidopsis* (a member of the Brassicaceae family, like canola) Asghar and others (2004) found that inoculation of auxin producing rhizobacteria onto *B. napus* seeds under gnotobiotic conditions increase seed yield and yield component parameters. Here we

showed that single application of auxin (4-Cl-IAA or 4-Me-IAA) to the canola plant has a cultivar specific ability to increase seed yield parameters under both normal and heat stress conditions, and under field conditions.

Table 3.1 Effect of 4-Cl-IAA concentration and number of 4-Cl-IAA applications on seed weight, number of pods, and number of racemes per plant of greenhouse-grown open-pollinated canola cv. Peace plants.

Auxin type	Hormone conc.[z]	Heat trt[y]	Number of applications[x]	Seed weight per plant (g)	Number of pods per plant	Number of racemes per plant
4-Cl-IAA	1×10^{-4}	-	1	2.997 ab[w]	100.2 b	7.8 a
4-Cl-IAA	1×10^{-5}	-	1	2.945 ab	183.3 a	12.8 a
4-Cl-IAA	1×10^{-6}	-	1	3.822 b	160.7 a	11.2 a
Tween 80	0	-	1	2.612 a	158.8 a	10.7 a
Mean		-	1	3.094 e	150.8 e	10.6 e
4-Cl-IAA	1×10^{-4}	+	1	2.615 a	141.2 a	11.3 a
4-Cl-IAA	1×10^{-5}	+	1	2.860 a	198.0 a	13.8 a
4-Cl-IAA	1×10^{-6}	+	1	4.145 b	214.7 a	13.3 a
Tween 80	0	+	1	2.738 a	185.0 a	13.3 a
Mean		+	1	3.090 e	184.7 f	12.9 f
4-Cl-IAA	1×10^{-4}	-	2	3.278 a	75.2 b	8.8 a
4-Cl-IAA	1×10^{-5}	-	2	4.006 a	123.0 a	12.6 a
4-Cl-IAA	1×10^{-6}	-	2	3.732 a	163.3 a	11.3 a
Tween 80	0	-	2	2.808 a	157.6 a	11.0 a
Mean		-	2	3.456 e	129.8 e	10.9 e
4-Cl-IAA	1×10^{-4}	+	2	3.214 a	93.0 b	8.2 b
4-Cl-IAA	1×10^{-5}	+	2	4.918 b	161.6 a	13.2 a
4-Cl-IAA	1×10^{-6}	+	2	5.534 b	180.2 a	14.8 a
Tween 80	0	+	2	4.406 b	173.0 a	14.3 a
Mean		+	2	4.518 f	151.9 e	12.6 e
4-Cl-IAA mean	1×10^{-4}			3.026 g	102.4 g	9.0 g
4-Cl-IAA mean	1×10^{-5}			3.682 g	166.5 h	13.1 h
4-Cl-IAA mean	1×10^{-6}			4.308 h	179.7 h	12.6 h
Tween 80 mean	0			3.141 g	168.6 h	12.3 h
Application 1 mean				-	167.7 m	11.8 m
Application 2 mean				-	140.9 n	11.8 m
Temperature means		+		-	168.3 r	12.8 r
		-		-	140.3 s	10.8 s
Temperature × application number				SD[v]	NS	NS

[z] Hormone concentration: aqueous solutions of 4-Cl-IAA ($1x10^{-6}$, $1x10^{-5}$ M and $1x10^{-4}$ M) in 0.1% Tween 80; Control adjuvant solution: 0.1% Tween 80 only.

[y] Heat treatment: The heat stress treatment was imposed by moving plants to receive the heat stress to a growth chamber (heat stress chamber) for 6 days, just after the hormone application. In the heat stress chamber, the light cycle began at 7:00 h at a 19°C air temperature. The heat treatment began at 11:00 h

(33^{o}C air temperature) and was maintained for 6 (until 17:00 h). Following the heat treatment, the remainder of the light cycle was maintained at a 22^{o}C air temperature. The dark cycle (began at 23:00 h) was maintained at 17^{o}C; photoperiod was 16 h light/8 h dark. After 6 days, the heat stress-treated plants were returned to the greenhouse maintained at non-heat stress conditions to develop to maturity.

[x] Number of applications: For one application, plants were sprayed to cover when the majority of the plants were at the BBCH 51 (green buds tightly clustered inside the young leaves); For two applications, plants were first sprayed to cover when the majority of the plants were at the BBCH 51 and again one week after the first application.

[w] The means of parameters with significantly different factorial treatment effects ($P \leq 0.05$) were separated using the LSD test. Means followed by a different letter are significantly different; (a and b) within heat treatment and application number, (e and f) between heat stress and control treatments within application number, (m and n) between one and two applications within same heat treatment, and (r and s) between heat stress and heat control treatments irrespective to the application number by the LSD test, $P \leq 0.05$.

[v] NS = non significant, SD = significant, at $P>0.05$.

Table 3.2 Effect of 4-Me-IAA concentration and number of 4-Me-IAA applications on seed weight, number of pods, and number of racemes per plant of greenhouse-grown open-pollinated canola cv. Peace plants.

Auxin type	Hormone conc.[z]	Heat trt[y]	Number of applications[x]	Seed weight per plant(g)	Number of pods per plant	Number of racemes per plant
4-Me-IAA	1×10^{-4}	-	1	3.087 a[w]	141.5 a	11.0 a
4-Me-IAA	1×10^{-5}	-	1	2.925 a	153.5 a	10.5 a
4-Me-IAA	1×10^{-6}	-	1	3.615 a	139.8 a	9.3 a
Tween 80	0	-	1	2.525 a	148.0 a	11.7 a
Mean		-	1	3.038 e	145.7 e	10.5 e
4-Me-IAA	1×10^{-4}	+	1	3.050 a	180.3 a	12.0 a
4-Me-IAA	1×10^{-5}	+	1	2.656 a	247.6 b	16.6 a
4-Me-IAA	1×10^{-6}	+	1	3.618 a	159.2 a	13.3 a
Tween 80	0	+	1	3.630 a	182.0 a	12.7 a
Mean		+	1	3.239 e	192.3 f	13.7 f
4-Me-IAA	1×10^{-4}	-	2	4.230 a	99.3 a	8.5 a
4-Me-IAA	1×10^{-5}	-	2	2.990 a	165.0 b	10.6 b
4-Me-IAA	1×10^{-6}	-	2	3.178 a	155.0 b	12.2 b
Tween 80	0	-	2	3.747 a	182.2 b	13.3 b
Mean		-	2	3.536 e	150.4 e	11.2 e
4-Me-IAA	1×10^{-4}	+	2	3.805 a	150.5 a	13.7 a
4-Me-IAA	1×10^{-5}	+	2	3.577 a	170.2 a	13.0 a
4-Me-IAA	1×10^{-6}	+	2	4.833 a	171.6 a	13.2 a
Tween 80	0	+	2	4.500 a	171.7 a	14.0 a
Mean		+	2	4.179 e	165.9 e	13.5 f
4-Me-IAA mean	1×10^{-4}			3.543 g	142.9 g	11.3 g
4-Me-IAA mean	1×10^{-5}			3.037 g	184.1 g	12.7 g
4-Me-IAA mean	1×10^{-6}			3.811 g	156.4 g	12.0 g
Tween 80 mean	0			3.600 g	170.9 g	12.8 g
Application 1 mean				3.138 m	168.9 m	12.1 m
Application 2 mean				3.858 n	158.2 m	12.3 m
Temperature means		+		3.709 r	179.1 r	13.6 r
		-		3.287 r	148.0 s	10.8 s
Temperature × application number				NS[v]	NS	NS

[z] Hormone concentration: aqueous solutions of 4-Cl-IAA ($1x10^{-6}$, $1x10^{-5}$ M and $1x10^{-4}$ M) in 0.1% Tween 80; Control adjuvant solution: 0.1% Tween 80 only.

[y] Heat treatment: The heat stress treatment was imposed by moving plants to receive the heat stress to a growth chamber (heat stress chamber) for 6 days, just after the hormone application. In the heat stress chamber, the light cycle began at 7:00 h at a 19^{o}C air temperature. The heat treatment began at 11:00 h (33^{o}C air temperature) and was maintained for 6 h (until 17:00 h). Following the heat treatment, the remainder of the light cycle was maintained at a 22^{o}C air temperature. The dark cycle (began at 23:00 h) was maintained at 17^{o}C; photoperiod was 16 h light/8 h dark. After 6 days, the heat stress-treated plants were returned to the greenhouse maintained at non-heat stress conditions to develop to maturity.

[x] Number of applications: For one application, plants were sprayed to cover when the majority of the plants were at the BBCH 51 (green buds tightly clustered inside the young leaves); For two applications, plants were first sprayed to cover when the majority of the plants were at the BBCH 51 and again one week after the first application.

[w] The means of parameters with significantly different factorial treatment effects ($P \leq 0.05$) were separated using the LSD test. Means followed by a different letter are significantly different; (a and b) within heat treatment and application number, (e and f) between heat stress and control treatments within application number, (m and n) between one and two applications within same heat treatment, and (r and s) between heat stress and heat control treatments irrespective to the application number by the LSD test, $P \leq 0.05$.

[v] NS = non significant, SD = significant, at $P>0.05$.

Table 3.3 Effect of 4-Me-IAA and type of adjuvant on seed weight, number of pods and number of racemes per plant of greenhouse-grown hybrid canola (cv. 45H21) plants.

Adjuvant	4-Me-IAA concentration[z]	Seed weight per plant (g)	Pods per plant	Number of racemes per plant
Adigor	1×10^{-7} M	10.795 a[w]	264 a	14.9 a
	1×10^{-6} M	10.035 a	265 a	14.2 a
	0	10.168 a	239 a	13.1 a
Mean		10.333 m	256 m	14.1 m
Agnique	1×10^{-7} M	10.967 a	283 a	15.3 a
	1×10^{-6} M	10.793 a	246 a	13.1 a
	0	10.472 a	253 a	12.6 a
Mean		10.744 m	261 m	13.6 m
Tween 80	1×10^{-7} M	10.830 a	256 a	13.8 a
	1×10^{-6} M	11.203 a	266 a	13.2 a
	0	9.655 a	250 a	14.7 a
Mean		10.563 m	257 m	13.9 m
4-Me-IAA	1×10^{-7} M mean	10.864 r	268 r	14.7 r
	1×10^{-6} M mean	10.677 r	259 r	13.5 r
	0 mean	10.098 r	247 r	13.5 r

[z] 4-Me-IAA concentration: aqueous solutions of 4-Me-IAA ($1x10^{-7}$ and $1x10^{-6}$ M) in 0.25% Adigor or 0.25% Agnique or 0.1% Tween80; Control adjuvant solutions: aqueous 0.25% Adigor or 0.25% Agnique or 0.1% Tween 80 only; one application sprayed on plants to cover when the majority of the plants were at the BBCH 50 to 52 developmental stage (Green buds present inside the leaves to green buds free from young leaves).

[w] The means of parameters with significantly different factorial treatment effects and/or significant interaction effects (at ($P\leq 0.05$) were separated using the LSD test. Means followed by a different letter are significantly different within sets (a: within adjuvant), (m), and (r) by the LSD test, $P < 0.05$.

Table 3.4 Effect of 4-Cl-IAA and 4-Me-IAA on seed yield of field-grown hybrid canola during the 2012 (cv. Canterra 1852 H) and 2013 (cv. SY 4114) field seasons.

		Seed yield (kg/ha)							
		Calgary		Red Deer		Regina		Saskatoon	
Trt[a]	Conc.	2012 (1852H)	2013 (SY4114)	2012 (1852H)	2013 (SY4114)	2012 (1852H)	2013 (SY4114)	2012 (1852H)	2013 (SY4114)
4-Cl-IAA	1×10^{-4} M	2777	3283	3967	1857	1816	nd[b]	2328	4477
	1×10^{-5} M	2938	3319	3930	1796	2061	nd	2381	3892
	1×10^{-6} M	3184	3310	4047	1921	1699	nd	2782	4478
	1×10^{-7} M	2914	3233	3999	1708	1808	nd	2437	4861
	0 (0.25% Adigor)	2848	3100	4232	1689	2027	nd	2685	3740
	0 (no treatment)	2939	3432	4066	2006	1850	nd	2502	4061
4-Me-IAA	1×10^{-4} M	2781	3347	4226	1736	1925	nd	2389	4994 *[z]
	1×10^{-5} M	2968	3345	3894	1509	1698	nd	2385	4486
	1×10^{-6} M	3144	3334	3709 *	1982	1745	nd	2571	4418
	1×10^{-7} M	3157 *	3306	3602 *	1723	1630	nd	2643	4159
	0 (0.25% Adigor)	2779	3099	4471	1676	2007	nd	2601	3680
	0 (no treatment)	2993	3433	3867	2031	1818	nd	2533	4059

[a] Hormone: aqueous solutions of 4-Me-IAA and 4-Cl-IAA ($1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$ and $1x10^{-4}$ M) in 0.25% Adigor; Control solutions aqueous 0.25% Adigor and control no solution; one application sprayed on plants to cover when the majority of the plants were at the BBCH 50-52 developmental stages (when flower buds are enclosed by leaves to flower buds free from the level of younger leaves).

[b] nd = not determined.

[z] Denotes means compared within hormone treatment that are different from the control (0.25% Adigor) at $P \leq 0.05$ using single degree of freedom contrast analysis.

Table 3.5 Effect of 4-Cl-IAA and 4-Me-IAA on seed size (thousand kernel weight) of field-grown hybrid canola during the 2012 (cv. Canterra 1852 H) and 2013 (cv. SY 4114) field seasons.

		Thousand kernel weight (g)							
		Calgary		Red Deer		Regina		Saskatoon	
Trt[a]	Conc.	2012 (1852H)	2013 (SY4114)	2012 (1852H)	2013 (SY4114)	2012 (1852H)	2013 (SY4114)	2012 (1852H)	2013 (SY4114)
4-Cl-IAA	1×10^{-4} M	3.41	3.83	4.22	4.89	3.51	nd[b]	3.55	3.56
	1×10^{-5} M	3.53	3.99	4.19	4.95	3.56	nd	3.56	3.76 *[z]
	1×10^{-6} M	3.49	3.78	4.37	4.95	3.58	nd	3.55	3.62
	1×10^{-7} M	3.46	3.86	4.15	4.89	3.61	nd	3.59	3.58
	0 (0.25% Adigor)	3.53	3.82	4.28	4.88	3.57	nd	3.59	3.56
	0 (no treatment)	3.33	3.84	4.47	4.91	3.57	nd	3.62	3.67
4-Me-IAA	1×10^{-4} M	3.40	3.74	4.39	4.89	3.65	nd	3.44	3.71 *
	1×10^{-5} M	3.35 *	3.82	4.31	4.92	3.58	nd	3.58	3.64
	1×10^{-6} M	3.54	3.84	4.36	4.89	3.53	nd	3.58	3.65
	1×10^{-7} M	3.43	3.74	4.35	4.92	3.54	nd	3.58	3.58
	0 (0.25% Adigor)	3.53	3.84	4.28	4.88	3.57	nd	3.59	3.56
	0 (no treatment)	3.33	3.82	4.47	4.91	3.57	nd	3.62	3.67

[a] Hormone: aqueous solutions of 4-Me-IAA and 4-Cl-IAA ($1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$ and $1x10^{-4}$ M) in 0.25% Adigor; Control solutions aqueous 0.25% Adigor and control no solution; one application sprayed on plants to cover when the majority of the plants were at the BBCH 50-52 developmental stages (when flower buds are enclosed by leaves to flower buds free from the level of younger leaves).

[b] nd = not determined.

[z] Denotes means compared within hormone treatment that are different from the control (0.25% Adigor) at $P \leq 0.05$ using single degree of freedom contrast analysis.

Table 3.6 Effect of 4-Cl-IAA and 4-Me-IAA on oil content (%) of field-grown hybrid canola during the 2012 (cv. Canterra 1852 H) and 2013 (cv. SY 4114) field seasons.

		Seed oil content (%)							
		Calgary		Red Deer		Regina		Saskatoon	
Trt[a]	Conc.	2012 (1852H)	2013 (SY4114)	2012 (1852H)	2013 (SY4114)	2012 (1852H)	2013 (SY4114)	2012 (1852H)	2013 (SY4114)
4-Cl-IAA	1×10^{-4} M	45.15	52.36	47.92*[z]	47.93	45.83	nd[b]	52.50	53.08
	1×10^{-5} M	45.80	52.73	47.90 *	48.66	47.41	nd	51.62	53.06
	1×10^{-6} M	45.62	52.71	47.51	48.89	46.02	nd	51.44	52.96
	1×10^{-7} M	46.23	52.76	47.69	48.82	46.51	nd	51.17	53.42
	0 (0.25% Adigor)	45.85	51.93	46.61	48.86	46.90	nd	51.26	53.11
	0 (no treatment)	45.94	52.93	47.66	47.97	46.26	nd	51.03	53.12
4-Me-IAA	1×10^{-4} M	45.91	52.72	48.55 *	48.68	45.96	nd	51.96	53.93
	1×10^{-5} M	45.49	52.57	48.11 *	48.11	46.19	nd	49.28	52.11
	1×10^{-6} M	46.01	53.31 *	47.82 *	48.68	45.93	nd	51.86	53.86
	1×10^{-7} M	45.51	51.89	48.16 *	48.47	46.07	nd	50.76	53.85
	0 (0.25% Adigor)	45.85	51.93	46.61	48.86	46.90	nd	51.26	53.11
	0 (no treatment)	45.94	52.93	47.66	47.97	46.26	nd	51.03	53.12

[a] Hormone: aqueous solutions of 4-Me-IAA and 4-Cl-IAA ($1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$ and $1x10^{-4}$ M) in 0.25% Adigor; Control solutions aqueous 0.25% Adigor and control no solution; one application sprayed on plants to cover when the majority of the plants were at the BBCH 50-52 developmental stages (when flower buds are enclosed by leaves to flower buds free from the level of younger leaves).

[b] nd = not determined.

[z] Denotes means compared within hormone treatment that are different from the control (0.25% Adigor) at $P \leq 0.05$ using single degree of freedom contrast analysis.

Table 3.7 Effect of 4-Cl-IAA and 4-Me-IAA on plant height of field-grown hybrid canola during the 2012 (cv. Canterra 1852 H) and 2013 (cv. SY 4114) field seasons.

		Plant height (cm)							
		Calgary		Red Deer		Regina		Saskatoon	
Trt[a]	Conc.	2012 (1852H)	2013 (SY4114)	2012 (1852H)	2013 (SY4114)	2012 (1852H)	2013 (SY4114)	2012 (1852H)	2013 (SY4114)
4-Cl-IAA	1×10^{-4} M	116	102	130	114	94	nd[b]	101	100
	1×10^{-5} M	119	101	129	109	98	nd	106	99
	1×10^{-6} M	115	100	128	113	98	nd	101	101
	1×10^{-7} M	113	100	128	110	95	nd	99	100
	0 (0.25% Adigor)	118	100	128	112	94	nd	106	101
	0 (no treatment)	117	102	130	116	94	nd	105	100
4-Me-IAA	1×10^{-4} M	121	99	130	111	96	nd	103	100
	1×10^{-5} M	116	98	129	109	96	nd	102	100
	1×10^{-6} M	117	102	127	117 *[z]	98	nd	104	100
	1×10^{-7} M	117	102	132	106 *	96	nd	101	101
	0 (0.25% Adigor)	118	100	128	112	94	nd	106	100
	0 (no treatment)	117	102	130	116	94	nd	105	100

[a] Hormone: aqueous solutions of 4-Me-IAA and 4-Cl-IAA ($1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$ and $1x10^{-4}$ M) in 0.25% Adigor; Control solutions aqueous 0.25% Adigor and control no solution; one application sprayed on plants to cover when the majority of the plants were at the BBCH 50-52 developmental stages (when flower buds are enclosed by leaves to flower buds free from the level of younger leaves).

[b] nd = not determined.

[z] Denotes means compared within hormone treatment that are different from the control (0.25% Adigor) at $P \leq 0.1$ using single degree of freedom contrast analysis.

Table 3.8 Effect of 4-Cl-IAA and 4-Me-IAA on number of pods of field-grown hybrid canola during the 2012 (cv. Canterra 1852 H) and 2013 (cv. SY 4114) field seasons.

Trt[a]	Conc.	Number of pods per plant							
		Calgary		Red Deer		Regina		Saskatoon	
		2012 (1852H)	2013 (SY4114)	2012 (1852H)	2013 (SY4114)	2012 (1852H)	2013 (SY4114)	2012 (1852H)	2013 (SY4114)
4-Cl-IAA	1×10^{-4} M	112	131	111	80	344	nd[b]	197	123
	1×10^{-5} M	104	129	106	76	288	nd	198	108
	1×10^{-6} M	102	119	104	74	320	nd	222	118
	1×10^{-7} M	109	117	126	68	267	nd	208	120
	0 (0.25% Adigor)	131	111	109	77	285	nd	219	114
	0 (no treatment)	95	125	100	75	270	nd	204	116
4-Me-IAA	1×10^{-4} M	112	131	117	71	263	nd	225	128
	1×10^{-5} M	117	119	101	71	292	nd	183	108
	1×10^{-6} M	96	129	99	78	325	nd	219	113
	1×10^{-7} M	110	138	126	65	300	nd	207	117
	0 (0.25% Adigor)	131	111	109	77	285	nd	219	116
	0 (no treatment)	95	125	100	75	270	nd	204	114

[a] Hormone: aqueous solutions of 4-Me-IAA and 4-Cl-IAA ($1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$ and $1x10^{-4}$ M) in 0.25% Adigor; Control solutions aqueous 0.25% Adigor and control no solution; one application sprayed on plants to cover when the majority of the plants were at the BBCH 50-52 developmental stages (when flower buds are enclosed by leaves to flower buds free from the level of younger leaves).

[b] nd = not determined.

Table 3.9 Effect of 4-Cl-IAA and 4-Me-IAA on number of racemes of field-grown hybrid canola during the 2012 (cv. Canterra 1852 H) and 2013 (cv. SY 4114) field seasons.

		Number of racemes per plant							
		Calgary		Red Deer		Regina		Saskatoon	
Trt[a]	Conc.	2012 (1852H)	2013 (SY4114)	2012 (1852H)	2013 (SY4114)	2012 (1852H)	2013 (SY4114)	2012 (1852H)	2013 (SY4114)
4-Cl-IAA	1×10^{-4} M	4.8	5.2	5.0	4.2	7.0	nd[b]	6.8	4.8
	1×10^{-5} M	5.0	4.8	5.0	4.2	6.8	nd	7.0	4.7
	1×10^{-6} M	5.0	5.0	5.0	4.2	6.5	nd	6.8	4.7
	1×10^{-7} M	4.5	4.7	4.8	4.3	5.8	nd	6.8	4.5
	0 (0.25% Adigor)	5.3	4.8	4.8	4.3	6.3	nd	6.8	4.8
	0 (no treatment)	4.5	5.2	4.8	4.5	6.3	nd	7.3	4.7
4-Me-IAA	1×10^{-4} M	4.8	5.2	4.8	4.3	6.0	nd	7.5	4.5
	1×10^{-5} M	4.5	5.2	4.5	4.3	6.3	nd	6.8	5.0
	1×10^{-6} M	4.8	5.7 *[z]	4.8	4.5	6.5	nd	7.5	4.7
	1×10^{-7} M	5.0	5.0	5.3	4.0	6.0	nd	7.3	4.5
	0 (0.25% Adigor)	5.3	4.8	4.8	4.3	6.3	nd	6.8	4.8
	0 (no treatment)	4.5	5.2	4.8	4.5	6.3	nd	7.3	4.7

[a] Hormone: aqueous solutions of 4-Me-IAA and 4-Cl-IAA ($1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$ and $1x10^{-4}$ M) in 0.25% Adigor; Control solutions aqueous 0.25% Adigor and control no solution; one application sprayed on plants to cover when the majority of the plants were at the BBCH 50-52 developmental stages (when flower buds are enclosed by leaves to flower buds free from the level of younger leaves).

[b] nd = not determined.

[z] Denotes means compared within hormone treatment that are different from the control (0.25% Adigor) at $P \leq 0.05$ using single degree of freedom contrast analysis. Mean difference at $P \leq 0.05$ is considered significantly different in the context of this experiment.

Chapter 4 – The effect of auxins on seed yield parameters in field pea cv. Carneval grown under controlled environment and western Canadian field conditions

4.1 Introduction

Field pea (*Pisum sativum* L.) is a cool season pulse crop belonging to the Fabaceae family. It is an annual crop grown in temperate zones and at high elevations in the tropics throughout the world including Russia, China, Canada, USA, Australia and Europe (Schatz and Endres 2009). Saskatchewan, Alberta, Manitoba and British Columbia are recognized as the major Canadian field pea growing areas, where Saskatchewan accounts for nearly two thirds, and Alberta nearly one third of the dry pea area (Agriculture and Agri-Food Canada 2014). Environmental stresses such as high or low temperatures, drought, and extreme levels of light reduce the quality and quantity of yield in many crops including field pea. On the western Canadian prairies, daytime temperature for best growth and development of pea ranges from 13 to 23^{o}C (Cutforth and others 2007). However, summer daytime temperatures exceeding 30^{o}C usually occur each growing season on the western Canadian prairies (cf. Bueckert and Clarke 2013). In pea and other legumes, temperatures above the optimal range (> 25^{o}C) at flowering can reduce seed yield (McDonald and Paulsen 1997; Jeuffroy and others 1990) through factors such as accelerating floral bud abortion, reducing fruit and seed set, reducing pollen or ovule viability, and anther dehiscence and embryo abortion (Guilioni and others 2003; Ahmed and others 1992; Devasirvatham and others 2012).

The use of plant growth regulators in order to ensure the efficient crop production under stress or non-stress conditions is becoming a popular research area in agriculture (Atkins and pigeaire 1993; Kagale and others 2007; Giannakoula and others 2012; Karcz and Burdach 2002). Plant growth regulators (plant hormones) are signal molecules that occur naturally within the plant or are synthetically produced and regulate many processes in vegetative and reproductive growth and development including cell division, cell enlargement, tropism, flowering, fruiting, seed formation and assimilate partitioning (Thimann 1938; Masuda 1990; Went 1942; Estelle 1996). Among the classes of plant growth regulators, auxins show some promising characteristics in regulating growth and yielding ability of specific crops under stress or non-

stress conditions. El-Saeid and others 2010 reported indole-3-acetic acid (IAA) application (at 25, 50 and 100 mg/L) to cowpea (*Vigna sinensis* L. cv. Cream 7) 10 days before and after flowering increased the number and weight of pods, and number and weight of seeds, at the lowest and moderate IAA concentrations. In faba bean (*Vicia faba* L. cv. Troy), Clifford and others (1992) reported that pod numbers were increased when the reproductive structures were sprayed with IAA. Sakata and others (2010) reported that YUCCA auxin biosynthesis gene expression was reduced under high temperature conditions, and this was correlated with data indicating reduction in anther auxin levels and pollen abortion in *Arabidopsis*. A single application of auxins (IAA, 1-naphthaleneacetic acid [NAA] at 1×10^{-7} and 1×10^{-6} M before increase the temperature) was able to reverse high temperature-induced pollen abortion in *Arabidopsis* (Sakata and others 2010). Shi and others (2014) worked on effect of IAA on ameliorating the drought stress in *Arabidopsis* and observed auxin might positively regulate drought stress resistance in *Arabidopsis* by maintaining the root architecture, and regulating stress responsive genes and reactive oxygen species metabolism. The auxin 4-Cl-IAA (naturally occurring in pea) has been implicated as a seed signaling molecule that is exported to the pericarp (ovary) where it a) stimulates pericarp gibberellin (GA) biosynthesis, and b) directly affects pericarp growth via auxin-related mechanisms stimulating pericarp growth (Ozga and Reinecke 2003; Ozga and others 2009). Among most auxin types used in excised tissue biological assays, 4-chloroindole-3-acetic acid (4-Cl-IAA) proved to be more effective in stimulating growth than IAA (Katekar and Geissler 1983: pea stem section and wheat coleoptile section assays). 4-Cl-IAA also is the most effective auxin in stimulating pea pericarp growth (Reinecke and others 1995), with 4-methylindole-3-acetic acid (4-Me-IAA) being the second most effective auxin in this whole tissue assay (Reinecke and others 1999).

In this study, we tested if a one-time application of the auxins, 4-Cl-IAA or 4-Me-IAA, to the foliage of pea plants immediately prior to or at the beginning of flowering affects seed yield or seed yield parameters when pea plants are exposed to non-stress or heat-stress conditions following auxin treatment in environmentally controlled conditions, or under field conditions.

4.2 Materials and methods

4.2.1 Experiment under controlled environmental conditions

4.2.1.1 Growth chamber experiment - 1 - Field pea cv. Carneval

This controlled environment experiment was designed and carried out by the Ozga/Reinecke lab. I completed the statistical analysis of these data that are presented in this chapter, in order to compare these results with those obtained in the field. 'Carneval' (*Pisum sativum* L.) was chosen as a model cultivar for semi-dwarf (*le*), semi-leafless (*af*) field pea, as these traits are extensively used in field pea production. 'Carneval' has white flowers and yellow cotyledons at maturity, begins to flower at about the 15 to 17th node under long day conditions. Seeds of 'Carneval' were planted at an approximate depth of 2.5 cm in 3-L plastic pots (4 seeds per pot and thinned to 2 plants per pot after approximately 2 weeks) in 1:4 Sunshine #4 potting mix (Sun Gro Horticulture, Vancouver, Canada) and sand. Pots were arranged in a completely randomized design and grown at the University of Alberta in a growth chamber set at 19^{o}C/17^{o}C (day/night) with a 16/8-h photoperiod under cool-white fluorescent lights (F54/I5/835/HO high fluorescent bulbs (Phillips, Holland) at 350 µmol m^{2} s^{-2}, measured with a LI-188 photometer, Li-Cor Biosciences, Lincoln, Nebraska). For the heat stress treatment, plants were placed in a separate growth chamber with a 16/8 h photoperiod under cool-white fluorescent lights (F54/I5/835/HO high fluorescent bulbs (Phillips, Holland) for 4 days where the temperature was cycled over a 24 h period as follows: 33-35^{o}C air temperature for 6 h per day (between 11:00 and 17:00 h) for 4 days during the light cycle; the remainder of the light cycle was maintained at a 22^{o}C air temperature; the dark cycle was maintained at 19^{o}C. After the 4 day heat treatment, the plants were returned to the same growth chamber they were originally grown in, where they were taken to maturity.

Aqueous 4-methylindole-3-acetic acid (4-Me-IAA) or 4-chloroindole-3-acetic acid (4-Cl-IAA) solutions at 1×10^{-7}, 1×10^{-6}, 1×10^{-5}, or 1×10^{-4} M in 0.1% (v/v) Tween 80 or aqueous 0.1% (v/v) Tween 80 (control) were applied as a spray to cover the entire plant when the first flowering node of the main stem was near or at anthesis. In addition to the application at the timing cited above, one application of 4-Me-IAA at 1×10^{-6} and 1×10^{-5} M was made when the

floral buds were tightly clustered inside the stipule leaves at the stem apex (floral buds not visible outside of stipule leaves; designated the 'Early' treatment) and compared with an aqueous 0.1% (v/v) Tween 80 (control). For the heat stress treatment, the hormone or control application was completed 16 h prior to the initiation of the first heat-stress cycle.

4.2.2. Field experiments

4.2.2.1 Field pea cv. Carneval dose response experiments

4-Cl-IAA and 4-Me-IAA dose-response field experiments were conducted in four locations in the prairies eco-zone in western Canada. There were two locations in Alberta (Calgary [Latitude: 50.7167/ Longitude: -113.3167 and Red Deer [Latitude: 52.24/ Longitude: -113.86]) and two in Saskatchewan (Regina [Latitude: 50.44 / Longitude: -104.37] and Saskatoon [Latitude: 52.42/ Longitude: -106.38]) in 2012 and 2013. Calgary and Red Deer sites were dominated with Black Chernozemic soils (Soil group map of Alberta 2014), where as Saskatoon sites had dark brown regosolic soil, and Regina sites had dark brown vertisolic soil (Saskatchewan Ministry of Agriculture 2009; Anderson 2010).

Apron Maxx RTA (at 325 mL per 100 kg of seed = 7.5 g Metalaxyl-M / 100kg seed + 2.5 g Fludioxonil / 100kg seed), plus Cruiser 5FS (at 20 gai per 100 kg seed) treated seeds of field pea cv. Carneval were sown into the soil at a 2.5 to 3 cm depth, using a calibrated cone seeder at a rate of 0.4 plants per square meter in 2012 and 0.9 plants per square meter in 2013. Inoculants were used to enhance rhizobium nodulation. In Calgary and Red Deer the inoculant was Nodulator (peat-based; Becker Underwood, Saskatoon, Canada) applied at a rate of 1.2 kg per 600 kg of seed. In Saskatoon and Regina the inoculant was TagTeam (peat-based from Novozymes BioAg, Saskatoon, Canada) applied at a rate of 1.6 g per kg seed. Plots were arranged in a Randomized Complete Block Design (RCBD) consisting of 10 treatments per replicate (4-Cl-IAA and 4-Me-IAA at 1×10^{-7} M, 1×10^{-6} M, 1×10^{-5} M and 1×10^{-4} M in aqueous 0.25% Adigor, 0.25% aqueous Adigor only control, and a no treatment control), with 4 (in 2012) or 6 (in 2013) replicates per treatment. Treatments were randomly applied to plots within each replicate. Individual treatment plot size was 6 m×1.5 m in 2012 and 7 m×1.5 m in 2013. Inter-plot spacing was 0.3 m, with a 2 m buffer between replicates. Each treatment plot consisted of 7

rows with an inter-row spacing of 18 cm from the middle of one row to the middle of the next row. Guard plots bordered the outermost treatment plots of the replicate row (Appendix A; Figure A.1. Plot illustration: 4-Me-IAA and 4-Cl-IAA dose response field experiments for wheat, pea and canola).

All the auxins and adjuvant solutions used were made by Dr. Dennis M. Reinecke, University of Alberta and stored in freezers in dark bottles and delivered to field locations under frozen and dark conditions. Frozen hormone and adjuvant control solutions were completely thawed in the dark, at ambient temperature, and mixed thoroughly just prior to field application. In all sites the solutions were applied at BBCH 50 (floral buds are observable within stipule leaves, but not beyond the stipule leaves). Hormones were sprayed to cover the plot plant canopy using a hand boom having 3 nozzles (110-02 low drift air induction nozzle with 275 kpa pressure) with a 90 degree angle to the crop at a rate of 200 L/ha spray volume. Application of herbicides, fungicides or any other chemical treatment for pest and or disease control was not done 7 days before or after the treatment application. Phenoxy or auxinic based products, Pyrasulfotole or other HPPD-inhibitors or bleachers were not used for any crop management purposes throughout the growing season. All of the other crop maintenance practices were completed following normal commercial field pea cultivation in western Canada (Alberta Agriculture and Rural Development 2012).

In 2012, plot plant density was determined by counting the number of plants in each plot after final plot trimming was completed. In 2013, plot plant density was estimated by counting the number of plants per meter (1 m per row in a diagonal pattern across the plot using the middle 5 rows) after the final plot trimming was completed. Five plants per plot were randomly selected from the inner rows of each plot for determination of plant dry weight, number of pods per plant, number of seeds per plant and plant height (excluding roots), when plants were at the BBCH 79 growth stage (when pods have reached typical mature size). For dry weights, plants (excluding roots) were dried at 45^{o}C for two days prior to weighing. Individual plots were harvested using a Wintersteiger Classic plot combine harvester with a 150 cm wide header. Plot seed yield as measured from the combine harvester was normalized for 16% moisture content and dockage weight. Weight of dockage per plot was estimated by calculating the percent dockage in 500 g seed subsamples from each plot (using a Clipper seed cleaner model 400:

Seedburo, IL, USA). To calculate Thousand Kernel Weight (TKW) per plot, 2 lots of 250 seeds were manually counted and weighed, then averaged and multiplied by the factor of 4. Hormone treatments were compared with adjuvant only (2.5% Adigor) control and no treated control.

Weather data were collected using an onsite weather station at the Calgary, Regina and Saskatoon field sites, while Red Deer weather data were collected from a Red Deer, Alberta, weather station WMO ID 71878. Soil moisture (vol%) was measured using a Watermark Soil Moisture Sensor (Spectrum Technologies, IL, USA) placed at a depth of 10 cm in Saskatoon and Regina. Soil moisture storage capacity (SMSC) was measured at the Calgary site using a Watchdog data logger with a Watermark Soil Moisture Sensor (Spectrum Technologies, IL, USA) placed at a depth of 20 cm.

4.2.3 Statistical analyses

4.2.3.1 Growth chamber experiment

Analyses were performed using the PROC MIXED procedure of SAS 9.3 software (SAS Institute Inc. Cary, NC, USA, 2010). To assess the effect of auxin concentration and temperature at flowering on reproductive parameters of pea, statistical significance of the data was determined by using a 5 (auxin concentrations)×2(temperatures) factorial analysis of variance for each auxin (4-Cl-IAA or 4-Me-IAA, independently). Additionally, to assess the effect of application timing on pea plant response to 4-Me-IAA, statistical significance of the data was determined by using a 2 (application times)×3(4-Me-IAA concentrations) factorial analysis of variance. Mean separation (main effects, treatments, and interactions) were determined using the Least Significant Difference (LSD) test.

4.2.3.2 Field experiments

For the dose response field trials, an analysis of variance (ANOVA) or analysis of covariance (ANCOVA) (for seed yield) was performed on field data within location and year using the PROC MIXED procedure of SAS 9.3 software (SAS Institute Inc. Cary, NC, USA, 2010). The one-way ANOVA analyses were carried out for yield component parameters (treatment level was taken as the main effect and analyses were carried out for 4-Me-IAA

treatments and controls as well as for 4-Cl-IAA treatments and controls, separately). Similarly, the one-way ANCOVA was carried out to analyze plot seed yield data with the treatment as the main effect and the number of plants per meter as the covariate. Preplanned single degree of freedom contrast analysis was performed between the adjuvant only control, 4-Me-IAA, and 4-Cl-IAA treatments, and the no treatment control, 4-Me-IAA, and 4-Cl-IAA treatments at each location and year. These contrasts were preplanned to examine specific treatment effects that would be of agronomic or biological importance. However, only yield parameters that showed significant differences compared with the no treatment control with respect to the hormone treatments were discussed. Statistical significance was declared at $P \leq 0.05$.

4.3 Results

4.3.1 Controlled environment experiments

4.3.1.1 Growth chamber experiment - 1 - Field pea cv. Carneval

This experiment was conducted to examine the effects of the auxins 4-Cl-IAA and 4-Me-IAA, heat stress, and application timing on seed yield and yield component parameters of the semi-dwarf field pea cv. Carneval. The highest concentration of 4-Cl-IAA (1×10^{-4} M) increased seed weight per plant by 38% when plants were grown under non-heat stress conditions and this seed weight increase was reflected in greater number of seeds per plant (47%) compared with the adjuvant only control (Table 4.1). Heat stress imposed during the initial flowering stage decreased the number of seeds per plant (compare temperature main effect means, Table 4.1). 4-Cl-IAA application at 1×10^{-4}, 1×10^{-5} and 1×10^{-6} M increased seed weight per plant by 38%, 56% and 40%, respectively, when plants were grown under heat stress conditions during initial flowering compared with the adjuvant only control (Table 4.1). The 4-Cl-IAA-induced seed yield increase in heat stress exposed plants was reflected in a greater number of pods per plant (47% at 1×10^{-4} M, 48% at 1×10^{-5} M and 41% at 1×10^{-6} M), as well as in a greater number of seeds per plant (48% at 1×10^{-4} M and 58% at 1×10^{-5} M) compared with the adjuvant only control (Table 4.1). 4-Cl-IAA-treated plants had more seeds develop to maturity on the main stem than the lateral stems compared with the adjuvant only control when plants were exposed to heat stress conditions (see lateral: main stem seed ratio; Table 4.1).

4-Me-IAA treatments increased the seed weight per plant (62% at 1×10^{-4} M, 61% at 1×10^{-5} M, 72% at 1×10^{-6} M and 79% at1×10^{-7} M) under non-heat stress conditions, and this seed weight increase was reflected in greater number of pods per plant (47% at 1×10^{-4} M, 45% at 1×10^{-5} M, 72% at 1×10^{-6} M and 80% at1×10^{-7} M) and greater number of seeds per plant (58% at 1×10^{-4} M, 70% at 1×10^{-5} M, 76% at 1×10^{-6} M and 79% at 1×10^{-7} M) compared with the adjuvant only control (Table 4.2). The increase in seed weight per plant, number of pods per plant and number of seeds per plant was higher at lower 4-Me-IAA concentrations (1×10^{-6} and 1×10^{-7} M) compared with the higher concentrations (1×10^{-4} and 1×10^{-5} M) (Table 4.2). In this experiment, the heat stress treatment did not affect seed weight or number of seeds per plant (compare temperature main effect means), and 4-Me-IAA application also had no effects on these parameters when exposed to the heat stress treatment (Table 4.2).

Application of 4-Me-IAA when the floral buds were tightly clustered inside the stipule leaves at the stem apex (early application treatment) increased the seed weight per plant (14%) and seed size (4.6%) compared with application at the later flowering stage (when the first flowering node of the main stem was near or at anthesis; compare application main effect means, Table 4.3). Application of 4-Me-IAA to the plants at the earlier timing (at both 1×10^{-5} and 1×10^{-6} M) increased the number of seeds produced on the lateral stems than that on the main stem, but this did not occur when 4-Me-IAA was applied at the later developmental stage (Table 4.3). The most effective auxin treatment for increasing seed yield in this experiment was early application of 4-Me-IAA at 1×10^{-6} M (Table 4.3).

4.3.2. Field dose-response experiments

4.2.3.2 Field pea cv. Carneval dose response experiments

A number of environmental and cultural management issues led to weak data for the pea field experiments for both the 2012 and 2013 field seasons. Substantial hail damage occurred at the Red Deer field site in 2012 making the plot yield data unreliable (however, the plant component data for this site was useable), and hail completely destroyed the pea plots in Regina in 2013. In 2012, the Saskatoon field site was lost due to a pea root rot infestation. Seeding was delayed (until early June) at Red Deer field site in 2013 due to seeder mechanical problems. Also

poor growth of pea plants at the Calgary field site in 2013 substantially reduced the yield compared with that in 2012 at the Calgary site. Lower plant density (4 plants per square feet) at all of the sites in the 2012 field season caused higher weed competition. Among the four sites over the 2012 and 2013 field seasons, the Calgary site in 2012 and 2013 and Saskatoon site in 2013 exhibited moderate heat stress (12 or more days at or above 25^{o}C daily maximum temperature) 14 days before and after hormone treatment (at BBCH 50 growth stage of pea), with adequate soil moisture conditions (Appendix C, Table C.1). Application of 4-Me-IAA at 1×10^{-7} M increased plot seed yield by 9.9% compared with the no treatment control ($P\leq0.05$ for single degree of freedom contrasts; Table 4.4) at the Red Deer site in 2013. The adjuvant only control treatment also showed higher seed yield compared with the no treatment control at the Red Deer and Calgary sites in 2013. Application of 4-Me-IAA at 1×10^{-4}, 1×10^{-6} and 1×10^{-7} M, and 4-Cl-IAA at 1×10^{-6} M increased seed size (TKW) of field pea (cv. Carneval) at the Saskatoon site in 2013 compared with the no treatment control (Table 4.5). However, since 4-Me-IAA at 1×10^{-4} and 1×10^{-6} M, and 4-Cl-IAA at 1×10^{-6} had lower number of seeds per plant compared with the no treatment control (at the Saskatoon site in 2013), the increase in seed size may be a reflection of a reduced seed number per plant (Table 4.7). The auxins had minimal to no effects on number of pods per plant (Table 4.6), plant dry weight (Table 4.8) and plant height (Table 4.9) in these field experiments.

4.4 Discussion

The effect of one foliar application of 4-Cl-IAA or 4-Me-IAA on field pea cv. Carneval seed yield parameters was assessed under controlled and field environments. The controlled environment experiments suggest that a one-time foliar application of 4-Me-IAA or 4-Cl-IAA can increase the fruit set and thereby the final seed number and seed weight of pea plants. Under non-stress controlled temperature conditions, both 4-Cl-IAA and 4-Me-IAA application at higher concentrations increased pea seed yield (Tables 4.1 and 4.2).

Guilioni and others (2003) reported that high temperature reduced the seed number (about 19%) in various field pea cultivars grown under field or controlled environment conditions in a temperature intensity dependent manner [used different combinations of high

temperature (from 28 to 36°C) and high temperature duration (4–6 h per day for 4 to 6 days)]. In both controlled environment studies with pea cv. Carneval, the average number of seeds per plant was lower for plants exposed to heat stress conditions during initial flowering (Tables 4.1 and 4.2, compare temperature main effect means); however, a significant reduction in seed number per plant (14%) was only observed in the study using 4-Cl-IAA (Table 4.1).

4-Cl-IAA application was more effective than 4-Me-IAA in increasing seed yield and yield component parameters over a range of temperatures during the early flowering stage (4-Cl-IAA increased seed yield parameters in plants exposed to normal and heat stress conditions at the initial flowering stage, where as 4-Me-IAA only increased seed yield under non-heat stress conditions). Under heat stress conditions, 4-Cl-IAA application increased the number of seeds produced on the main stem compared with that on the lateral stems (Table 4.1), suggesting that 4-Cl-IAA increased the sink strength of the pods present at the time of the heat stress.

4-Cl-IAA is more active than 4-Me-IAA in stimulating deseeded pea pericarp growth (Reinecke and others 1999). Since auxins are labile at high temperatures, it is possible that the greater auxin biological activity of 4-Cl-IAA was necessary to induce fruit set at the higher temperatures.

With respect to application timing, the most effective auxin treatment for increasing seed yield was an early application (when the floral buds were tightly clustered inside the stipule leaves at the stem apex) of 4-Me-IAA at 1×10^{-6} M (Table 4.3). The early auxin treatment led to an increase in the number of seeds produced on the lateral stems greater than that on the main stem, suggesting that the auxin treatment at this earlier stage can affect the apical meristem for increased lateral stem and lateral stem pod production (Table 4.3).

The effect of 4-Cl-IAA and 4-Me-IAA on seed yield of cv. Carneval plants under field conditions was inconclusive due to many uncontrolled factors influencing seed yield, independent of the auxin treatments. However, at one site (Red Deer in 2013) 4-Me-IAA at 1×10^{-7} M increased plot seed yield by 9.9%, suggesting that 4-Me-IAA may be effective in increasing seed yeild in pea under field conditions.

In summary, our data suggest that both, 4-Cl-IAA and 4-Me-IAA can be effective in increasing seed yield in field pea cv. Carneval. Further studies are required to determine if these auxins can increase pea seed yield under field conditions.

Table 4.1 Effect of 4-Cl-IAA concentration and heat stress at flowering on seed weight, number of seeds, and number of pods per plant, seed size, and the ratio of seeds produced on the lateral stems to that on the main stem of growth chamber-grown field pea cultivar Carneval.

Auxin type	Hormone conc.[z]	Heat trt[y]	Seed weight per plant (g)	Seed size (mg)	Number of pods per plant	Number of seeds per plant	Lateral: main stem seed ratio
4-Cl-IAA	1×10^{-4}	-	17.522 a[w]	268 a	16.50 a	66.7 a	0.448 a
4-Cl-IAA	1×10^{-5}	-	16.460 b	275 a	16.60 a	60.1 ab	0.573 a
4-Cl-IAA	1×10^{-6}	-	12.588 b	271 a	14.00 a	46.4 b	0.472 a
4-Cl-IAA	1×10^{-7}	-	15.805 b	262 a	16.00 a	60.5 a	0.602 a
Tween 80	0	-	12.679 b	280 a	13.40 a	45.5 b	0.579 a
Temperature Mean		-	15.011 r	271 r	15.30 r	55.8 r	0.535 r
4-Cl-IAA	1×10^{-4}	+	14.579 a	289 ab	15.40 a	51.4 a	0.369 a
4-Cl-IAA	1×10^{-5}	+	16.407 a	298 ab	15.50 a	55.1 a	0.335 a
4-Cl-IAA	1×10^{-6}	+	14.748 a	310 a	14.78 a	54.8 ab	0.502 a
4-Cl-IAA	1×10^{-7}	+	12.315 ab	280 b	11.60 b	43.4 ab	0.537 a
Tween 80	0	+	10.544 b	308 ab	10.50 b	34.8 b	0.827 b
Temperature Mean		+	13.719 r	297 s	13.56 r	47.9 s	0.514 r
4-Cl-IAA mean	1×10^{-4}		16.051 m	279 mn	15.95 m	59.1 m	0.409 a
4-Cl-IAA mean	1×10^{-5}		16.434 m	287 mn	16.05 m	57.6 m	0.454 a
4-Cl-IAA mean	1×10^{-6}		13.668 mn	291 m	14.39 mn	50.6 mn	0.487 a
4-Cl-IAA mean	1×10^{-7}		14.060 mn	271 n	13.80 mn	52.0 m	0.570 ab
Tween 80 mean	0		11.612 n	294 m	11.95 n	40.2 n	0.703 b
Hormone concentration ×Temperature			NS[v]	NS	NS	NS	NS

[z] Hormone concentration: aqueous solutions of 4-Cl-IAA ($1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$ M and $1x10^{-4}$ M) in 0.1% Tween80; Control adjuvant solution: 0.1% Tween 80 only applied as a spray to cover the entire plant when the first flowering node of the main stem was near or at anthesis.

[y] Heat treatment: The heat stress treatment was imposed by moving plants to receive the heat stress to a growth chamber (heat stress chamber) for 4 days, just after the hormone application. Growth chamber was maintained with a 16/8 h photoperiod under cool-white fluorescent lights (F54/I5/835/HO high fluorescent bulbs, Phillips, Holland) where the temperature was cycled over a 24 h period as follows: 33-35^{o}C air temperature for 6 h per day (between 11:00 and 17:00 h) for 4 days during the light cycle; the remainder of the light cycle was maintained at a 22^{o}C air temperature; the dark cycle was maintained at 19^{o}C. After the 4 day heat treatment, the plants were returned to another growth chamber and maintained at non-heat stress conditions to develop to maturity.

[w] Means followed by a different letter are significantly different within parameter and sets (a, b, c: among auxin concentrations within temperature treatment), (r, s: temperature main effect means) and (m and n: auxin concentration main effect means) by the LSD test, $P\leq0.05$. [v]NS = non significant.

Table 4.2 Effect of 4-Me-IAA concentration and heat stress at flowering on seed weight, number of seeds, and number of pods per plant, seed size, and the ratio of seeds produced on the lateral stems to that on the main stems of growth chamber-grown field pea cultivar Carneval.

Auxin type	Hormone conc.[z]	Heat trt[y]	Seed weight per plant (g)	Seed size (mg)	Number of pods per plant	Number of seeds per plant	Lateral: main stem seed ratio
4-Me-IAA	1×10^{-4}	-	13.996 a[w]	248 a	14.00 a	56.1 a	0.329 a
4-Me-IAA	1×10^{-5}	-	13.952 a	231 a	13.80 ab	60.3 a	0.455 a
4-Me-IAA	1×10^{-6}	-	14.883 a	236 a	16.30 a	62.6 a	0.455 a
4-Me-IAA	1×10^{-7}	-	15.521 a	245 a	17.10 ab	63.5 a	0.390 a
Tween 80	0	-	8.665 b	247 a	9.50 c	35. 5 b	0.430 a
Temperature mean		-	13.403 r	241 r	14.14	55.6 r	0.412 r
4-Me-IAA	1×10^{-4}	+	14.463 a	261 a	13.90 a	54.1 a	0.613 a
4-Me-IAA	1×10^{-5}	+	13.750 a	273 a	13.44 a	45.6 a	1.047 b
4-Me-IAA	1×10^{-6}	+	12.924 a	277 a	10.90 a	45.8 a	0.687 a
4-Me-IAA	1×10^{-7}	+	15.288 a	273 a	13.40 a	55.8 a	0.757 a
Tween 80	0	+	11.907 a	274 a	11.10 a	43.2 a	0.615 a
Temperature mean		+	13.666 r	272 s	12.55	48.9 r	0.744 s
4-Me-IAA mean	1×10^{-4}		14.230 m	255 m	13.95	55.1 m	0.471 a
4-Me-IAA mean	1×10^{-5}		13.851 m	252 m	13.62	53.0 m	0.751 b
4-Me-IAA mean	1×10^{-6}		13.903 m	257 m	13.60	54.2 m	0.571 ab
4-Me-IAA mean	1×10^{-7}		15.404 m	259 m	15.25	59.7 m	0.574 ab
Tween 80 mean	0		10.286 n	261 m	10.30	39.4 n	0.523 a
Hormone concentration ×Temperature			NS[v]	NS	SD	NS	NS

[z] Hormone concentration: aqueous solutions of 4-Me-IAA ($1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$ M and $1x10^{-4}$ M) in 0.1% Tween80; Control adjuvant solution: 0.1% Tween 80 only applied as a spray to cover the entire plant when the first flowering node of the main stem was near or at anthesis.

[y] Heat treatment: The heat stress treatment was imposed by moving plants to receive the heat stress to a growth chamber (heat stress chamber) for 4 days, just after the hormone application. Growth chamber was maintained with a 16/8 h photoperiod under cool-white fluorescent lights (F54/I5/835/HO high fluorescent bulbs, Phillips, Holland) where the temperature was cycled over a 24 h period as follows: 33-35^{o}C air temperature for 6 h per day (between 11:00 and 17:00 h) for 4 days during the light cycle; the remainder of the light cycle was maintained at a 22^{o}C air temperature; the dark cycle was maintained at 19^{o}C. After the 4 day heat treatment, the plants were returned to another growth chamber and maintained at non-heat stress conditions to develop to maturity.

[w] Means followed by a different letter are significantly different within parameter and sets (a, b, c: among auxin concentrations within temperature treatment), (r, s: temperature main effect means) and (m and n:auxin concentration main effect means)) by the LSD test, $P\leq0.05$.
[v] NS = non significant, SD = significant, at $P>0.05$.

Table 4.3 Effect of 4-Me-IAA concentration, auxin application time, and heat stress at flowering on seed weight, number of seeds, and number of pods per plant, seed size, and the ratio of seeds produced on the lateral stems to that on the main stem of growth chamber-grown field pea cultivar Carneval.

Auxin type	Hormone conc.[z]	Application time[y]	Seed weight per plant (g)	Seed size (mg)	Number of pods per plant	Number of seeds per plant	Lateral: main stem seed ratio
4-Me-IAA	1×10^{-5}	Early	16.213 a[w]	253 a	16.30 a	64.9 a	0.766 a
4-Me-IAA	1×10^{-6}	Early	18.201 a	249 a	18.50 a	74.4 a	0.679 a
4-Me-IAA	0	Early	8.665 b	247 a	9.50 b	35.8 b	0.43 b
Application mean (Early)			14.360 m	249 m	14.80 m	58.3 m	0.625 m
4-Me-IAA	1×10^{-5}	Late	13.952 a	231 a	13.80 a	60.3 a	0.455 a
4-Me-IAA	1×10^{-6}	Late	14.883 a	236 a	16.30 a	62.6 a	0.455 a
4-Me-IAA	0	Late	8.665 b	247 a	9.50 b	35.8 b	0.43 a
Application mean (Late)			12.5 n	238 n	13.20 m	52.8 m	0.447 n
4-Me-IAA mean	1×10^{-5}		15.083 r	242 r	15.00 r	62.5 r	0.611 r
4-Me-IAA mean	1×10^{-6}		16.542 r	242 r	17.40 s	68.5 r	0.567 rs
4-Me-IAA mean	0		8.665 s	247 r	9.50 t	35.8 s	0.430 s
4-Me-IAA	1×10^{-5}	Early	16.213 p	253 p	16.3 p	64.9 p	0.766 p
	1×10^{-5}	Late	13.952 p	231 q	13.8 p	60.3 p	0.455 q
4-Me-IAA	1×10^{-6}	Early	18.201 p	249 p	18.5 p	74.4 p	0.679 p
	1×10^{-6}	Late	14.883 q	236 p	16.3 p	62.6 p	0.455 p
Hormone concentration × application time			NS[v]	NS	NS	NS	NS

[z] Hormone concentration: aqueous solutions of 4-Me-IAA ($1x10^{-6}$ and $1x10^{-5}$M) in 0.1% Tween 80 and Control adjuvant only solution: 0.1% Tween 80 applied as a spray to cover the entire plant when the first flowering node of the main stem was near or at anthesis (Late time) and when the floral buds were tightly clustered inside the stipule leaves at the stem apex (floral buds not visible outside of stipule leaves).

[y] Application time: The early application time was when the floral buds were tightly clustered inside the stipule leaves at the stem apex (floral buds not visible outside of stipule leaves; BBCH 50). Late application time was when the first flowering node of the main stem was near or at anthesis (BBCH 60)

Means followed by a different letter are significantly different within parameter and sets (a, b: within hormone concentration and application time), (r, s and t: auxin concentration main effect means) and (m, n: application time main effect means) by the LSD test, $P\leq0.05$.

[v] NS = non significant.

Table 4.4 Effect of 4-Cl-IAA and 4-Me-IAA on seed yield when applied to field-grown pea (cv. Carneval) at Calgary, Red Deer, Regina and Saskatoon sites during the 2012 and 2013 field seasons.

		Yield (kg/ha)							
		Calgary		Red Deer		Regina		Saskatoon	
Hormone[a]	Concentration	2012	2013	2012	2013	2012	2013	2012	2013
4-Cl-IAA	$1x10^{-4}$ M	6909	2624	1492	3063	3699	nd[b]	nd	5407
	$1x10^{-5}$ M	6209	2667	2204*[c]	2924	3635	nd	nd	5306
	$1x10^{-6}$ M	6594	2560	1562	2984	3464	nd	nd	5489
	$1x10^{-7}$ M	6666	2360	1853	2970	3556	nd	nd	5422
	0 (2.5% Adigor)	6680	2729	1606	3370	3428	nd	nd	5259
	0 (no treatment)	6288	2476	1659	2957	3737	nd	nd	5351
4-Me-IAA	$1*10^{-4}$ M	7211	2595	1508	2896	3609	nd	nd	5541
	$1*10^{-5}$ M	6455	2562	1809	3060	3909	nd	nd	5467
	$1*10^{-6}$ M	6914	2307	1984	3148	3710	nd	nd	5419
	$1*10^{-7}$ M	6222	2574	1413	3223*	3605	nd	nd	5497
	0 (2.5% Adigor)	6656	2727*	1634	3385*	3398	nd	nd	5247
	0 (no treatment)	6304	2478	1690	2934	3741	nd	nd	5375

[a] Hormone: aqueous solutions of 4-Me-IAA and 4-Cl-IAA ($1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$ and $1x10^{-4}$ M) in 0.25% Adigor; Control solutions aqueous 0.25% Adigor and control no solution; one application sprayed on plants to cover when the majority of the plants were at the BBCH 50 (when floral buds are observable within stipule leaves, but not beyond the stipule leaves).

[b] nd = not determined.

[c] Denotes means compared within hormone that are different from the no treatment control at $P \leq 0.05$ using single degree of freedom contrast analysis.

Table 4.5 Effect of 4-Cl-IAA and 4-Me-IAA on seed size (thousand kernel weight) when applied to field-grown pea (cv. Carneval) at Calgary, Red Deer, Regina and Saskatoon sites during the 2012 and 2013 field seasons.

		Thousand kernel weight (g)							
		Calgary		Red Deer		Regina		Saskatoon	
Hormone[a]	Concentration	2012	2013	2012	2013	2012	2013	2012	2013
4-Cl-IAA	$1x10^{-4}$ M	215	161	187	201	213	nd[b]	nd	207
	$1x10^{-5}$ M	218	166	187	203	213	nd	nd	208
	$1x10^{-6}$ M	217	155*[c]	188	201	218	nd	nd	210*
	$1x10^{-7}$ M	219	162	189	199	213	nd	nd	206
	0 (2.5% Adigor)	219	166	185	200	212	nd	nd	203
	0 (no treatment)	220	165	187	200	214	nd	nd	202
4-Me-IAA	$1*10^{-4}$ M	219	164	192	200	213	nd	nd	210*
	$1*10^{-5}$ M	218	168	183	200	207*	nd	nd	208
	$1*10^{-6}$ M	220	166	185	200	215	nd	nd	209*
	$1*10^{-7}$ M	215	168	186	198	219	nd	nd	210*
	0 (2.5% Adigor)	219	166	185	200	212	nd	nd	203
	0 (no treatment)	220	165	187	200	214	nd	nd	202

[a] Hormone: aqueous solutions of 4-Me-IAA and 4-Cl-IAA ($1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$ and $1x10^{-4}$ M) in 0.25% Adigor; Control solutions aqueous 0.25% Adigor and control no solution; one application sprayed on plants to cover when the majority of the plants were at the BBCH 50 (when floral buds are observable within stipule leaves, but not beyond the stipule leaves).

[b] nd = not determined.

[c] Denotes means compared within hormone that are different from the no treatment control at $P \leq 0.05$ using single degree of freedom contrast analysis.

Table 4.6 Effect of 4-Cl-IAA and 4-Me-IAA on number of pods per plant when applied to field-grown pea (cv. Carneval) at Calgary, Red Deer, Regina and Saskatoon sites during the 2012 and 2013 field seasons.

		Number of pods per plant							
		Calgary		Red Deer		Regina		Saskatoon	
Hormone[a]	Concentration	2012	2013	2012	2013	2012	2013	2012	2013
4-Cl-IAA	$1x10^{-4}$ M	10.8	9.0	26.0	5.7	13.8	nd[b]	nd	7.2
	$1x10^{-5}$ M	11.0	9.0	26.3	4.7	12.0	nd	nd	8.0
	$1x10^{-6}$ M	11.5	9.0	29.0	4.7	15.0	nd	nd	7.0
	$1x10^{-7}$ M	12.5	8.2	28.8	5.3	14.3	nd	nd	6.3
	0 (2.5% Adigor)	12.5	9.5	25.8	5.8	13.3	nd	nd	6.8
	0 (no treatment)	13.0	8.8	30.0	5.2	14.8	nd	nd	7.8
4-Me-IAA	$1*10^{-4}$ M	12.8	8.3	19.8 *[c]	5.5	17.0	nd	nd	6.3*
	$1*10^{-5}$ M	13.5	9.3	30.5	5.5	14.8	nd	nd	7.3
	$1*10^{-6}$ M	12.8	6.2	24.8	7.0	12.0	nd	nd	6.8
	$1*10^{-7}$ M	12.5	6.8	21.5	5.5	14.8	nd	nd	8.0
	0 (2.5% Adigor)	12.5	9.5	25.8	5.8	13.3	nd	nd	6.8
	0 (no treatment)	13.0	8.8	30.0	5.2	14.8	nd	nd	7.8

[a] Hormone: aqueous solutions of 4-Me-IAA and 4-Cl-IAA ($1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$ and $1x10^{-4}$ M) in 0.25% Adigor; Control solutions aqueous 0.25% Adigor and control no solution; one application sprayed on plants to cover when the majority of the plants were at the BBCH 50 (when floral buds are observable within stipule leaves, but not beyond the stipule leaves).

[b] nd = not determined.

[c] Denotes means compared within hormone that are different from the no treatment control at $P \leq 0.05$ using single degree of freedom contrast analysis.

Table 4.7 Effect of 4-Cl-IAA and 4-Me-IAA on number of seeds per plant when applied to field-grown pea (cv. Carneval) at Calgary, Red Deer, Regina and Saskatoon sites during the 2012 and 2013 field seasons.

		Number of seeds per plant							
		Calgary		Red Deer		Regina		Saskatoon	
Hormone[a]	Concentration	2012	2013	2012	2013	2012	2013	2012	2013
4-Cl-IAA	$1x10^{-4}$ M	54	26	nd[b]	23	72	nd	nd	34
	$1x10^{-5}$ M	56	28	nd	19	61	nd	nd	36
	$1x10^{-6}$ M	56	25	nd	18	79	nd	nd	31*[c]
	$1x10^{-7}$ M	62	26	nd	19	70	nd	nd	30*
	0 (2.5% Adigor)	64	31	nd	24	67	nd	nd	30*
	0 (no treatment)	66	28	nd	19	75	nd	nd	38
4-Me-IAA	$1*10^{-4}$ M	60	24	nd	18	85	nd	nd	29*
	$1*10^{-5}$ M	66	30	nd	21	73	nd	nd	33
	$1*10^{-6}$ M	66	19*	nd	23	61	nd	nd	30*
	$1*10^{-7}$ M	60	23	nd	20	66	nd	nd	38
	0 (2.5% Adigor)	64	31	nd	24	67	nd	nd	30*
	0 (no treatment)	66	28	nd	19	75	nd	nd	38

[a] Hormone: aqueous solutions of 4-Me-IAA and 4-Cl-IAA ($1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$ and $1x10^{-4}$ M) in 0.25% Adigor; Control solutions aqueous 0.25% Adigor and control no solution; one application sprayed on plants to cover when the majority of the plants were at the BBCH 50 (when floral buds are observable within stipule leaves, but not beyond the stipule leaves).

[b] nd = not determined.

[c] Denotes means compared within hormone that are different from the no treatment control at $P \leq 0.05$ using single degree of freedom contrast analysis.

Table 4.8 Effect of 4-Cl-IAA and 4-Me-IAA on plant dry weight when applied to field-grown pea (cv. Carneval) at Calgary, Red Deer, Regina and Saskatoon sites during the 2012 and 2013 field seasons.

		Plant dry weight (g)							
		Calgary		Red Deer		Regina		Saskatoon	
Hormone[a]	Concentration	2012	2013	2012	2013	2012	2013	2012	2013
4-Cl-IAA	$1x10^{-4}$ M	10.12	6.7	nd[b]	7.38	8.9	nd	nd	5.31
	$1x10^{-5}$ M	9.47*[c]	6.54	nd	6.29	7.45	nd	nd	5.91*
	$1x10^{-6}$ M	10.48	6.52	nd	6.98	9.38	nd	nd	5.39
	$1x10^{-7}$ M	11.23	6.31	nd	6.88	8.21	nd	nd	4.86*
	0 (2.5% Adigor)	10.36	6.45	nd	7.58	8.5	nd	nd	5.64
	0 (no treatment)	11.47	5.91	nd	7.26	9.26	nd	nd	6.04
4-Me-IAA	$1*10^{-4}$ M	10.07	5.68	nd	7.5	10.13	nd	nd	5.38
	$1*10^{-5}$ M	10.89	6.17	nd	7.53	8.38	nd	nd	5.65
	$1*10^{-6}$ M	10.94	5.31	nd	7.91	7.58	nd	nd	4.96
	$1*10^{-7}$ M	10.83	5.53	nd	7.36	9.07	nd	nd	5.76
	0 (2.5% Adigor)	10.36	6.45	nd	7.58	8.5	nd	nd	5.64
	0 (no treatment)	11.47	5.91	nd	7.26	9.26	nd	nd	6.04

[a] Hormone: aqueous solutions of 4-Me-IAA and 4-Cl-IAA ($1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$ and $1x10^{-4}$ M) in 0.25% Adigor; Control solutions aqueous 0.25% Adigor and control no solution; one application sprayed on plants to cover when the majority of the plants were at the BBCH 50 (when floral buds are observable within stipule leaves, but not beyond the stipule leaves).

[b] nd = not determined.

[c] Denotes means compared within hormone that are different from the no treatment control at $P \leq 0.05$ using single degree of freedom contrast analysis.

Table 4.9 Effect of 4-Cl-IAA and 4-Me-IAA on plant height when applied to field-grown pea (cv. Carneval) at Calgary, Red Deer, Regina and Saskatoon sites during the 2012 and 2013 field seasons.

		Plant height (cm)					
		Calgary		Red Deer		Saskatoon	
Hormone[a]	Concentration	2012	2013	2012	2013	2012	2013
4-Cl-IAA	$1x10^{-4}$ M	67.4	65.7	106.5	106.7	81.0	88.8
	$1x10^{-5}$ M	67.0	62.9	104.7	100.8	76.5	88.8
	$1x10^{-6}$ M	68.2	62.0	103.1	103.3	80.4	87.9
	$1x10^{-7}$ M	65.4	63.1	102.7	106.0	80.9	84.8
	0 (2.5% Adigor)	67.1	59.0	102.1	109.6	78.4	86.6
	0 (no treatment)	67.8	57.8	97.3	104.8	81.2	89.4
4-Me-IAA	$1*10^{-4}$ M	63.0	58.7	103.5	105.6	83.1	86.0
	$1*10^{-5}$ M	66.2	58.7	101.3	108.1	81.5	86.7
	$1*10^{-6}$ M	70.3	59.1	106.6	109.7	79.2	82.1
	$1*10^{-7}$ M	66.1	57.5	102.4	106.8	78.5	89.2
	0 (2.5% Adigor)	67.1	59.0	102.1	109.6	78.4	86.6
	0 (no treatment)	67.8	57.8	97.3	104.8	81.2	89.4

[a] Hormone: aqueous solutions of 4-Me-IAA and 4-Cl-IAA ($1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$ and $1x10^{-4}$ M) in 0.25% Adigor; Control solutions aqueous 0.25% Adigor and control no solution; one application sprayed on plants to cover when the majority of the plants were at the BBCH 50 (when floral buds are observable within stipule leaves, but not beyond the stipule leaves).

Chapter 5 – Summary and conclusion

Seed and fruit set are major determinants of overall crop yield. They are determined by the agronomic interaction between plant morphology, physiology and growing conditions. Of those factors, limited photoassimilate supply to reproductive organs is likely the primary factor contributing to flower, fruit and seed abortion (Charles-Edwards 1984; Reynolds and others 2009; Rijven 1986; Jeuffroy and Devienne 1995). Flower, fruit and seed abortion are accelerated by high temperature stress and water deficit (Saini and Aspinall 1982; Saini and others 1983; Ferris and others 1998). These stresses reduce pollen or ovule viability, anther dehiscence and enhance embryo abortion (Ahmed and others 1992; Sakata and others 2010; Pressman and others 2002). However, the response to these stresses is genotype/cultivar specific (Rahman and others 2009; Balla and others 2009; Ferris and others 1998).

Auxins are plant hormones that help regulate vegetative and reproductive growth and development. Auxins regulate photoassimilate partitioning into reproductive organs (Cole and Patrick 1998; Bangerth and others 1985). They are also involved in phloem translocation (Borkovec and others 1994) and male and female reproductive growth (Aloni and others 2006; Cecchetti and others 2008). Sakata and others (2010) reported endogenous auxin levels significantly decreased in developing anthers of *Arabidopsis* and barley when they were exposed to high temperature stress. Multiple applications of auxin completely reversed male sterility in both plant species. This important finding indicates that a reduction in auxin biosynthesis, at high temperatures, is a major cause of male sterility. It appears that balanced and well-coordinated auxin signaling is necessary for male reproductive organ development.

Experiments described in this thesis were designed to investigate the effects of foliar auxin applications on wheat, canola and pea. Auxins, 4-Cl-IAA or 4-Me-IAA, were applied immediately before or at the onset of flowering to determine their ability to affect seed yield and yield components under non-stress or heat-stress conditions. Experiments were conducted under both environmentally controlled conditions and western Canadian field conditions. In general, experiments showed that 4-Cl-IAA and 4-Me-IAA had the ability to increase seed yield and yield components; however, results were genotype specific.

5.1 The effect of auxins on grain yield parameters in wheat grown under controlled environment and western Canadian field conditions.

Controlled environment experiments on hard red spring wheat showed that auxin induced grain yield parameters varied with: the type of auxin applied; plant growth stage of the auxin application; genotype; adjuvant and temperature at flowering.

Cultivar Harvest experiments with different adjuvants: Adigor (petroleum hydrocarbon and surfactant blend); Agnique (alcohol ethoxylated surfactant) and Tween 80 (polyoxyethylenesorbitan monooleate surfactant) performed differently under heat stress and non heat stress conditions with auxins. These results suggest that some adjuvants improve auxin uptake and the most suitable adjuvant depends on the auxin form and the temperature received after application. In our controlled environment experiments Adigor and Agnique perform well compared to the Tween 80 under heat stressed and heat controlled conditions.

Controlled environment experiments with the cv. Harvest also showed auxin application is more effective at the reproductive development stage (BBCH 45 [late-boot] or BBCH 59 [end of heading]) rather than at the vegetative stage (BBCH 24 [four tiller stage]). A higher auxin level may coordinate auxin signaling which sustains or enhances the yielding ability of wheat during reproductive development. It is thought that exogenously applied auxins are absorbed by the vascular pathways, which then induce phloem loading and translocation. This stimulates assimilate partitioning into developing reproductive organs resulting in increased grain set. However, our experiments did not investigate the effect of auxin applications made at the vegetative growth stage under high temperature stress.

Under field conditions, the cv. Harvest did not increase seed yield with either 4-Me-IAA or 4-Cl-IAA. Future experiments should be conducted, under controlled environmental conditions, testing application of both 4-Me-IAA and 4-Cl-IAA at lower (1×10^{-6} and 1×10^{-7} M) and higher concentrations (1×10^{-5} and 1×10^{-4} M) when plants are at early reproductive development stage (BBCH 43-45), under stressed (high heat stress >33^{o}C) or non-stressed conditions to determine if auxins can impact seed yield of the cv. Harvest.

Controlled environmental and field experiments on the wheat cv. CDC Go found auxin (4-Cl-IAA or 4-Me-IAA at 1×10^{-6} M) application at BBCH 43-45 increased grain yield and grain

set under range of temperatures and/or lower moisture conditions. Those experiments also suggest that 4-Cl-IAA is more effective than 4-Me-IAA. Concentration of 4-Cl-IAA at 1×10^{-6} M is most effective at increasing grain yield under stress conditions. The percent yield increases under non-stress field conditions (based on weather data Table A.2) was not statistically significant. Future studies with larger plot sizes or more replicates may help to determine significant differences between treatments. Field trial data interpretation was also complicated by the fact that heat stress and low moisture condition occurred concurrently. Future controlled environmental studies which impose individual heat or water stress, and combinations of both heat and water stress may help to explain the effect of those stresses on auxin response.

The different responses of Harvest and CDC Go, to the auxin treatments, in the controlled environment experiments may be partially attributed to differences in the imposed heat stresses. For the cv. Harvest, the high heat stress treatment was 33^{o}C for 6 h per day for 6 days followed by average temperature of 21^{o}C day/19^{o}C night until maturity. For the cv. CDC Go, plants were subjected to 35^{o}C for 6 h per day for 6 days followed by maximum day temperature of 30^{o}C and average minimum night temperature of 17^{o}C until maturity. Short duration (6 h per day for 6 days), high temperature stress followed by moderate heat stress conditions during flowering may have increased the auxin benefit on grain yield and yield component parameters in the cv. CDC Go.

A number of environmental and cultural management issues resulted in poor data for the cv. 5604 HR CL dose response field experiments. Hail damage occurred at the Red Deer field site in 2013 during grain filling reduced the yield data accuracy. At Regina in 2013, hail completely destroyed the wheat plots. In 2012, the Calgary field site was reduced to three replicates instead of four, and plot size of the remaining three replicates was reduced due to herbicide drift. The 2013 Calgary plots received the auxin treatments at a later crop growth stage (at BBCH 58 [80% of inflorescence emerged]) and data could not be compared with other sites. From the reliable data, application of 4-Cl-IAA at 1×10^{-6} M or 1×10^{-7} M increased grain yield per plot in the site which had moderate heat stress and low soil moisture during auxin application time.

In summary, both 4-Cl-IAA and 4-Me-IAA can be effective in increasing grain yield in hard red spring wheat. 4-Cl-IAA at 1×10^{-6} M found to be more effective than other concentrations of 4-Cl-IAA or similar concentrations of 4-Me-IAA.

5.2 The effect of auxins on seed yield parameters in canola grown under controlled environment and western Canadian field conditions

Controlled environment and field experiments suggest auxin induced canola seed yield increases depend on cultivar, auxin type, and environmental conditions.

In controlled environmental experiments, a single application of 4-Cl-IAA at 1×10^{-6} M (applied at BBCH 51) increased seed yield per plant in the canola cv. Peace. Yield increases were observed under heat stress and non-heat stress conditions, but the increased seed yield was not associated with pod number. The percent seed yield increase was higher under heat stress treatment compared with non-heat stress treatment. Rather the increased seed yield per plant may be attributed to an increased seed number per pod and/or increased seed size (TKW); however, those parameters were not assessed. 4-Me-IAA had no effect in increasing seed yield in cv. Peace. Future studies should be conducted with 4-Cl-IAA to determine its ability to affect: number of pods per plant; number of seeds per pod; and seed weight per plant.

Pod number per plant was reduced, but not total seed yield per plant, when high concentrations of auxin were applied under controlled environmental conditions. Specifically, this occurred in the cv. Peace with the single application of 1×10^{-4} M, 4-Cl-IAA (non-heat stress conditions) or with 4-Me-IAA (heat stress conditions) at BBCH 51. It also occurred with two auxin applications when 4-Cl-IAA was applied 1 week after the first application. This suggests that high auxin concentrations induce flower or pod abscission and reduce the final pod number per plant. Clifford and others in 1992 found higher auxin accumulation in the upper stem as a result of blockage of auxin transport through the stem with auxin-transport inhibitors (2,3,5-triiodobenzoic acid (TIBA) or methyl-2-chloro-9-hydroxyfluorene-9-carboxylate (CF, chlorflurenol)) reduced the bean pod number in the upper stem of faba bean (*Vicia faba* L. cv. Troy). They suggested that ethylene was produced in response to the accumulated high auxin concentrations which caused increased flower and pod abscission. Our data suggests that auxin

may enhance the seed size in the remaining pods by allocating more photoassimilates to those remaining pods and therefore seeds.

No effect was found with single applications of 4-Me-IAA at lower concentrations (1×10^{-6} and 1×10^{-7} M), regardless of the surfactant used (Adigor or Agnique or Tween 80) on the cv. 45H21 (when applied at BBCH 50 to 52) under non- heat stress conditions. Future studies on this canola cultivar, with auxins, 4-Me-IAA and 4-Cl-IAA, and heat treatments may be worthwhile to understand the effect of auxin.

In field experiments, 4-Me-IAA at 1×10^{-7} M induced a seed yield increase in the canola cv. 1852 H. This occurred at the Calgary (2012) site when low temperatures followed the hormone application. At the Saskatoon site (2013), 4-Me-IAA at 1×10^{-4} M increased seed yield in the cv. SY4114 when high temperatures followed the hormone application. Temperature differences that occurred after the hormone application may partially explain the large discrepancy between the effective concentrations that increased seed yield at these two sites. Alternately, differences in effective concentration may be explained by different responses of the different cultivars.

Environmental conditions and cultural management issues reduced the reliability of data at some field locations. Waterlogged conditions at Red Deer in 2013 and weed pressure at Saskatoon in 2012 resulted in poor quality data. Hail completely destroyed the canola plots at Regina in 2013.

In summary, both controlled environment and field experiments suggest 4-Cl-IAA and/or 4-Me-IAA could increase canola seed yield. The effective auxin concentration for increasing yield depends on the temperature following the auxin application and the cultivar. Further studies with the application of labeled auxins may broaden our knowledge of the effect of auxins on seed yield and yield component parameters.

5.3 The effect of auxins on seed yield parameters in field pea cv. Carneval grown under controlled environment and western Canadian field conditions

Auxin increased field pea seed yield and yield components in controlled environment studies with the cv. Carneval. Environmental and cultural management issues mentioned in the

results section of chapter 4 made field data inconclusive for most of the field sites. However, 4-Me-IAA (at 1×10^{-7} M) increased seed yield at Red Deer in 2013 which suggests that lower concentrations can be effective at increasing pea seed yield under field conditions. However, this must be substantiated with additional site years of high quality field data.

Controlled environmental experiments on the field pea cv. Carneval suggest that a single foliar application (made when the first flowering node of the main stem was near or at anthesis) of all concentrations of 4-Me-IAA (under non-heat stress condition) or 4-Cl-IAA (at 1×10^{-4} M, under non-heat stress condition or at 1×10^{-6}, 1×10^{-5} or 1×10^{-4} M, under heat stress condition) can increase the fruit set and/or seed number and therefore seed yield. Interestingly, the highest concentration of both 4-Me-IAA and 4-Cl-IAA increased seed yield and yield component parameters in field pea cv. Carneval under controlled environmental conditions. These results suggest that auxin can sustain or enhance the yielding ability of field pea (cv. Carneval) by increasing pod and seed set. These yield increases may be attributed to increase assimilate partitioning to developing seeds and/or reducing male and female flower sterility under heat stress or non-heat stress condition. Furthermore, our data suggest that a 4-Cl-IAA application was more effective than 4-Me-IAA in increasing seed yield and yield components over a range of temperatures during the early flowering stage. Furthermore, under heat stress conditions, 4-Cl-IAA increased the number of seeds produced on the main stem compared with that on the lateral stems. It is worthwhile to note that most lateral stems were at early stages of development when the auxin and heat stress treatments were applied. In some instances, lateral stems did not form until after the treatments were applied. This suggests that 4-Cl-IAA increased the sink strength of the pods present at the time of the heat stress treatment.

Application timing experiments (early timing: when the floral buds were tightly clustered inside the stipule leaves in the stem apex; late timing: when the first flowering node of the main stem was near or at anthesis) suggest that an early application of 4-Me-IAA (at 1×10^{-6} M) is more effective in increasing seed yield than 4-Cl-IAA at the same concentration applied at the late timing. Furthermore, early auxin applications resulted in higher seed set on the lateral stems compared with the main stem. These data suggest that an early auxin application could increase both vegetative and reproductive growth of pea plants by increasing the number of lateral stems with productive pods.

In summary, controlled environment and field data suggest that 4-Cl-IAA and 4-Me-IAA can increase seed yield and yield component parameters in the field pea cv. Carneval. 4-Cl-IAA was more effective under a range of temperatures and environmental conditions. 4-Me-IAA seems to be more effective under field conditions, although, more field experiments are needed to make this conclusion. All pea experiments were conducted on one field pea cultivar and experiments with different cultivars (including determinate and indeterminate types) may give us a better understanding of how auxin applications increase field pea yields.

5.4 General conclusion

Charles-Edwards 1984; Reynolds and others 2009; Rijven 1986, and Jeuffroy and Devienne 1995 suggest limited photoassimilate supply to the reproductive organs is likely the major factor of fruit and seed abortion in many crops. Plaut and others (2004) suggested high temperature and water deficit considerably reduce the dry matter accumulation in wheat. Therefore, fruit and seed abortion, and the subsequent reduction in seed/grain production is generally observed in plants under stress conditions. The pea and wheat controlled environmental experiments described in this thesis also showed reduced seed weight, seed number and pod/spike number under high temperature stress conditions. Sakata and others (2010) found that multiple applications of auxins (IAA, NAA, or 2,4-D) ameliorated the effects of high temperature stress on male sterility/anther abortion and seed set in barley and *Arabidopsis*. Here we show that a single foliar application of auxin (4-Cl-IAA or 4-Me-IAA) to wheat, canola and pea plants immediately before or at flowering has an ability to increase yield component parameters and thereby seed yield under heat stressed conditions. However, the magnitude of the yield increase depends on the intensity of the temperature stress.

Previous research showed that exogenous IAA is involved in increasing yield and/or yield component parameters in various crops under non-stress conditions (El-Saeid and others 2010; Clifford and others 1992). In our experiments, we showed a single foliar application of auxin (4-Cl-IAA or 4-Me-IAA) on wheat, canola and pea immediately before or at flowering has an ability to increase yield component parameters and thereby seed/grain yield under non-stressed conditions both in controlled or field environments.

Reinecke and others (1999) found that 4-Cl-IAA was more effective in stimulating pea pericarp growth than 4-Me-IAA. Here, we also found that 4-Cl-IAA was more effective than 4-Me-IAA, at the same concentration, under a range of temperatures, at increasing seed yield and yield component parameters in wheat, canola and pea. These results were clearly demonstrated in controlled environment experiments. However, field experiments suggest that the effect of auxin type and concentration depend on the cultivar and the environmental conditions during the time of auxin application. Our study found that lower concentrations of 4-Cl-IAA are the best means of increasing seed yield and yield component parameters in hard red spring wheat. For pea and canola, 4-Cl-IAA induced seed yield increases in controlled environments; however, 4-Me-IAA was better at inducing seed yield increases under field conditions. Furthermore, wheat, canola and pea experiments showed auxin effects are highly dependent on genotype and the plant growth stage of auxin application. Effects were more obvious when auxins were applied immediately before or at the flowering time.

In our experiments, canola (under controlled environment conditions) treated with high concentrations of 4-Cl-IAA and 4-Me-IAA reduced seed yield. In contrast, 4-Cl-IAA and 4-Me-IAA at high concentrations (1×10^{-4} M) increased seed yield and yield components in the field pea cv. Carneval (under non-heat stress and/or heat stress conditions in controlled environment studies). This suggests auxin response is genotype specific.

Further testing the effects of 4-Cl-IAA and 4-Me-IAA on wheat, canola and pea under both normal and heat stress conditions, and under field conditions, would broaden our knowledge of the conditions and genotypes that would optimize auxin use to increase yields. Future studies with labeled auxins will give us more knowledge on auxin involvement in increasing seed yield and yield component parameters. Controlled environment experiments involving both heat and water stress conditions would help to explain the different trends observed in controlled environment and field studies. In future studies it would be better to use canopy temperature rather than air temperature, to determine the true heat stress conditions. More studies are required with other synthetic or natural auxins (including 4-Cl-IAA and 4-Me-IAA) to determine the best type of auxin to increase seed yield and yield component parameters. Therefore, Further testing of the effects of auxins on the ability to increase seed/grain yield under

both normal and stress conditions, and under field and controlled environment conditions, would broaden our understanding of how auxins can be used to increase seed yield.

References

Agriculture and Agri Food Canada (2014) Statistics and market information: Outlook for principal field crops. Available at http://www.agr.gc.ca/eng/industry-markets-and-trade/statistics-and-market-information/?id=1361289956531 Access date 20th February, 2015

Ahmed FE, Hall AE, Demason DA (1992) Heat injury during floral development in cowpea (*Vigna unguiculata*). Am J Bot 79:784-791

Aitkins CA, Pigeaire A (1993) Application of cytokinins to flowers increase pod set in *Lupinus angustifolius* L. Aust J Agric Res 44:1799-181

Akemo MC, Regnier EE, Bennett MA (2000) Weed suppression in spring-sown rye (*Secale cereale*)-pea (*Pisum sativum*) cover crop mixes. Weed Technol 14:545-549

Aksouh NM, Jacobs BC, Stoddard FL, Miller RJ (2001) Response of canola to different heat stresses. Aust J Agric Res 52:817-824

Aksouh-Harradj NM, Campbell LC, Mailer RJ (2006) Canola response to high and moderately high temperature stresses during seed maturation. Can J Plant Sci 86:967-980

Alberta Agriculture and Rural Development (2012) Cereal crop management. Available at http://www.agriculture.alberta.ca/app21/infopage?cat1=Crops&cat2=Cereals Access date 15th February, 2012

Alberta Agriculture and Rural Development (2012) Peas. Available at http://www.agriculture.alberta.ca/app21/infopage?cat1=Crops&cat2=Peas Access date 15th February, 2012

Alberta Agriculture and Rural Development (2014) Crop decision making tool: Variety information. Available at http://www.mastinseeds.com/media/pdfs/cdc-go-variety-information.pdf Access date 20th January, 2014

Aloni R, Aloni E, Langhans M, Ullrich CI (2006) Role of auxin in regulating *Arabidopsis* flower development. Planta 223:315-328

Anderson D (2010) Vertisolic soils of the prairie region. Prairie soils & crops J 3:29-36

Angadi SV, Cutforth HW, Miller PR, McConkey BG, Entz MH, Brandt SA, Volkmar KM (2000) Response of three *Brassica* species to high temperature stress during reproductive growth. Can J Plant Sci 80:693-701

Arfan M, Athar HR, Ashraf M (2007) Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress? J Plant Physiol 164:685-694

Asghar HN, Zahir ZA, Arshad M (2004) Screening rhizobacteria for improving the growth, yield, and oil content of canola (*Brassica napus* L.). Aust J Agric Res 55:187-194

Ashley RO, Robertson LD, Seyedbagheri MM (2001) Water requirements of hard red spring wheat for final irrigations. University of Idaho. CES Bulletin 833. Available at http://www.cals.uidaho.edu/edcomm/pdf/BUL/BUL0833.pdf Access date 14th July, 2014

Bajguz A, Hayat S (2009) Effects of brassinosteroids on the plant responses to environmental stresses. Plant Physiol Biochem 47:1-8

Balla K, Karsai I, Veisz O (2009) Analysis of the quality of wheat varieties at extremely high temperatures. Alps-Adria scientific workshop, Neum, Bosnia-Herzegovina. 37:13-16 Available at http://docs.agrisafe.eu/Publications/Balla2.pdf Access date 16th December, 2012

Bangerth F, Aufhammer W, Baum O (1985) IAA level and dry matter accumulation at different positions within a wheat ear. Physiol Plant 63:121-125

Bartel B (1997) Auxin biosynthesis. Annu Rev Plant Physiol Plant Mol Biol 48:51-66

Bauermeister A, Dale JE, Williams EJ, Scobie J (1980) Movement of ^{14}C and ^{11}C-labelled assimilate in wheat leaves: The effect of IAA. J Exp Bot 31:1199-1209

Bell JM (1982) From rape seed to canola: A brief history of research for superior meal and edible oil production in Canada, feeding trial. Poult Sci 61:613-622

Bentley JA (1958) The naturally occurring auxins and inhibitors. Annu Rev Plant Physiol 9:47-80

Bérard R, Brandt S, McGregor I (2001) Determining the barriers to higher canola yields in Saskatchewan: An extensive analysis and a closer look at heat and water stress. Final report to the Saskatchewan Canola Development Commission, Saskatoon, SK, pp 75. Available at http://www.saskcanola.com/quadrant/System/research/reports/report-Brandt-higheryields-long.pdf Access date 25th March, 2015

Beynon JL, beringer JE and Johnston AWB (1980) Plasmids and host-range in *Rhizobium Leguminosarum* and *Rhizobium phaseoli.* J Gen Appl Microbiol 120:421-429

Blake TJ, Reid DM, Rood SB (1983) Ethylene, indoleacetic acid and apical dominance in peas: A reappraisal. Physiol Plant 59: 481-487

Boogaard RVD, Boer MD, Veneklaas EJ, Lanibers H (1996) Relative growth rate, biomass allocation pattern and water use efficiency of three wheat cultivars during early ontogeny as dependent on water availability. Physiol Plant 98:493-504

Borkovec V, Didehvar F, Baker DA (1994) The biosynthesis and translocation of ^{14}C-IAA in *Ricinus communis*. Plant Growth Reg 15:137-141

Böttger M, Engvild KC, Soll H (1978) Growth of *Avena* coleoptiles and pH drop of protoplast suspensions induced by chlorinated indoleacetic acids. Planta 140:89-92

Brisson N and 17 others (2001) STICS: A generic model for the simulation of crops and their water and nitrogen balances. I. Theory and parametrization applied to wheat and maize. J Agron 18:311–346

Bueckert RA, Clarke JM (2013) Review: Annual crop adaptation to abiotic stress on the Canadian prairies: Six case studies. Can J Plant Sci 93:375-385

Butler JD, Byrne PF, Mohammadi V, Chapman PL, Haley SD (2005) Agronomic performance of *Rht* alleles in a spring wheat population across a range of moisture levels. Crop Sci 45:939-947

Canadian food inspection agency (2015) Crop reports. Available at http://www.inspection.gc.ca/english/plaveg/pbrpov/cropreport/whe/app00007650e.shtml Access date 30th January, 2015

Canola Council of Canada (2012) Canola grower's Mannual. Available at http://www.canolacouncil.org/crop-production/canola-grower's-manual-contents Access date 15th February, 2012

Canola Council of Canada (2014) Canola in Canada, canola industry. Available at http://www.canola-council.org/canadian_canola_industry.aspx. Access date 15th October, 2014

Canola performance experiments (2013) Available at http://www.canolaperformanceexperiments.ca/Booklet-2013.pdf Access date 12th March, 2013

Canola performance experiments and grower directory (2011) Available at http://www.seed.ab.ca/pdf/seed_spring2011_58_OBC_REVISED04.pdf Access date 12th March, 2013

Cecchetti V, Altamura MM, Falasca G, Costantino P, Cardarelli M (2008) Auxin regulates *Arabidopsis* anther dehiscence, pollen maturation, and filament elongation. Plant Cell 20:1760-1774

Charles-Edwards DA (1984) On the ordered development of plants: An hypothesis. Ann Bot 53: 699-707

Clifford PE, Pentland BS, Baylis AD (1992) Effects of growth regulators on reproductive abscission in faba bean *(Vicia faba* cv. Troy). J Agric Sci 119:71-78

Cole DMA, Patrick JW (1998) Auxin control of photoassimilate transport to and within developing grains of wheat. Aust J Plant Physiol 25:69-77

Cutforth HW, McGinn SM, McPhee KE, Miller PR (2007) Adaptation of pulse crops to the changing climate of the northern Great Plains. Agron J 99:1684-1699

Davis WP (2003) A historical perspective from the green revolution to the gene revolution. Nutr Res Rev 61:124-134

DePauw RM, Townley-Smith TF, Humphreys G, Knox RE, Clarke FR, Clarke JM (2004) Cultivar description: Lilian hard red spring wheat. Can J Plant Sci 85:397-401

Devasirvatham V, Gaur PM, Mallikarjuna N, Tokachichu RN, Trethowan RM, Tan DKY (2012) Effect of high temperature on the reproductive development of chickpea genotypes under controlled environments. Open access repository: International Crops Research Institute for semi arid tropics. Available at http://oar.icrisat.org/6090/1/Chickpea_controlled_environments_FP12033%20%28R3%29.pdf. Access date 24th May, 2015

Downey RK, Klassen AJ, McAnsh J (1974) Rapeseed: Canada's "Cinderella" crop. Available at http://wdm.ca/skteacherguide/WDMResearch/CanolaResearchPaper.pdf. Access date 14th November, 2014

Dupont FM, Hurkman WJ, Vensel WH, Tanaka CK, Kothari KM, Chung OK, Altenbach SB (2006) Protein accumulation and composition in wheat grains: Effects of mineral nutrients and high temperature. Eur J Agron 25: 96–107

Dupont J, White PJ, Johnston KM, Heggtveit HA. McDonald BE, Grundy SM, Bonanome A (1989) Food safety and health effects of canola oil. J Am Coll Nut 8:360-375

Dupont Pioneer (2014) Canola products. Available at http://www.pioneer.com/home/site/about/products/crops/canola/. Access date 12th March, 2015

Edwards GF (2008) Mustard, cabbage and broccoli: The healthy *Brassicaceae* family. Nat Life Mag 121:31-33

Ellis M, Spielmeyer W, Gale K, Rebetzke G, Richards R (2002) "Perfect" markers for the *Rht-B1b* and *Rht-D1b* dwarfing genes in wheat. Theor Appl Genet 105: 1038-1042

El-Saeid HM, Abou-Hussein SD, El-Tohamy WA (2010) Growth characters, yield and endogenous hormones of cowpea plants in response to IAA application. Res J Agric & Biol Sci 6: 27-31

Estelle M (1996) Plant tropisms: The ins and outs of auxin. Curr Biol 6:1589–1591

FAO crop water information (2013) Wheat. Available at http://www.fao.org/nr/water/cropinfo_wheat.html. Access date 15th August, 2014

Faraji A, Latifi N, Soltani A, Rad AHS (2009) Seed yield and water use efficiency of canola (*Brassica napus* L.) as affected by high temperature stress and supplemental irrigation. Agric Water Manag 96:132-140

Farooq M, Bramley H, Palta JA, Siddique KHM (2011) Heat stress in wheat during reproductive and grain-filling phases. Crit Rev Plant Sci 30:491-507

Ferris R, Ellis RH, Wheeler TR, Hadley P (1998) Effect of high temperature stress at anthesis on grain yield and biomass of field-grown crops of wheat. Ann Bot 82:631-639

Fischer RA, Stockman YM (1986) Increased kernel number in Norin 10-derived dwarf wheat: Evaluation of the cause. Aust J Plant Physiol 13:767-784

Fowler DB, Downey RK (1970) Lipid and morphological changes in developing rapeseed (*Brassica napus*). Can J Plant Sci 50:233-247

Fox SL, McKenzie RIH, Lamb RJ, Wise IL, Smith MAH, Humphreys DG, Brown PD, Townley-Smith TF, McCallum BD, Fetch TG, Menzies JG, Gilbert JA, Fernandez MR, Despins T, Lukow O, Niziol D (2009) Cultivar description: Unity hard red spring wheat. Can J Plant Sci 90:71-78

Fox SL, Townley-Smith TF, Thomas JB, Humphreys DG, Brown PD, McCallum BD, Fetch TG, Menzies JG, Gilbert JA, Fernandez MR, Gaudet DA, Noll JS (2010) Cultivar description: Harvest hard red spring wheat. Can J Plant Sci 90:503-509

Gale MD, Law CN, Gregory RS, Quick J (1981) Norin 10 semi-dwarfism in tetraploid wheat and associated effects on yield. Euphytica 30:347-354

Gan Y, Angadi SV, Cutforth H, Potts D, Angadi VV, McDonald CL (2004) Canola and mustard response to short periods of temperature and water stress at different developmental stages. Can J Plant Sci 84:697-704

Giannakoula AE, Ilias LF , Maksimovic´ JJD, Maksimovic´ VM, Zivanovic´ BD (2012) The effects of plant growth regulators on growth, yield, and phenolic profile of lentil plants. J Food Compos Anal 28:46-53

Gibson LR, Paulsen GM (1999) Yield components of wheat grown under high temperature stress during reproductive growth. Crop Sci 39:1841-1846

Guilioni L, Wéry J, Lecoeur J (2003) High temperature and water deficit may reduce seed number in field pea purely by decreasing plant growth rate. Funct Plant Biol 30:1151-1164

Guilioni L, Wery J, Tardieu F (1997) Heat stress-induced abortion of buds and flowers in pea: Is sensitivity linked to organ age or to relations between reproductive organs? Ann Bot 80:159-168

Gupta P, Kulwal P, Rustgi S (2005) Wheat cytogenetics in the genomics era and its relevance to breeding. Cytogenet Genome Res 109:315-327

Hauggaard-Nielsen H, Ambus P, Jensen ES (2001) Inter-specific competition, N use and interference with weeds in pea-barley intercropping. Field Crops Res 70:101-109

Hiron RWP, Wright STC (1973) The role of endogenous abscisic acid in the response of plants to stress. J Exp Bot 24:769-781

Hussein MM, Balbaa LK, Gaballah MS (2007) Salicylic acid and salinity effects on growth of maize plants. Res J Agric & Biol Sci 3:321-328

Iannucci A, Terribile MR, Martiniello P (2008) Effects of temperature and photoperiod on flowering time of forage legumes in a Mediterranean environment. Field Crops Res 106:156-162

Jacob WP (1952) The role of auxin in differentiation of xylem around a wound. Am J Bot 39: 301-309

Jeuffroy MH, Duthion C, Meynard J M, Pigeaire A (1990) Effect of a short period of high day temperatures during flowering on the seed number per pod of pea (*Pisum sativum* L.). Agron J 2:139-145

Johnson RC, Kanemasu ET (1983) Yield and development of winter wheat at elevated temperatures. Agron J 75:561-565

Jones PD, New M, Martin S, Rigor IG (1999) Surface air temperature and its changes over the past 150 years. Rev Geophys 37:173-199

Kagale S, Divi UK, Krochko JE, Keller WA, Krishna P (2007) Brassinosteroid confers tolerance in *Arabidopsis thaliana* and *Brassica napus* to a range of abiotic stresses. Planta 225:353-364

Kalapos T, Boogaard VDR, Lambers H (1996) Effect of soil drying on growth biomass allocation and leaf gas exchange of two annual grass species. Plant Soil 185:137-149

Karcz W, Burdach Z (2002) A comparison of the effects of IAA and 4-Cl-IAA on growth, proton secretion and membrane potential in maize coleoptiles segments. J Exp Bot 53:1089-1098

Katekar GF, Geissler AE (1983) Structure-activity differences between indoleacetic acid auxins on pea and wheat. Phytochem 22: 27-31

Khush GS (1999) Green revolution: Preparing for the 21st century. Genome 42:646-655

Kurepin LV, Ozga JA, Zaman M and Pharis RP (2013) The physiology of plant hormones in cereal, oilseed and pulse crops. Prairie Soil Crops J 6:7-23

Leyser O (2005) The fall and rise of apical dominance. Curr Opin Genet Dev 15:468-471

Lindemann WC, Glover CR (2003) Nitrogen fixation by legumes. Available at http://aces.nmsu.edu/pubs/_a/A129/ Access date 21st April, 2015

Marumo SH Abe HH Munakata K (1968) Isolation of a novel auxin, methyl 4-chloroindoleacetate from immature seeds of *Pisum sativum*. Agric Biol Chem 32:117-118

Masuda Y (1990) Auxin induced cell elongation and cell wall changes. Bot Mag Tokyo 103:345-370

Mathews KL, Chapman SC, Trethowan R, Singh RP, Crossa J, Pfeiffer W, van Ginkel M, DeLacy I (2006) Global adaptation of spring bread and durum wheat lines near-isogenic for major reduced height genes. Crop Sci 46:603-613

Mattsson J, Ckurshumova W, Berleth T (2003) Auxin signaling in *Arabidopsis* leaf vascular development. Plant physiol 131:1327-1329

McDonald GK, Paulsen GM (1997) High temperature effects on photosynthesis and water relations of grain legumes. Plant Soil 196:47-58

Mohammadi M, Sharifi P, Karimizadeh R, Shefazadeh MK (2012) Relationship between grain yield and yield components in bread wheat under different water availability (Dry land and supplemental irrigation conditions). Not Bot Horti Agrobo 40:195-200

Morrison MJ (1993) Heat stress during reproduction in summer rape. Can J Bot 71:303- 308

Morrison MJ, Stewart DW (2002) Heat stress during flowering in summer *Brassica*. Crop Sci 42:797–803

Muchow RC, Sinclair TR (1986) Water and nitrogen limitations in soybean grain production. Field Crops Res 15:143-156

Nayirfa W, Harron B (2001) Assessment of climate change on the agricultural resources of the Canadian prairies. Available at http://www.parc.ca/pdf/research_publications/agriculture4.pdf. Access date 20th January, 2013

Nielsen DC (1997) Water use and yield of canola under dry land conditions in the central Great Plains. J Prod Agric 10:307-313

Nuttal WF, Moulin AP, Townley-Smith LJ (1992) Yield response of canola to nitrogen, phosphorus, precipitation and temperature. J Agron 84:765-768

Onyibe JE (2005) Effect of irrigation regime on growth and development of two wheat cultivars (*Triticum aestivum* L.) in the Nigerian savanna. J Agric Rural Dev Trop Subtrop 106:177–192

Ozga JA, Reinecke DM, Ayele BT, Ngo P, Nadeau C, Wickramarathna AD (2009) Developmental and hormonal regulation of gibberellin biosynthesis and catabolism in pea fruit. Plant Physiol 150:448-462

Ozga JA, Yu J, Reinecke DM (2003) Pollination, development, and auxin-specific regulation of gibberellin 3ß-hydroxylase gene expression in pea fruit and seed. Plant Physiol 131:1137-1146

Peng J, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE, Beales J, Fish LJ, Worland AJ, Pelica F, Sudhakar D, Christou P, Snape JW, Gale MD, Harberd NP (1999) ′Green revolution′ genes encode mutant gibberellin response modulators. Nature 400:256-261

Plaut Z, Butow BJ, Blumenthal CS, Wrigley CW (2004) Transport of dry matter into developing wheat kernels and its contribution to grain yield under post-anthesis water deficit and elevated temperature. 86:185- 198

Polowick PL, Sawhney VK (1988) High temperature induced male and female sterility in canola (*Brassica napus* L). Ann Bot 62:83-86

Porter JR, Gawith M (1999) Temperatures and the growth and development of wheat: A review. Eur J Agron 10:23-36

Prasad PVV, Staggenborg SA, Ristic Z (2008) Impacts of drought and/or heat stress on physiological, developmental, growth, and yield processes of crop plants. Adv Agric Sys Model 1:301-355

Pressman E, Peet MM, Pharr DM (2002) The effect of heat stress on tomato pollen characteristics is associated with changes in carbohydrate concentration in the developing anthers. Ann Bot 90:631-636

Rahman MA, Chikushi J, Yoshida S, Karim AJMS (2009) Growth and yield components of wheat genotypes exposed to high temperature stress under control environment. Bangladesh J Agric Res 34:361-372

Raymer PL (2002) Canola: An emerging oilseed crop. In: Janick J and Whipkey A (eds.), Trends in new crops and new uses. Available at https://www.hort.purdue.edu/newcrop/ncnu02/v5-122.html. Access date 28th March, 2015

Reinecke DM (1999) 4-Chloroindole-3-acetic acid and plant growth. Plant Growth Regul 27:3-13

Reinecke DM, Ozga JA, Ilic N, Magnus V, Kojic-Prodi B (1999) Molecular properties of 4-substituted indole-3-acetic acids affecting pea pericarp elongation. Plant Growth Regul 27: 39-48

Reinecke DM, Ozga JA, Magnus V (1995) Effect of halogen substitution of indole-3-acetic acid on biological activity in pea fruit. Phytochem 40:1361-1366

Reinecke DM, Wickramarathna AD, Ozga JA, Kurepin LV, Jin AL, Good AG, Pharis RP (2013) Gibberellin 3-oxidase gene expression patterns influence gibberellin biosynthesis, growth, and development in pea. Plant Physiol. 163:929-45

Reynolds M, Foulkes MJ, Slafer GA, Berry P, Parry MAJ, Snape JW, Angus JW (2009) Raising yield potential in wheat. J Exp Bot 60:1899-1918

Rijven AHGC (1986) Heat inactivation of starch synthase in wheat endosperm tissue. Plant Physiol 81:448-453

Saini HS, Aspinall D (1982) Abnormal sporogenesis in wheat (*Triticum aestivum* L.) induced by short periods of high temperature. Ann Bot 49: 835-846

Saini HS, Sedgley M, Aspinall D (1983) Effect of heat stress during floral development on pollen tube growth and ovary anatomy in wheat *(Triticum aestivum* L.). Aust J Plant Physiol 10:137-44

Sairam RK (1994) Effects of homobrassinolide application on plant metabolism and grain yield under irrigated and moisture-stress conditions of two wheat varieties. Plant Growth Regul 14: 173-181

Sakata T, Oshino T, Miura S, Tomabechi M, Tsunaga Y, Higashitani N, Miyazawa Y, Takahashi H, Watanabe N, Higashitani A (2010). Auxins reverse plant male sterility caused by high temperatures. Proc Natl Acad Sci USA 107: 8569-8574

Sarkar PK, Haque MS, Karim MA (2002) Effects of GA3 and IAA and their frequency of application on morphology, yield contributing characters and yield of soybean. Pak J Agron 1:119-122

Saskatchewan Ministry of Agriculture (2009) Soil zones in southern Saskatchewan. Available at http://www.agriculture.gov.sk.ca/soil_zones_map. Access date 25th July, 2014

Sato S, Peet MM, Tomas JF (2000) Physiological factors limit fruit set of tomato (*Lycopersicon esculentum* Mill.) under chronic, mild heat stress. Plant Cell Environ 23:719-726

Schatz B, Endres G (2009) Field pea production: NDSU Extension Service, North Dakota State University, Fargo, North Dakota. Available at http://www.ndsu.edu/pubweb/pulse-info/resources-pdf/Fieldpea%20production%20guide.pdf. Access date 10th June, 2014

Schneider EA, Kazakoff CW, Wightman F (1985) Gas chromatography-mass spectrometry evidence for several endogenous auxins in pea seedling organs. Planta 165:232-241

Schnyder H (1993) The role of carbohydrate storage and redistribution in the source-sink relations of wheat and barley during grain filling- A review. New Physiol 123:233-245

Shahid MR , Amjad M , Ziaf K, Jahangir MM , Ahmad S , Iqbal Q, Nawaz A (2013) Growth, yield and seed production of okara as influenced by different growth regulators. Pak J Agric Sci 50:387-392

Sharp RE, Wu Y, Voetberg GS, Saab IN, LeNoble ME (1994) Confirmation that abscisic acid accumulation is required for maize primary root elongation at low water potential. J Exp Bot 45:1743-1751

Shi H, Chen L, Ye T, Liu X, Ding K, Chan Z (2014) Modulation of auxin content in *Arabidopsis* confers improved drought stress resistance. Plant Physiol Biochem 82:209-21

Simon S, Petrasek J (2011) Why plants need more than one type of auxin. Plant Sci 180:454-460

Simpson BB, Conner-Ogorzaly M (1986) Economic Botany: Plants in our world. McGraw-Hill, New York pp 76-125

Singh RP, Huerta-Espino J, Rajaram S, Crossa J (2001) Grain yield and other traits of tall and dwarf isolines of modern bread and durum wheat. Euphytica 119:241-244

Soil group map of Alberta (2014). Available at http://www1.agric.gov.ab.ca/soils/soils.nsf/soilgroupmap?readform. Access date 25th June, 2014

Solomon S, Qin D, Manning M, Marquis M, Averyt K, Tignor MMB, Miller HL, Cheng Z (2007) Climate change 2007: The Physical Science basis: Contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change. New York, Cambridge University Press, pp1-50

Spiertz JHJ, Hamer RJ, Xu H, Primo-Martin C, Don C, van der Putten PEL (2006). Heat stress in wheat (*Triticum aestivum* L.): Effects on grain growth and quality traits. Eur J Agron 25:89-95

Statista (2014) Leading wheat producers. Available at http://www.statista.com/statistics/237908/global-top-wheat-producing-countries/. Access date 16th July, 2014

Statistics Canada (2013) Production of principal field crops. Available at http://www.statcan.gc.ca/daily-quotidien/140821/dq140821b-eng.htm. Access date 8th November, 2013

Stevenson FC, van Kessel C (1996) A landscape-scale assessment of the nitrogen and non-nitrogen rotation benefits of pea. Soil Sci Soc Am J 60:1797-1805

Stone PJ, Nicolas ME (1994) Wheat cultivars vary widely in their responses of grain yield and quality to short periods of post-anthesis heat stress. Aust J Plant Physiol 21:887-900

Thimann KV (1938) Hormones and the analysis of growth. Plant Physiol 13:437-449

Townley-Smith L, Slinkard AE, Bailey LD, Biederbeck VO, Rice WA (1993) Productivity, water use and nitrogen fixation of annual-legume green-manure crops in the dark brown soil zone of Saskatchewan. Can J Plant Sci 73:139-148

Ullah F, Bano A, Nosheen A (2012) Effects of plant growth regulators on growth and oil quality of canola (*Brassica napus* L.) under drought stress. Pak J Bot 44:1873-1880

United Nations Department of Economic and Social Affairs (2014) Concise report on the world population situation. Available at http://www.un.org/en/development/desa/population/publications/trends/concisereport2014.shtml. Access date 26th May, 2015

United States Department of Agriculture (2012) Soybeans and oil crops: Canola. Available at http://www.ers.usda.gov/topics/crops/soybeans-oil-crops/canola.aspx. Access date 22nd March 2015

United States Department of Agriculture (2014) Grain: World markets and trade. Available at http://apps.fas.usda.gov/psdonline/circulars/grain.pdf. Access date 18th September, 2014

United States Department of Agriculture (2014) Wheat data: Economic research unit. Available at http://www.ers.usda.gov/data-products/wheat-data.aspx#25184. Access date 25th June, 2014

University of Saskatchewan (2014). Winter wheat production manual: Growth stages of wheat. Available at http://www.usask.ca/agriculture/plantsci/winter_cereals/winter-wheat-production-manual/chapter-10.php. Access date 18th September, 2014

Wang N, Daun JK (2004) The chemical composition and nutritive value of Canadian pulses. Available at

http://www.pulsecanada.com/uploads/c4/91/c491f652f0cf53390d9a5b86aa63aeea/The-Chemical-Composition-and-Nutritive-Value-of-Canadian-Pulses.pdf. Access date 9th August

Went FW (1942) Growth, auxin and tropisms in decapitated *Avena* coleoptiles. Plant Physiol 17:236-49

Wightman F, Lighty DL (1982) Identificatioii of phenylacetic acid as a natural auxin in the shoots of higher plants. Physiol Plant 55:17-24

Young LW, Wilen RW, Bonham-Smith PC (2004) High temperature stress of *Brassica napus* during flowering reduces micro- and megagametophyte fertility, induces fruit abortion, and disrupts seed production. J Exp Bot 55:485-495

Zhang X, Brown R, Vincent L, Skinner W, Feng Y, Mekis E (2011) Canadian climate trends, 1950-2007. Canadian biodiversity: Ecosystem status and trends 2010, Technical Thematic Report No. 5. Canadian Councils of Resource Ministers. Ottawa, ON. Available at http://www.biodivcanada.ca/FBAC4E7F75F46A6971DD844E265CD2/4876No.5_General%20Climate%20July%202011_E.pdf Access date 23rd October, 2013

Zhao Y (2010) Auxin Biosynthesis and its role in plant development. Annu Rev Plant Biol 61:49-64

Zinn KE, Tunc-Ozdemir M, Harper JF (2010) Temperature stress and plant sexual reproduction: Uncovering the weakest links. J Exp Bot 61:1959-1968

Appendix

Appendix A

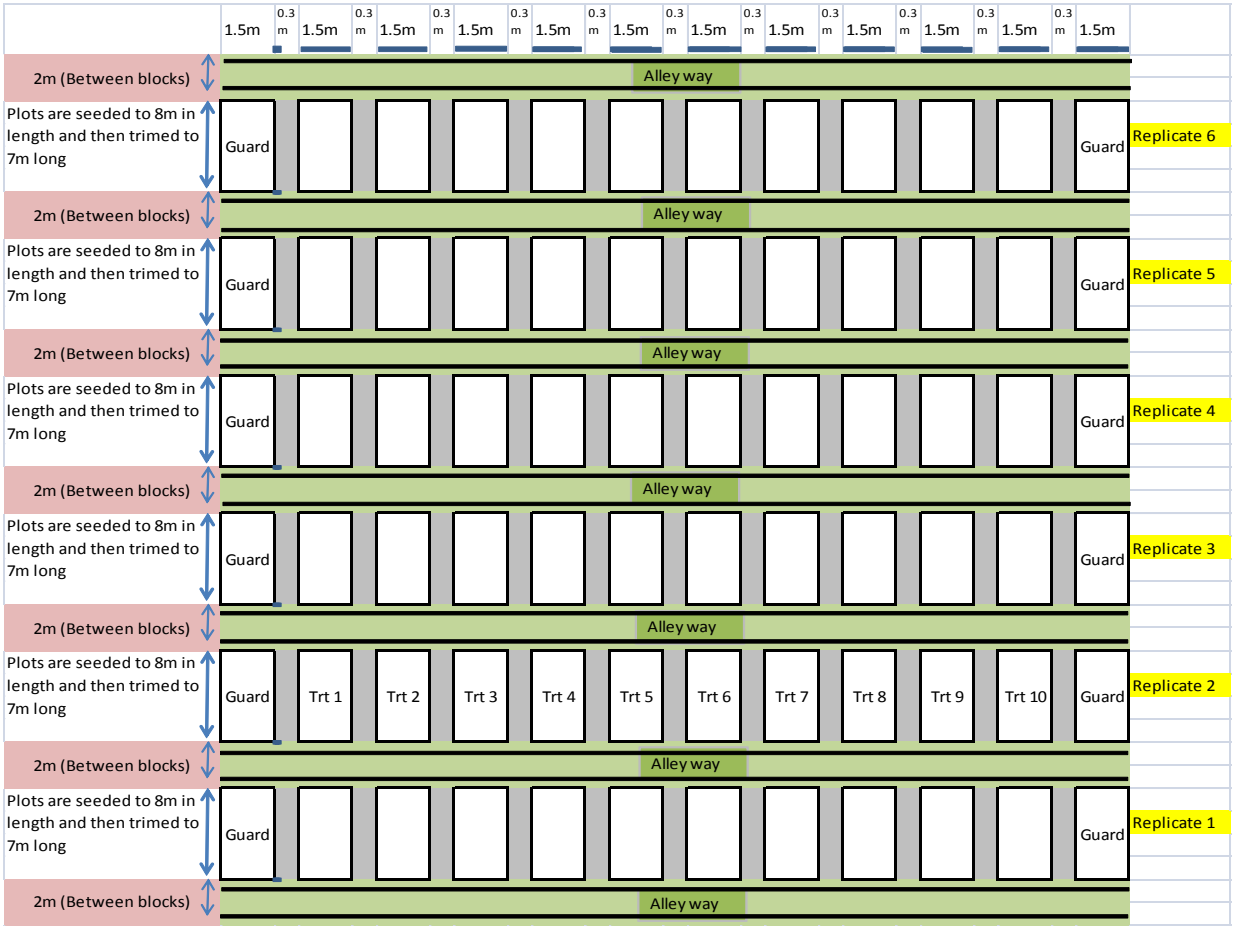


Figure A.1 Plot illustration – 4-Me-IAA and 4-Cl-IAA dose response field experiments for Wheat, Canola and Pea.

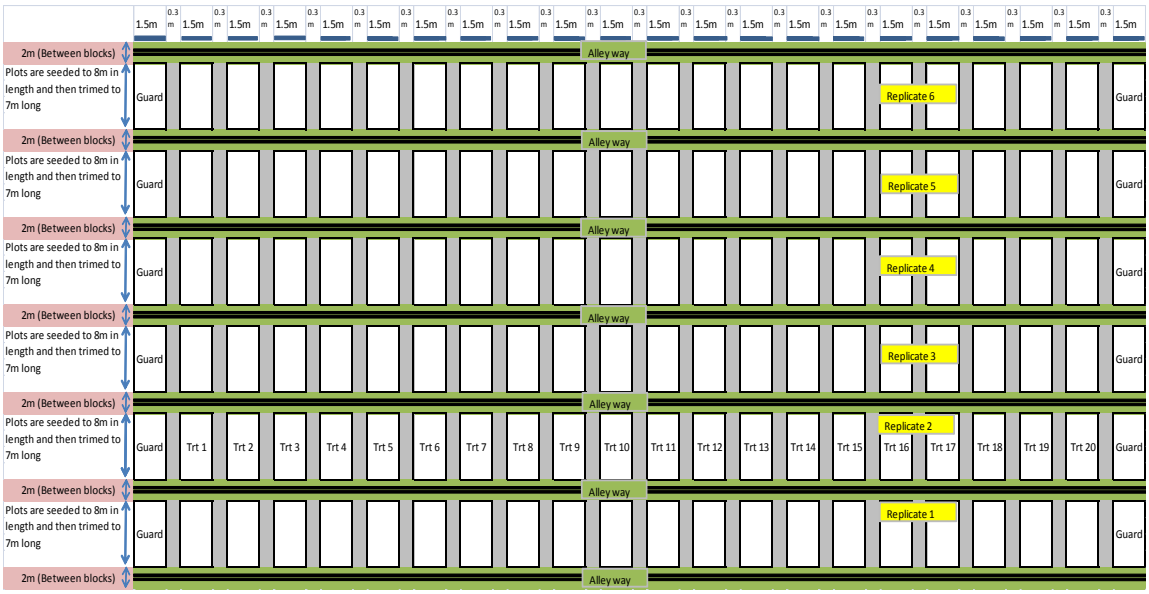


Figure A.2 Plot illustration – Wheat cultivar response field trial.

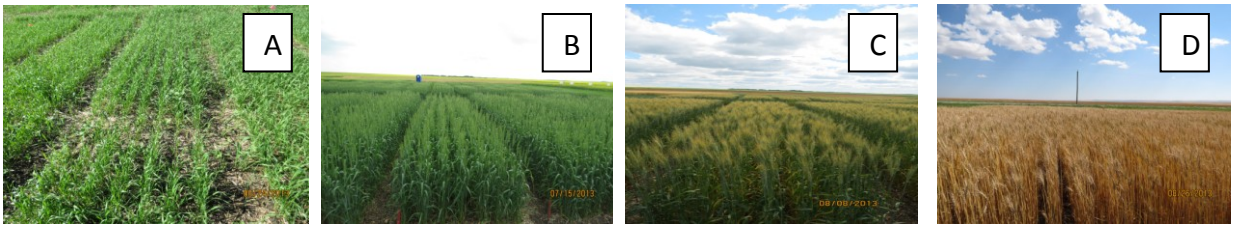


Figure A.3 Saskatoon wheat plots in the 2013 field season. (A) Plants at the tillering stage, (B) plants at the beginning of flowering, (C) plants at the early dough stage, and (D) plants at the fully mature stage.

Figure A.4 Treatment solutions were applied to the wheat field plots at the mid to late boot developmental stage (BBCH 43-45).

A.1 Environmental and cultural management issues observed in the wheat field experimental sites in the 2012 and 2013 field seasons.

In 2012, the Calgary wheat field site was reduced from four to three replicates, and plot size of the remaining three replicates was reduced from 6 m up to 3.5 m depending on the damage due to early and late season herbicide drift damage (Figs. A.5 and A.6).

Figure A.5 Early season herbicide drift damage in Calgary wheat field site in the 2012 field season.

Figure A.6 Late season herbicide drift damage in Calgary wheat field site in the 2012 field season (the stunted wheat plants in the lower right corner of the figure).

In 2013, some Calgary wheat field plots (11 plots out of 60) showed uneven germination mainly due to uneven seeding (Figs. A.7 and A.8)

Figure A.7 Medium to large size rocks in the wheat field site make difficulties in seeding at the Calgary 2013 field site.

Figure A.8 Uneven germination was observed in many plots at the Calgary wheat field site in the 2013 field season.

In 2013, Calgary plots received the auxin treatments at a later crop growth stage (at BBCH 58 [80% of inflorescence emerged]) than that outlined in the experimental design (Fig. A.9).

Figure A.9 BBCH 58 plant growth stage, the wheat plant developmental stage at the time of treatment application for the Calgary site in 2013

At Regina in 2013, hail completely destroyed the wheat plots (Fig. A.10).

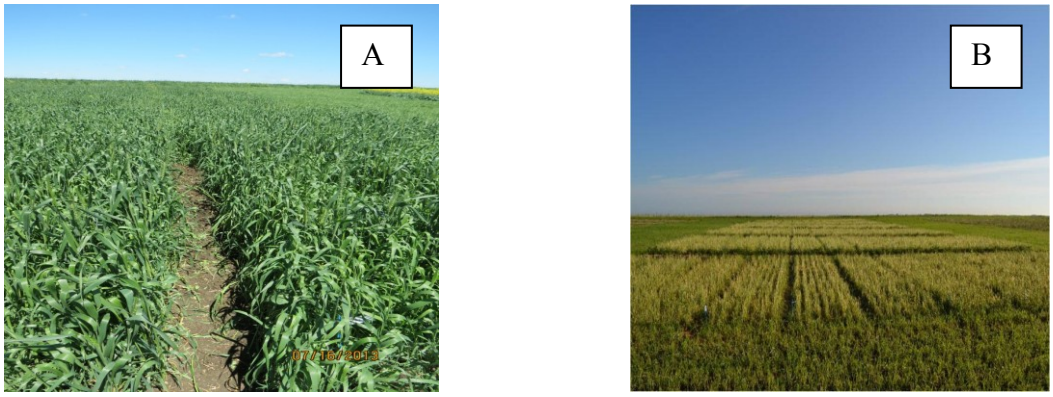


Figure A.10 Regina wheat field plots in 2013 before (A) and after (B) hail on 19th July

Table A.1 Maximum and minimum temperature (^{o}C) data inside the greenhouse during hormone application time in greenhouse experiment 2 (Auxin response on cultivars with different growth habit).

Date	Temperature max (^{0}C)	Temperature min (^{0}C)	Date	Temperature max (^{0}C)	Temperature min (^{0}C)
28th June[a]	NA[z]	NA	15th July	33.3	20
29th June	NA	NA	16th July	32.5	20
30th June	28.7	18	17th July[c]	33.1	19
1st July	31	18	18th July	31.8	19
2nd July	31.3	19	19th July	32.3	19
3rd July	31.4	19	20th July	31.5	19
4th July	30.4	25	21st July	30.1	17
5th July	25	17	22nd July	29.2	19
6th July	26.5	18	23rd July	31.7	19
7th July	32.6	19	24th July	32.8	20
8th July	33.2	19	25th July	25	16
9th July[b]	33.1	20	26th July	30.2	18
10th July	31.3	18	27th July	30.8	18
11th July	NA	NA	28th July	31.7	18
12th July	32.5	19	29th July	34.6	18
13th July	33.2	17			
14th July	32.9	19			

[a] Denotes the hormone application date of Nesser tall and Nesser dwarf wheat cultivars.

[b] Denotes the hormone application date of Pavon tall and dwarf wheat cultivars.

[c] Denotes the hormone application date of CDC GO wheat cultivar.

[z] NA = not available.

Table A.2 Maximum temperature (°C) and soil moisture content (volume %) data for two weeks before and two weeks after hormone application in Red Deer, Calgary, Saskatoon and Regina in 2012 and 2013 field seasons.

	Red Deer				Calgary				Saskatoon				Regina	
	2012		2013		2012		2013		2012		2013		2012	
	Max Temp[a] (°C)	Soil Moisture[b] (vol%)	Max Temp (°C)	Soil Moisture (vol%)	Max Temp (°C)	Soil Moisture (SMSC)	Max Temp (°C)	Soil Moisture (SMSC)	Max Temp (°C)	Soil Moisture (vol%)	Max Temp (°C)	Soil Moisture (vol%)	Max Temp (°C)	Soil Moisture (vol%)
14 DBA[c]	23	nd[d]	19	nd	21	10	25	nd	17	22	23	32	22	29
13 DBA	22	nd	15	nd	22	10	26	nd	23	20	22	31	24	29
12 DBA	20	nd	19	nd	25	12	26	nd	25	19	25	31	26	29
11 DBA	22	nd	19	nd	25	14	25	nd	25	18	25	30	24	29
10 DBA	19	nd	22	nd	23	11	28	nd	22	17	26	29	29	28
9 DBA	21	nd	22	nd	23	10	33	nd	25	21	26	28	32	28
8 DBA	22	nd	18	nd	20	12	24	nd	23	19	25	27	22	28
7 DBA	23	nd	23	nd	22	13	25	nd	20	18	29	26	25	27
6 DBA	25	nd	23	nd	23	9	20	nd	24	17	30	25	28	27
5 DBA	22	nd	26	nd	24	10	19	nd	26	16	25	24	26	26
4 DBA	23	nd	27	nd	27	12	21	nd	28	16	26	24	29	26
3 DBA	18	nd	26	nd	29	13	17	nd	27	15	25	23	26	26
2 DBA	17	nd	26	nd	32	15	25	nd	29	15	18	23	29	27
1 DBA	22	nd	31	nd	32	9	28	nd	32	16	21	23	23	27
HAD[e]	23	nd	24	nd	29	8	26	nd	30	15	21	23	23	26
1 DAA[f]	27	nd	22	nd	28	10	22	nd	29	15	24	23	26	26
2 DAA	29	nd	21	nd	27	9	20	nd	27	15	28	23	26	26
3 DAA	31	nd	17	nd	25	8	24	nd	23	28	29	22	27	26
4 DAA	30	nd	22	nd	18	9	17	nd	21	26	22	22	29	26

5 DAA	27	nd	18	nd	22	10	23	nd	17	26	19	21	33	26
6 DAA	27	nd	27	nd	25	11	26	nd	19	26	21	21	24	26
7 DAA	27	nd	27	nd	28	13	27	nd	28	24	25	21	27	26
8 DAA	24	nd	22	nd	26	11	27	nd	25	23	23	21	29	26
9 DAA	19	nd	21	nd	24	10	27	nd	24	20	26	21	24	26
10 DAA	20	nd	19	nd	28	12	24	nd	28	18	25	21	21	26
11 DAA	24	nd	22	nd	26	13	24	nd	25	17	20	21	26	26
12 DAA	28	nd	16	nd	23	14	24	nd	26	16	20	21	23	26
13 DAA	23	nd	23	nd	21	15	24	nd	25	16	18	21	29	27
14 DAA	23	nd	22	nd	23	15	20	nd	22	16	22	21	30	27

[a] Maximum temperature data were measured using on site weather stations in Calgary, Saskatoon and Regina sites; Red Deer data were collected from hourly data report of Red Deer, Alberta, weather station WMO ID 71878

[b] In Saskatoon and Regina, soil moisture was measured using a Watermark Soil Moisture Sensor from Spectrum Technologies Inc. , placed at a depth of 10 cm; In Calgary soil moisture storage capacity (SMSC) was measured using Watchdog data logger with a Watermark Soil Moisture Sensor made from Spectrum Technologies, placed at a depth of 8 inches.

[c] DBA = days before hormone application; [d] nd = not determined ; [e] HAD = hormone application date; [f] DAA = days after application

Table A.3 Effect of 4-Cl-IAA and 4-Me-IAA on total spike weight when applied to field-grown wheat (cv. 5604 HR CL) at Calgary, Red Deer, Regina and Saskatoon sites during the 2012 and 2013 field seasons.

		Total spike weight (g)							
		Calgary		Red Deer		Regina		Saskatoon	
Hormone[a]	Concentration	2012	2013	2012	2013	2012	2013	2012	2013
4-Cl-IAA	1×10^{-4} M	3.44*[c]	3.23	2.63	1.68	3.06	nd[b]	3.48	2.17
	1×10^{-5} M	2.95	3.33	2.42*	1.75	3.34*	nd	3.88	2.28
	1×10^{-6} M	2.79	3.16	2.48	1.58	3.43*	nd	3.49	2.24
	1×10^{-7} M	2.82	2.98	2.34*	1.54	3.02	nd	3.70	2.31
	0 (0.25% Adigor)	3.16	3.38	2.39*	1.55	2.80	nd	3.40	2.26
	0 (no treatment)	2.86	3.21	2.78	1.64	2.78	nd	3.50	2.28
4-Me-IAA	1×10^{-4} M	3.31*	3.20	2.36	1.58	3.06	nd	3.85	2.28
	1×10^{-5} M	3.35*	3.17	2.53	1.69	2.93	nd	3.58	2.28
	1×10^{-6} M	3.33*	3.45	3.02	1.73	2.91	nd	3.26	2.52
	1×10^{-7} M	2.96	3.39	2.76	1.65	2.88	nd	3.77	2.70
	0 (0.25% Adigor)	3.16*	3.38	2.39	1.55	2.80	nd	3.40	2.26
	0 (no treatment)	2.86	3.21	2.78	1.64	2.78	nd	3.50	2.28

[a] Hormone: aqueous solutions of 4-Me-IAA and 4-Cl-IAA ($1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$ and $1x10^{-4}$ M) in 0.25% Adigor; Control solutions aqueous 0.25% Adigor and control no solution; one application sprayed on plants to cover when the majority of the plants were at the BBCH scale 43-45 developmental stages (mid-boot stage to late-boot stage).

[b] nd = not determined.

[c] Denotes means compared within hormone that are different from the no treatment control and adjuvant only (0.25% Adigor) control at $P \leq 0.05$ using single degree of freedom contrast analysis.

Table A.4 Effect of 4-Cl-IAA and 4-Me-IAA on plant height when applied to field-grown wheat (cv. 5604 HR CL) at Calgary, Red Deer, and Saskatoon sites during the 2012 and 2013 field seasons.

		Plant height (cm)					
		Calgary		Red Deer		Saskatoon	
Hormone[a]	Concentration	2012	2013	2012	2013	2012	2013
4-Cl-IAA	1×10^{-4} M	85*[c]	109	107	116	101	102
	1×10^{-5} M	85*	108	107	117	103	102
	1×10^{-6} M	84	107	108	116	102	102
	1×10^{-7} M	82	109	108	117	101	101
	0 (0.25% Adigor)	85*	108	108	117	100	102
	0 (no treatment)	80	107	108	117	100	101
4-Me-IAA	1×10^{-4} M	85	107	107	116	101	102
	1×10^{-5} M	84	107	107	115	101	101
	1×10^{-6} M	84	108	108	116	101	101
	1×10^{-7} M	86*	109	108	116	100	100
	0 (0.25% Adigor)	85	108	108	117	100	102
	0 (no treatment)	80	107	108	117	100	101

[a] Hormone: aqueous solutions of 4-Me-IAA and 4-Cl-IAA ($1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$ and $1x10^{-4}$ M) in 0.25% Adigor; Control solutions aqueous 0.25% Adigor and control no solution; one application sprayed on plants to cover when the majority of the plants were at the BBCH scale 43-45 developmental stages (mid-boot stage to late-boot stage).

[c] Denotes means compared within hormone that are different from the no treatment control and adjuvant only (0.25% Adigor) control at $P \leq 0.05$ using single degree of freedom contrast analysis.

Table A.5 Effect of 4-Cl-IAA and 4-Me-IAA on plant dry weight when applied to field-grown wheat (cv. 5604 HR CL) at Calgary, Red Deer, Regina and Saskatoon sites during the 2012 and 2013 field seasons.

		Plant dry weight (g)							
		Calgary		Red Deer		Regina		Saskatoon	
Hormone[a]	Concentration	2012	2013	2012	2013	2012	2013	2012	2013
4-Cl-IAA	1×10^{-4} M	3.08	4.53	4.51	5.14	3.79	nd[b]	3.28	3.70
	1×10^{-5} M	2.50	4.78	4.33	5.60	3.83	nd	3.58	3.79
	1×10^{-6} M	2.92	4.41	4.06	4.98	4.30*[c]	nd	3.71	3.80
	1×10^{-7} M	3.04	4.27	3.68*	4.96	4.15*	nd	3.36	3.93
	0 (0.25% Adigor)	2.81	4.48	4.25	4.76	3.69	nd	3.75	3.86
	0 (no treatment)	2.56	4.61	4.57	5.15	3.77	nd	3.68	3.87
4-Me-IAA	1×10^{-4} M	2.87	4.35	4.28	5.22	3.78	nd	3.40	3.85
	1×10^{-5} M	2.87	4.36	4.45	5.31	3.67	nd	3.48	3.83
	1×10^{-6} M	3.39*	4.85	4.81	5.57	3.73	nd	3.74	3.83
	1×10^{-7} M	2.67	4.47	4.42	5.46	3.99	nd	3.51	4.02
	0 (0.25% Adigor)	2.81	4.48	4.25	4.76	3.69	nd	3.75	3.86
	0 (no treatment)	2.56	4.61	4.57	5.15	3.77	nd	3.68	3.87

[a] Hormone: aqueous solutions of 4-Me-IAA and 4-Cl-IAA ($1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$ and $1x10^{-4}$ M) in 0.25% Adigor; Control solutions aqueous 0.25% Adigor and control no solution; one application sprayed on plants to cover when the majority of the plants were at the BBCH scale 43-45 developmental stages (mid-boot stage to late-boot stage).

[b] nd = not determined.

[c] Denotes means compared within hormone that are different from the no treatment control and adjuvant only (0.25% Adigor) control at $P \leq 0.05$ using single degree of freedom contrast analysis.

Table A.6 Effect of 4-Cl-IAA and 4-Me-IAA on number of grains per plant when applied to field-grown wheat (cv. 5604 HR CL) at Calgary, Red Deer, Regina and Saskatoon sites during the 2012 and 2013 field seasons.

		Number of grains per plant							
		Calgary		Red Deer		Regina		Saskatoon	
Hormone[a]	Concentration	2012	2013	2012	2013	2012	2013	2012	2013
4-Cl-IAA	1×10^{-4} M	94 *[c]	87	105	93	73	nd[b]	84	67
	1×10^{-5} M	80	92	96 *	94	77	nd	88	71
	1×10^{-6} M	74	85	95*	88	80	nd	80	71
	1×10^{-7} M	77	82	93*	85	70	nd	90	71
	0 (0.25% Adigor)	83	89	103	85	70	nd	80	70
	0 (no treatment)	76	87	112	87	71	nd	83	72
4-Me-IAA	1×10^{-4} M	90	87	100	87	74	nd	88	70
	1×10^{-5} M	88	85	103	93	71	nd	84	71
	1×10^{-6} M	85	94	115	96	72	nd	78	67
	1×10^{-7} M	81	91	103	93	71	nd	86	72
	0 (0.25% Adigor)	83	89	103	85	70	nd	80	70
	0 (no treatment)	76	87	112	87	71	nd	83	72

[a] Hormone: aqueous solutions of 4-Me-IAA and 4-Cl-IAA ($1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$ and $1x10^{-4}$ M) in 0.25% Adigor; Control solutions aqueous 0.25% Adigor and control no solution; one application sprayed on plants to cover when the majority of the plants were at the BBCH scale 43-45 developmental stages (mid-boot stage to late-boot stage).

[b] nd = not determined.

[c] Denotes means compared within hormone that are different from the no treatment control and adjuvant only (0.25% Adigor) control at $P \leq 0.05$ using single degree of freedom contrast analysis.

Table A.7 Effect of 4-Cl-IAA and 4-Me-IAA on number of grains per spike when applied to field-grown wheat (cv. 5604 HR CL) at Calgary, Red Deer, Regina and Saskatoon sites during the 2012 and 2013 field seasons.

		Number of grains per spike							
		Calgary		Red Deer		Regina		Saskatoon	
Hormone[a]	Concentration	2012	2013	2012	2013	2012	2013	2012	2013
4-Cl-IAA	1×10^{-4} M	31	31	26	25	26	nd[b]	25	28
	1×10^{-5} M	30	31	24	25	26	nd	27	28
	1×10^{-6} M	27	31	25	25	25	nd	25	27
	1×10^{-7} M	26	30	25	24	23	nd	26	28
	0 (0.25% Adigor)	29	33	26	23	23	nd	25	28
	0 (no treatment)	28	31	26	24	22	nd	26	28
4-Me-IAA	1×10^{-4} M	30	31	26	24	25	nd	27	28
	1×10^{-5} M	29	31	24	25	23	nd	26	28
	1×10^{-6} M	29	32	25	24	24	nd	25	28
	1×10^{-7} M	29	32	26	24	23	nd	27	27
	0 (0.25% Adigor)	29	33	26	23	23	nd	25	28

[a] Hormone: aqueous solutions of 4-Me-IAA and 4-Cl-IAA ($1x10^{-7}$, $1x10^{-6}$, $1x10^{-5}$ and $1x10^{-4}$ M) in 0.25% Adigor; Control solutions aqueous 0.25% Adigor and control no solution; one application sprayed on plants to cover when the majority of the plants were at the BBCH scale 43-45 developmental stages (mid-boot stage to late-boot stage).

[b] nd = not determined.

Table A.8 Effect of 4-Cl-IAA and 4-Me-IAA on total spike weight and plant dry weight of six field-grown wheat cultivars (cv. 5604 HR CL, AC Lillian, AC Unity, CDC Go, Harvest, and WR859 CL) at Red Deer and Saskatoon sites during the 2012 and 2013 field seasons.

		Total spike weight (g)				Plant dry weight (g)			
		Red Deer		Saskatoon		Red Deer		Saskatoon	
Variety	Hormone treatment[a] (1×10^{-6} M)	2012	2013	2012	2013	2012	2013	2012	2013
5604 HR CL	4-Cl-IAA	2.92	2.67	3.69	4.34 *	5.07	3.82	3.16	4.70
	4-Me-IAA	3.32	3.07 *	3.53	3.67	5.08	4.20	3.13	3.82
	0 (0.25% Adigor)	2.98	2.50	3.69	3.98	4.86	3.83	3.03	4.69
	0 (no treatment)	nd[b]	2.58	nd	3.67	nd	3.86	nd	4.64
AC Lillian	4-Cl-IAA	2.98	2.37	3.59	3.67 *	4.95	4.54	3.44	4.36
	4-Me-IAA	2.60	2.52	3.56	3.66 *	6.82	4.75 *	3.20	4.67 *
	0 (0.25% Adigor)	2.85	2.20	3.44	3.10	5.30	3.99	3.33	3.78
	0 (no treatment)	nd	2.28	nd	3.22	nd	4.11	nd	4.58
AC Unity	4-Cl-IAA	2.91	2.06	3.31 *	3.70	4.90	4.29	3.04	4.44
	4-Me-IAA	3.01	2.64	3.66	3.35	4.81	4.67	3.49	4.10
	0 (0.25% Adigor)	2.93	2.19	3.80	3.59	4.40	4.34	3.39	4.73
	0 (no treatment)	nd	2.8	nd	3.71	nd	4.48	nd	4.60
CDC Go	4-Cl-IAA	3.05	2.80	3.76	4.26 *	4.5 *	3.54	3.06	3.44
	4-Me-IAA	2.80	2.66	3.59	3.71	5.01	3.11	3.25	3.35
	0 (0.25% Adigor)	2.81	2.68	3.52	3.75	5.45	3.17	2.99	3.25
	0 (no treatment)	nd	2.65	nd	3.75	nd	3.27	nd	3.46
Harvest	4-Cl-IAA	2.69	2.41	3.61	3.71	3.63	3.67	3.12	4.32
	4-Me-IAA	2.87	2.35	3.27	3.75	4.97	3.82	2.95	3.96
	0 (0.25% Adigor)	3.14	2.29	3.36	3.56	5.10	3.73	2.54	3.58

	0 (no treatment)	nd	2.11	nd	3.39	nd	3.61	nd	3.62
WR859 CL	4-Cl-IAA	2.92	nd	3.50	nd	4.93	nd	3.28	nd
	4-Me-IAA	3.16	nd	3.36	nd	5.01	nd	2.97	nd
	0 (0.25% Adigor)	2.76	nd	3.43	nd	5.24	nd	2.63	nd
	0 (no treatment)	nd	nd	nd	nd	nd	nd	nd	nd

[a] Hormone treatments: aqueous solutions of 4-Me-IAA and 4-Cl-IAA ($1x10^{-6}$ M) in 0.25% Adigor; Control solutions aqueous 0.25% Adigor and control no solution; one application sprayed on plants to cover when the majority of the plants were at the BBCH scale 43-45 developmental stages (mid-boot stage to late-boot stage) in each variety.

[b] nd = not determined.

[c] Denotes means compared within cultivar that are different from the 0.25% Adigor control at $P \leq 0.05$ using single degree of freedom contrast analysis.

Appendix A: Materials and Method for the St. Albert wheat cultivar response experiment

A cultivar response field experiment was conducted in 2012 at St. Albert, (Latitude: 55.34/ Longitude: -113.31), Alberta using the same six wheat cultivars (AC Lilian, AC Harvest, CDC GO, AC Unity, 5604 HR CL and WR859 CL) described for the Alberta and Saskatchewan sites in 2012. The St. Albert site was dominated with Black Chernozemic soil (Alberta Agriculture Food and Rural Development, 2002). Grains (treated with CruiserMaxx® at 325 ml/100 kg grain) were seeded into a 5cm depth (using Fabro cone seeder by Swift Current, SK, Canada) on May 16th 2012, at a seeding rate of 32 plants per meter. Plots were arranged in RCBD design with 24 plots per replicate (6 cultivars × 4 treatments [4-Me-IAA at 1 x 10^{-7} M, 1 x 10^{-6} M and 1 x 10^{-5} M in aqueous 0.25% Adigor and no treatment control]) with three replicates per treatment. Treatments were randomly applied to plots within each replicate. Individual treatment plot size was 4 m × 0.9 m. Inter-plot spacing was 0.3m, with a 1.8 m buffer between replicates. Each treatment plot consisted of 4 rows with an inter-row spacing of 0.233 m from the middle of one row to the middle of next row. (Appendix A; Figure A.11; Plot illustration – Wheat cultivar response trial- St. Albert). Hormone application, field maintenance, harvesting and data collection were done similar to other wheat dose response and cultivar response field experiments.

Statistical analysis

Analysis of variance (ANOVA) and analysis of covariance (ANCOVA) (for grain yield) were performed on field data using the PROC MIXED procedure of SAS 9.3 software (SAS Institute Inc. Cary, NC, USA, 2010). The one-way ANOVA was carried out with the treatment levels including no treatment control, 4-Me-IAA and 4-Cl-IAA treatments. Similarly, the one-way ANCOVA was carried out with treatment as the main effect and the number of spikes per meter (the number of plants per meter data was not accurate as it was counted after the tillering stage) as a covariate to account for the variation in plot plant density. Prior to the experiment, we already planned the following single degree of freedom contrasts; no treatment control vs. 4-Me-IAA treatments. These contrasts were preplanned to examine specific treatment effects that would be of agronomic or biological importance.

Appendix A: Results and discussion for the St. Albert wheat cultivar response experiment

4-Me-IAA concentrations did not increase the grain yield, spike weight and number of spikes per plant in cvs. AC Lilian, AC Harvest, CDC Go, AC Unity, 5604 HR CL and WR859 CL used in St. Albert field trial (Table A.9). Small plot sizes in St. Albert field may not enough to find significant treatment effects. Some yield component parameters showed 4-Me-IAA induced increases in some cultivars. 1×10^{-6} M of 4-Me-IAA increased the thousand kernel weight in cv. CDC GO (Table A.9). 4-Me-IAA treated plants showed increase in plant dry weight in cv. 5604 HR CL compared to the control (Table A.9).

Appendix A: Conclusion for the St. Albert wheat cultivar response experiment

Hard red spring wheat cultivars grown in small plots did not show yield increasing effect with 4-Me-IAA concentrations under St. Albert weather conditions. Genotypic variations in response to 4-Me-IAA could observe in thousand kernel weight and plant dry weight.

Table A.9 Effect of 4-Me-IAA on grain yield and yield component parameters of six field-grown wheat cultivars (cv. 5604 HR CL, AC Lillian, AC Unity, CDC Go, Harvest and WR859 CL) at St. Albert research field during the 2012 field season.

Variety	4-Me-IAA hormone concentration[a]	Grain yield (kg/ha)	Thousand kernel weight (g)	Individual spike weight (g)	Plant height (cm)	Number of spikes per plant	Plant dry weight (g)
5604 HR CL	1×10^{-5} M	4300	32.2	0.9	103	3.67	3.51 *[c]
	1×10^{-6} M	4144	31.43	0.84	102	3.67	3.79 *
	1×10^{-7} M	3953	29.9	0.8	103	3.33	3.49 *
	0 (no treatment)	4307	30.63	0.81	102	3.33	2.97
AC Unity	1×10^{-5} M	4235	34.2	0.72	103	4	3.45
	1×10^{-6} M	4343	33.27	0.64	102	4.67	3.47
	1×10^{-7} M	4322	34.33	0.74	99	4.67	4.01
	0 (no treatment)	4175	34	0.67	104	3.33	3.16
AC Lilian	1×10^{-5} M	3935	34.43	0.68	102	4	3.39
	1×10^{-6} M	3898	34.03	0.64	102	3.67	3.82
	1×10^{-7} M	3876	34.2	0.61	103	4.33	4.09
	0 (no treatment)	3818	33.67	0.6	100	4	3.8
CDC Go	1×10^{-5} M	4380	38.73	0.76	90	4.33	3.54
	1×10^{-6} M	4239	39.53*	0.83	91	3.67	2.57
	1×10^{-7} M	4273	37.8	0.72	91	4.67	3.93
	0 (no treatment)	4229	35.63	0.68	91	3.67	3.16
Harvest	1×10^{-5} M	3657	32.23	0.78	98 *	4	3.75
	1×10^{-6} M	3778	34.67	0.8	99 *	4	2.39 *
	1×10^{-7} M	3977	33.1	0.72	98 *	4	3.76
	0 (no treatment)	4089	33.03	0.83	102	4.33	3.82
WR859 CL	1×10^{-5} M	3757	29.25	0.62	96	4.33	3.15
	1×10^{-6} M	4022	30.87	0.71	95	4.67	3.76
	1×10^{-7} M	3946	30.7	0.59	95	4	3.1
	0 (no treatment)	4213	29.97	0.61	96	4	3.17

[a] 4-Me-IAA hormone concentration: aqueous solutions of 4-Me-IAA ($1x10^{-5}$, $1x10^{-6}$, $1x10^{-7}$ M) in 0.25% Adigor; Control no solution; one application sprayed on plants to cover when the majority of the plants were at the BBCH scale 43-45 developmental stages (mid-boot stage to late-boot stage) in each variety.

[c] Denotes means compared within cultivar that are different from the 0.25% Adigor control at $P \leq 0.05$ using single degree of freedom contrast analysis.

Figure A.11 Plot illustration – Wheat cultivar response field trial – St. Albert

Appendix B

Figure B.1 Saskatoon canola plots in 2013. (A) Plants at the 4 to 6 leaf development stage (B) plants during flowering, and (C) plants with pods at the beginning of seed ripening.

Figure B.2 Treatment solutions were applied to the canola field plots when flower buds were either enclosed by leaves or flower buds were free from the younger leaves (BBCH 50-52). The canola plant in this figure is at the BBCH 52 crop growth stage.

B.1 Environmental and cultural management issues observed in canola field experimental sites in the 2012 and 2013 field seasons.

High weed pressure was present at the Saskatoon canola field plots in 2012 (Fig. B.3).

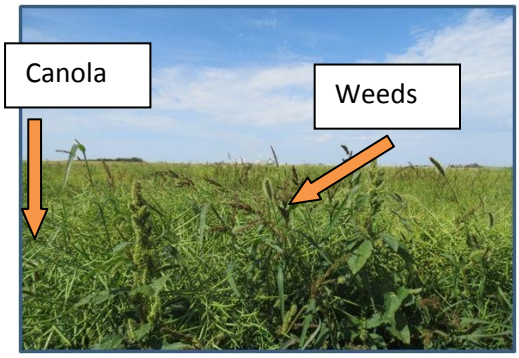


Figure B.3 Weed pressure in one canola plot at the Saskatoon 2012 field site.

Saskatoon 2013 canola plots were sprayed at BBCH 53 [flower buds raised above the youngest leaves] to BBCH 57 [individual flower buds visible, but still closed] (Figs. B.4 and B.5) than at BBCH 50 to 52 due to weather conditions at time of solution application.

Figure B.4 Canola plants at BBCH 53, the plant developmental stage at the time of treatment application for the Calgary site in 2013

Figure B.5 Canola plants at BBCH 57, the plant developmental stage at the time of treatment application for the Calgary

Early to mid season waterlogged conditions were present at the Red Deer 2013 canola site which resulted in variable plant plot density across the site (Fig. B.6).

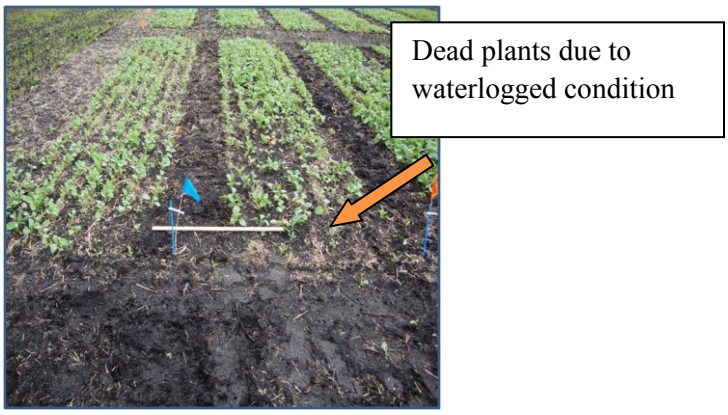


Figure B.6 Early season waterlogged conditions at the Red Deer 2013 canola site led to variable plant establishment within the plots.

Hail completely destroyed the canola plots at Regina in 2013 (Fig. B.7).

Figure B.7 Regina canola field site in 2013 before (A) and after (B) hail on 19th July.

Table B.1 Maximum temperature (°C) and soil moisture content (volume %) data for two weeks before and two weeks after hormone application in Red Deer, Calgary, Saskatoon and Regina in 2012 and 2013 field seasons.

	Red Deer				Calgary				Saskatoon				Regina	
	2012		2013		2012		2013		2012		2013		2012	
	Max Temp[a] (°C)	Soil Moisture[b] (vol%)	Max Temp (°C)	Soil Moisture (vol%)	Max Temp (°C)	Soil Moisture (SMSC)	Max Temp (°C)	Soil Moisture (SMSC)	Max Temp (°C)	Soil Moisture (vol%)	Max Temp (°C)	Soil Moisture (vol%)	Max Temp (°C)	Soil Moisture (vol%)
14 DBA[c]	17.2	nd[d]	16.8	nd	22.2	10.00	15.8	nd	20	25.90	19.2	30.96	20.5	29.60
13 DBA	22.9	nd	19.6	nd	24.4	10.00	20.4	nd	19	24.90	16.7	31.71	20.3	29.36
12 DBA	18.4	nd	16.4	nd	19.5	12.00	20.9	nd	20	22.30	18.6	32.87	20.3	29.09
11 DBA	17.2	nd	22.1	nd	19.4	14.00	20.6	nd	19	27.70	19.7	32.46	16.8	28.86
10 DBA	16.3	nd	19.8	nd	17.8	11.00	23.3	nd	15	25.70	21.1	32.12	14.9	28.74
9 DBA	19.5	nd	18.7	nd	24.2	10.00	23.9	nd	17	24.90	23.3	31.74	20.4	28.74
8 DBA	22.9	nd	15.3	nd	21.7	12.00	24.7	nd	16	26.60	22.1	31.08	22.3	28.76
7 DBA	23.4	nd	18.7	nd	13.3	13.00	25.6	nd	19	25.10	24.6	30.82	23.7	28.77
6 DBA	21.6	nd	19.4	nd	17.5	9.00	26.2	nd	21	23.10	25.1	30.00	25.5	28.75
5 DBA	19.8	nd	21.7	nd	19.5	10.00	24.7	nd	23	20.80	25.7	28.91	23.9	28.55
4 DBA	21.7	nd	21.7	nd	22.6	12.00	27.6	nd	24	20.10	25.7	27.73	29.3	28.14
3 DBA	19.4	nd	18.4	nd	21.3	13.00	33.0	nd	24	19.70	25.4	26.76	31.9	27.90
2 DBA	21.3	nd	23.3	nd	19.1	15.00	23.6	nd	30	21.60	28.6	25.89	21.7	27.61
1 DBA	22.1	nd	23.4	nd	19.0	9.00	25.2	nd	24	23.90	30.3	24.76	24.6	26.97
HA[e]	22.5	nd	26.4	nd	24.0	8.00	20.0	nd	17	21.60	25.4	24.01	27.8	26.58
1 DAA[f]	25.1	nd	26.4	nd	14.0	10.00	18.6	nd	23	19.90	26.0	23.55	26.3	26.40
2 DAA	21.6	nd	26.2	nd	20.8	9.00	21.2	nd	25	18.60	24.5	23.19	29.3	26.22
3 DAA	23.3	nd	26.4	nd	21.6	8.00	17.0	nd	25	17.90	17.6	23.05	26.2	26.33
4 DAA	17.8	nd	31.2	nd	24.9	9.00	25.2	nd	22	17.20	21.3	23.08	28.6	26.52
5 DAA	16.9	nd	24.0	nd	25.1	10.00	27.6	nd	25	20.80	20.7	23.00	23.0	26.51
6 DAA	21.7	nd	22.2	nd	23.3	11.00	26.5	nd	23	19.40	24.0	22.96	23.4	26.29
7 DAA	23.3	nd	21.0	nd	23.4	13.00	22.4	nd	20	17.70	27.6	22.81	26.3	26.22

8 DAA	26.7	nd	16.7	nd	19.8	11.00	19.6	nd	24	16.90	29.0	22.46	25.9	26.24
9 DAA	29.1	nd	22.0	nd	21.9	10.00	24.4	nd	26	16.20	21.6	21.94	27.0	26.13
10 DAA	31.0	nd	18.1	nd	22.8	12.00	17.1	nd	28	15.70	19.3	21.49	28.5	25.97
11 DAA	29.6	nd	26.8	nd	23.9	13.00	23.0	nd	27	15.40	21.2	21.35	32.9	25.93
12 DAA	27.2	nd	26.8	nd	26.6	14.00	25.8	nd	29	15.20	24.7	21.38	24.2	26.08
13 DAA	27.3	nd	21.9	nd	28.6	15.00	27.0	nd	32	15.80	22.7	21.29	27.4	26.15
14 DAA	27.0	nd	20.5	nd	32.2	15.00	27.0	nd	30	15.40	25.8	21.15	28.6	26.22

[a] Maximum temperature data were measured using on site weather stations in Calgary, Saskatoon and Regina sites; Red Deer data were collected from hourly data report of Red Deer, Alberta, weather station WMO ID 71878

[b] In Saskatoon and Regina, soil moisture was measured using a Watermark Soil Moisture Sensor from Spectrum Technologies Inc. , placed at a depth of 10 cm; In Calgary soil moisture storage capacity (SMSC) was measured using Watchdog data logger with a Watermark Soil Moisture Sensor made from Spectrum Technologies, placed at a depth of 8 inches.

[c] DBA = days before hormone application; [d] nd = not determined ; [e] HAD = hormone application date; [f] DAA = days after application

Appendix C

Figure C. 1 Saskatoon field pea plots in 2013. (A) Plants at the vegetative stage, (B) Plants at full flowering and (C) plants with developing green pods.

Figure C. 2 Treatment solutions were applied to the pea field plots when floral buds were present but not visible outside of stipule leaves (BBCH 50).

C.1 Environmental and cultural management issues observed in pea field experimental sites in the 2012 and 2013 field seasons.

In 2012, the Saskatoon pea field site was lost due to *Fusarium* root rot infestation (Fig. C.3).

Figure C.3 (A) *Fusarium* root rot-infested pea plants in the field and (B) individual plants grown at the Saskatoon 2012 field site.

Lower plant density (4 plants per square feet) at all pea sites in the 2012 field season led to greater weed competition during the growing season (Figs. C.4 and C.5).

Figure C.4 Lower pea plant density at the Regina pea field site in 2012.

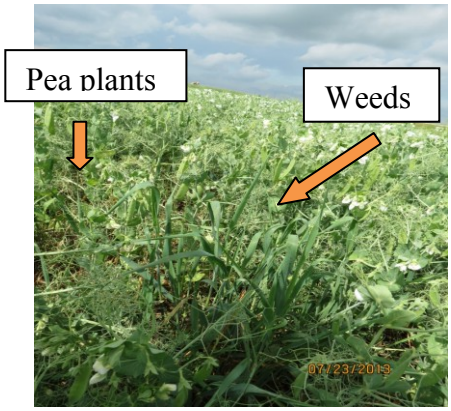


Figure C.5 Higher weed density at Red Deer pea field site in 2012.

Substantial late season (8th August) hail damage at the Red Deer field site in 2012 making the plot yield data unreliable (Figs. C.6 and C.7).

Figure C.6 Hail damage on pea pods at Red Deer site in 2012.

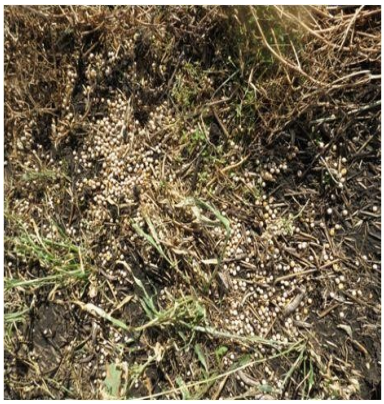


Figure C.7 Lost of pea seeds due to hail at Red Deer pea field site in 2012.

Hail completely destroyed the pea plots in Regina in 2013 (Fig. C.8).

Figure C.8 Regina pea field 2013 site before (A) and after (B) hail on 19th July.

Table C.1 Maximum temperature (°C) and soil moisture content (volume %) data for two weeks before and two weeks after hormone application in Red Deer, Calgary, Saskatoon and Regina in 2012 and 2013 field seasons.

	Red Deer				Calgary				Saskatoon				Regina	
	2012		2013		2012		2013		2012		2013		2012	
	Max Temp[a] (°C)	Soil Moisture[b] (vol%)	Max Temp (°C)	Soil Moisture (vol%)	Max Temp (°C)	Soil Moisture (SMSC)	Max Temp (°C)	Soil Moisture (SMSC)	Max Temp (°C)	Soil Moisture (vol%)	Max Temp (°C)	Soil Moisture (vol%)	Max Temp (°C)	Soil Moisture (vol%)
14 DBA[c]	19	nd[d]	20.23	nd	24	8	16	nd	nd	nd	21.1	32.12	15	28.76
13 DBA	21	nd	18.14	nd	14	10	20	nd	nd	nd	23.3	31.74	16	28.77
12 DBA	22	nd	20.63	nd	21	9	21	nd	nd	nd	22.1	31.08	18	28.75
11 DBA	23	nd	15.46	nd	22	8	21	nd	nd	nd	24.6	30.82	18	28.55
10 DBA	25	nd	22.25	nd	25	9	23	nd	nd	nd	25.1	30.00	22	28.14
9 DBA	22	nd	21.13	nd	25	10	24	nd	nd	nd	25.7	28.91	23	27.90
8 DBA	23	nd	26.69	nd	23	11	25	nd	nd	nd	25.7	27.73	16	27.61
7 DBA	18	nd	25.53	nd	23	13	26	nd	nd	nd	25.4	26.76	18	26.97
6 DBA	17	nd	25.37	nd	20	11	26	nd	nd	nd	28.6	25.89	18	26.58
5 DBA	22	nd	21.08	nd	22	10	25	nd	nd	nd	30.3	24.76	20	26.40
4 DBA	23	nd	21.15	nd	23	12	28	nd	nd	nd	25.4	24.01	21	26.22
3 DBA	27	nd	22.84	nd	24	13	33	nd	nd	nd	26	23.55	20	26.33
2 DBA	29	nd	22.85	nd	27	14	24	nd	nd	nd	24.5	23.19	21	26.52
1 DBA	31	nd	16.39	nd	29	15	25	nd	nd	nd	17.6	23.05	18	26.51
HA[e]	30	nd	22.06	nd	32	15	20	nd	nd	nd	21.3	23.08	17	26.29
1 DAA[f]	27	nd	17.31	nd	32	15	19	nd	nd	nd	20.7	23.00	19	26.22
2 DAA	27	nd	18.86	nd	29	16	21	nd	nd	nd	24	22.96	20	26.24
3 DAA	27	nd	15	nd	28	17	17	nd	nd	nd	27.6	22.81	19	26.13
4 DAA	24	nd	19.4	nd	27	18	25	nd	nd	nd	29	22.46	21	25.97
5 DAA	19	nd	22.92	nd	25	19	28	nd	nd	nd	21.6	21.94	24	25.93
6 DAA	20	nd	21.56	nd	18	20	26	nd	nd	nd	19.3	21.49	19	26.08
7 DAA	24	nd	21.96	nd	22	20	22	nd	nd	nd	21.2	21.35	20	26.15

8 DAA	28	nd	21.46	nd	25	19	20	nd	nd	nd	24.7	21.38	20	26.22
9 DAA	23	nd	22.97	nd	28	19	24	nd	nd	nd	22.7	21.29	20	26.22
10 DAA	23	nd	21.41	nd	26	19	17	nd	nd	nd	25.8	21.15	19	26.06
11 DAA	23	nd	21	nd	24	20	23	nd	nd	nd	25.2	21.14	20	26.03
12 DAA	25	nd	20.65	nd	28	9	26	nd	nd	nd	20	20.93	19	26.26
13 DAA	24	nd	13.17	nd	26	13	27	nd	nd	nd	20.1	20.86	22	26.58
14 DAA	23	nd	20.48	nd	23	16	27	nd	nd	nd	18	20.92	22	26.82

[a] Maximum temperature data were measured using on site weather stations in Calgary, Saskatoon and Regina sites; Red Deer data were collected from hourly data report of Red Deer, Alberta, weather station WMO ID 71878

[b] In Saskatoon and Regina, soil moisture was measured using a Watermark Soil Moisture Sensor from Spectrum Technologies Inc., placed at a depth of 10 cm; In Calgary soil moisture storage capacity (SMSC) was measured using Watchdog data logger with a Watermark Soil Moisture Sensor made from Spectrum Technologies, placed at a depth of 8 inches.

[c] DBA = days before hormone application; [d] nd = not determined ; [e] HAD = hormone application date; [f] DAA = days after application

Appendix D

Executive summary

The experiments described in this thesis test the hypothesis that application of auxins 4-chloroindole-3-acetic acid (4-Cl-IAA) and 4-methylindole-3-acetic acid (4-Me-IAA) as a foliar spray when plants were at early reproductive stage of three major agricultural crops grown in western Canada [wheat (*Triticum aestivum* L.) canola (*Brassica napus* L.) and field pea (*Pisum sativum* L.)], will increase seed yield under normal environmental conditions, and reduce the effects of heat stress on crop yield when the stress occurs during reproductive development. 4-Cl-IAA and/or 4-Me-IAA application near or at the flowering stage increased seed yield under controlled environmental conditions in all three crops tested, often under both non-stress and heat stress conditions. In well managed field trails that did not experience hail damage or biotic stress conditions, auxin application was most effective at increasing seed yield at the sites that experienced higher temperatures during the flowering period for wheat and canola. The effect of genotype on auxin response was only systematically tested in wheat (hard red spring- type), where one of five cultivars was found to be the most responsive to auxin-induced (4-Cl-IAA) increases in seed yield under both controlled environment and field conditions. Overall, these studies suggest that application of 4-Cl-IAA or 4-Me-IAA to wheat, canola and pea crops is a promising yield enhancing technology. Further studies are required to confirm the yield-enhancing effects of 4-Cl-IAA and 4-Me-IAA under field conditions and to understand the mechanisms involved in auxin-induced increases in seed yield in these crops.