The Physiology of Plant Hormones in Cereal, Oilseed and Pulse Crops

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Summary

Plant hormones regulate virtually all aspects of plant growth and development, as well as plant responses to biotic and abiotic signals. Herein, we discuss three hormone groups, gibberellins, auxin and ethylene. These plant hormones influence seed germination, root, stem and leaf growth, flowering, and fruit and seed growth. In so doing, the hormone groups often interact with each other and with the plant's environment. This review discusses the regulatory roles that these three plant hormone groups play in the physiology of cereal, oilseed and pulse crops and also discusses the usefulness of applications of hormones, and of other plant growth regulators. Additionally, it discusses the potential uses of plant growth promoting rhizosphere (root-zone) microorganisms (PGPRs) in order to obtain yield improvement, or to alleviate abiotic stress.

Introduction to plant hormones

Plant hormones occur naturally in crop plants and regulate both vegetative and reproductive growth and development. There are at least six classes of hormones: gibberellins (GAs), auxins, brassinosteroids (BRs), cytokinins (CKs), ethylene and abscisic acid (ABA). This review will concentrate on three hormone groups with proven track records in applied crop physiology, i.e. GAs, auxins and ethylene (Fig. 1).
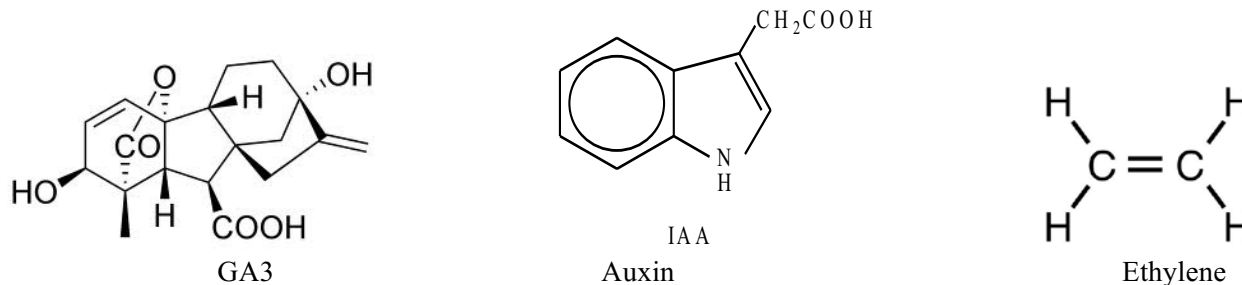

Figure 1. Chemical structures of GA_3 (a native GA in many crop plants, which is highly bioactive and commercially available), IAA (the major native auxin in crop plants), and ethylene (a ubiquitous gaseous hormone).

Gibberellins regulate seed germination, growth of the root and shoot (leaves and stem), the transition from the vegetative to the reproductive state, including floral apex induction, sex expression, organ growth within the developing flower and fruit set. Pollen germination and pollen tube growth are also controlled by GAs, as is fruit growth. Finally, the GAs can delay fruit senescence and regulate seed and embryo growth[36,54,67]. Gibberellins are also important regulators of flowering in cereals and grasses[36]. In Persian darnel, an annual Lolium species (*Lolium temulentum* L.), exogenous application of GAs induced flowering and/or

associated stem elongation in a structure-dependant manner[24].

Plant growth retardants are a group of plant growth regulators (PGRs; Fig. 2) and they are, to date, not known to occur naturally in higher plants. Rather, they are synthetic chemicals which mainly modify or inhibit the biosynthesis of GAs in both agronomic and horticultural crops[55]. They usually reduce vegetative growth with few or no deleterious effects, while preserving or enhancing crop yield per plant and per hectare. Most of the plant growth retardants are also

fungitoxic[29]. In fact, the triazole class of growth retardants, which includes paclobutrazol (PP333 or Cultar), was initially developed for fungicide use.

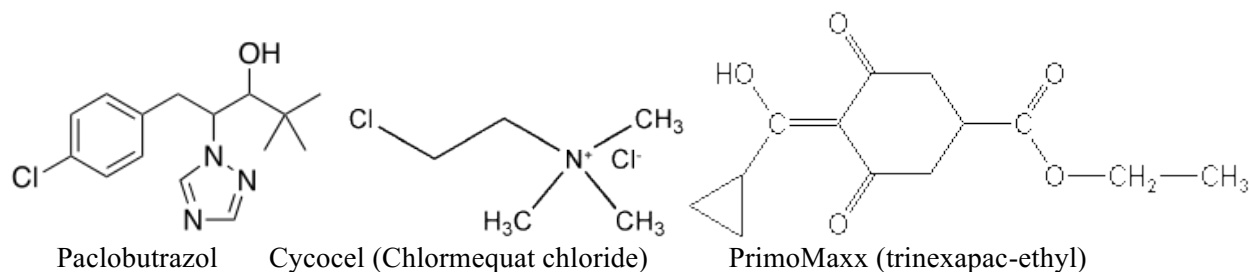Paclobutrazol   Cycocel (Chlormequat chloride)   PrimoMaxx (trinexapac-ethyl)

Figure 2. Chemical structures of several PGRs which have plant growth retardant abilities by inhibiting GA biosynthesis.

Auxins regulate a wide variety of growth and developmental processes in higher plants, including cell elongation and thus the stimulation of shoot growth. However, roots are very sensitive to auxins and even low concentrations can inhibit root growth. Auxins also regulate differentiation of vascular tissue (phloem and xylem), and induce adventitious root initiation on shoot and root cuttings used for propagation. Shoot growth toward light (photo-tropic) and toward (roots) or away from (shoots) gravity (gravi-tropic) is also regulated by auxin. Auxins maintain dominance of the main root over lateral root growth and also the apical dominance of the main shoot over the growth of lateral buds and tillers. The herbicide 2,4-D can also yield auxin-like activity at low concentrations[64]. Auxins can delay leaf senescence, but at high concentrations auxins increase ethylene production. Ethylene then triggers leaf and early fruit drop (abscission) and inhibits stem growth. Ethylene can also promote femaleness in some dioecious plants, i.e. plants where female and male flowers occur on separate plants[18].

Ethylene is a gaseous plant hormone, one that is often associated with plant responses to biotic and abiotic stresses[1]. As noted above, ethylene production can be promoted by auxin. Ethylene is also involved in lateral and adventitious root development[42], and red- and far-red light-mediated shoot development[37,41]. Ethylene also promotes ripening of many fruits[15].

Cereal crops
Cereal grain production was significantly lower prior to the "Green Revolution", when varieties of wheat and rice plants with dwarf shoot phenotypes were introduced, and became a crucial component of increased grain yield[63], including facilitating increased crop fertilizer (N) inputs. These dwarfed wheat varieties had altered GA biosynthesis (an example is shown in Fig. 3) or a modified sensitivity to endogenous GA (See Fig. 4). Research with transgenic lines of wheat has confirmed that plants with reduced levels of growth-active GAs have shorter stems, thereby reducing lodging, which also improved grain yield[53,62]. Manipulation of endogenous GA biosynthesis through the use of retardant PGRs, (primarily in Western Europe) and the utilization of 'dwarfing' mutants (often used in the Prairie provinces) have become common tools for increasing seed and grain yield in commercially important cereals[63].

Figure 3. Courtesy of Professor Peter Hedden, Rothamsted Research, Harpenden, Herts, UK. The wheat lines with reduced shoot length were obtained by Professor Hedden by increasing the expression of a gene (*PcGA2ox1*), that codes for a class of enzymes called GA-2-oxidases. The GA-2 oxidases are enzymes that deactivate the growth-promotive GAs in wheat[4].
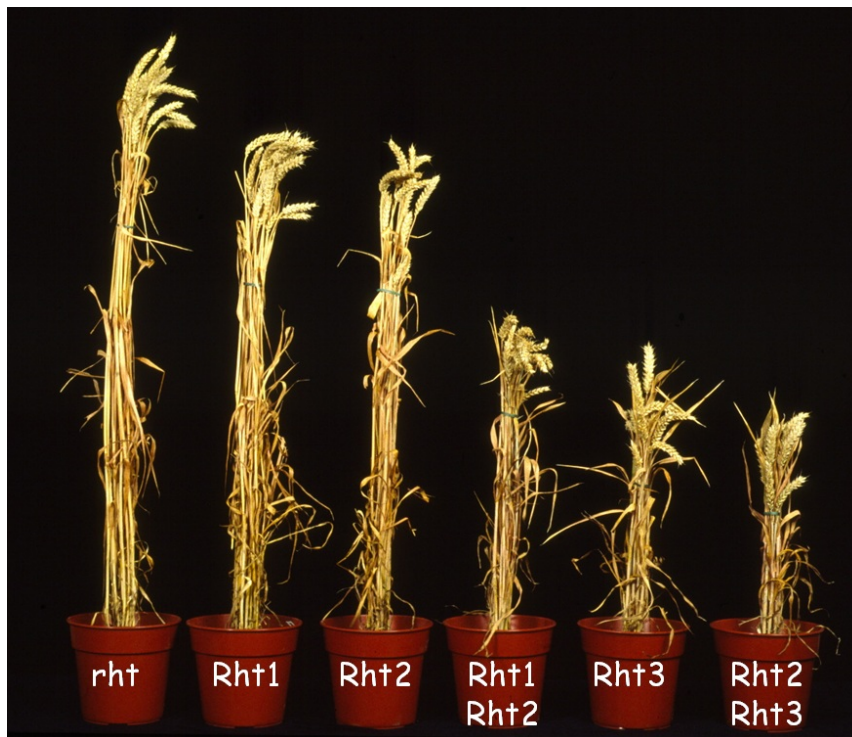

Figure 4. Courtesy of Professor Peter Hedden, Rothamsted Research, Harpenden, Herts, UK. The shorter plants (with varying degrees of dwarfing) are "Reduced Height" (*Rht1*, *Rht2*, *Rht3* etc.) mutations, genotypes which have reduced sensitivity to the growth-promotive GAs that are produced by the plant[53].

Examples of a GA-deficient shoot dwarfing that was caused by repeated inbreeding in maize are shown in Figs. 5A & 5B. Here, the dwarf phenotype could be partially "cured" by application of GA_3, or by cross-breeding with other inbred maize parents, which then yielded a high frequency of hybrid lines exhibiting heterosis (hybrid vigour)[59].
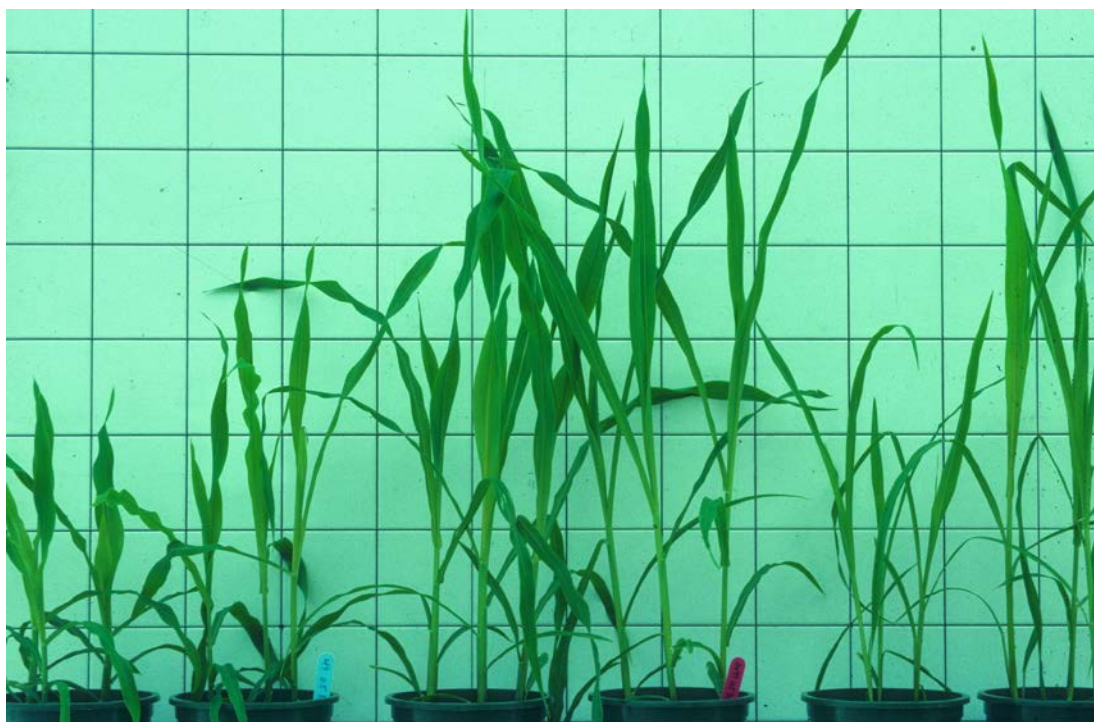

Figure 5A. Courtesy of Professor Stewart B. Rood, Biological Sciences Department, U. Lethbridge. Two maize genotypes which are exhibiting a dwarf phenotype due to 'inbreeding depression'. Seedlings of one inbred parent (CM49 - 'Canada Mordan' (MB)) are planted in the 2 pots on the left, and seedlings of the other inbred parent (CM7) are in the 2 pots on the right. Seedlings of their hybrid progeny (CM7 [male] x CM49), which exhibit heterosis (hybrid vigour) for height and shoot biomass are shown in the middle two pots. Grain yield of the hybrid can be 3-fold greater than either parental inbred. Plants were treated with 0.5 milligrams (mg) of GA_3 (2nd, 4th, and 6th pot from the left) and compared to non-treated plants (1st, 3rd and 5th pot from the left). NOTE that each (dwarfed) inbred parental genotype shows a good visual response to the applied GA_3. In contrast, the hybrid genotype plants show only a slight response in internode length, although leaf blade growth is appreciably enhanced by the applied GA_3[58,59].

Figure 5B. Courtesy of Professor Stewart B. Rood, Biological Sciences Department, U. Lethbridge. A maize genotype (CM49) is shown on the left. It exhibits a dwarf phenotype, due to inbreeding depression. Another seedling of the CM49 genotype is shown on the right. It has been treated throughout the summer (in Lethbridge, Alberta) with a solution of GA_3. Both plants are the same age. Note that the dwarf phenotype of the control (no GA_3) inbred CM49 genotype has been changed by the GA_3 treatment to a tall phenotype, one very similar to that seen for a hybrid maize genotype exhibiting heterosis. There were problems, though, with tassel and cob development, and thus grain yield by the +GA_3-treated CM49 plant. Subsequent research demonstrated that dwarfing caused by inbreeding depression is due to a deficiency in endogenous growth-active GA_1[59].

Plant growth retarding chemicals can be applied to control lodging of tall wheat genotypes, but their effect can be both dose- and development stage-dependant[23]. The most common inhibitors of GA biosynthesis are CCC (commercially available in western Europe as Cycocel), trinexapac-ethyl (PrimoMaxx or Moddus) and paclobutrazol (Cultar). Cycocel and trinexapac-ethyl are used on cereal grains and are usually applied at early vegetative growth stages in order to give increased grain yield, and they accomplish this primarily by reducing lodging caused by heavy rain or hail.

Later applications of the growth retardants – during the reproductive phase of the cereal plant's development, can reduce grain yield. The long history of growth-retarding PGR use in western Europe reinforces the concept that optimal levels of endogenous GAs are important for maximal shoot growth and also for development of the seed in cereal grain species. In fact, a foliar spray of GA_3 [100 ppm at 3 mL, (300 micrograms) per plant] to the foliage of wheat plants (at spike initiation) significantly increased grain yield, by ca. 5 to 10%[8].

As noted above, grain yield and quality losses for cereal crops, especially barley and oats, often occur as a result of lodging from heavy rains and hail. Foliar application of ethephon (a compound which produces ethylene when applied at a physiological (acidic) pH) can reduce lodging, although studies have produced conflicting results[19]. Foliar application of ethephon at a rate of 0.55 kg per hectare (ha^{-1}) decreased stem growth and thus controlled plant height and lodging, while increasing the yield of a tall 'weak-stemmed' cultivar of barley (*Hordeum vulgare* L.). A slightly higher concentration of ethephon (0.84 kg ha^{-1}) also significantly inhibited stem elongation in a semi-dwarf variety of wheat, but there was no associated increase in per hectare grain yield[19]. Application of ethephon at 0.3 and 0.6 kg ha^{-1} to commercial cultivars of spring barley in a two year field trial showed that tillering and yield can be promoted by ethephon treatment, although

the response depends on the cultivar, dose rate and time of application[26,27].

Another larger scale study on the effects of ethylene and ethephon on individual plant grain yield was performed in greenhouse trials on agriculturally important crops in Western Canada. These included barley and wheat plants[5]. Plants were treated with either ethylene (as a gas) or ethephon sprays (200 litres per hectare at a 40 milli-Molar [mM] concentration) on to the plants at one of 7 different growth stages. Ethylene gas and ethephon sprays yielded similar effects. Application of ethephon during early vegetative stages inhibited plant growth, but had no effect on yield unless further ethephon applications were administered at later growth stages. Finally, a decrease in plant yield was observed for both barley and wheat when ethephon was applied at very late vegetative and early reproductive (boot swelling, spike emerging and early anthesis) stages[5].

Application of L- tryptophan, a biosynthesis precursor of auxin (indole-3-acetic acid; IAA) to fertile soil for field-grown wheat plants was reported to significantly increase shoot growth and grain yield[74]. This effect may have involved plant growth promoting rhizobacteria (PGPR) since these bacteria are capable of synthesizing IAA, possibly from the soil-applied L-tryptophan[45]. Also, by applying L-tryptophan a significant increase in an auxin-like compound (identification not definitive) produced by the PGPR was reported[6]. Increases in auxin-like compounds by PGPRs has also been reported to be responsible for increased shoot growth and seed yield in other crop plants, such as wheat, sorghum and corn[30,34,61,72,76]. Also, for cereal grain species inoculation with *Azospirillum* sp. caused increased grain yield and shoot dry matter[33,52,57]. In one field experiment simultaneous application of both *Azotobacter* and *L*-tryptophan (10^{-3} and 10^{-4} Molar solutions) to soil, which had been seeded with wheat, increased both grain and straw yields, and per 1000-grain weight, by 22%, 21%, and 6%, respectively, relative to yields for untreated controls[35]. Further experiments are required, though, to determine if these increases in yield are associated with an auxin-mediated mechanism.

Some PGPR organisms carry a gene that encodes for the ACC deaminase enzyme. Activity by ACC deaminase significantly reduces plant ethylene production by decreasing the endogenous levels of ethylene's immediate biosynthesis precursor, ACC (see p. 651[67]). Inoculation of field-grown wheat plants with an 'organic' fertilizer containing a PGPR (from *Pseudomonas* spp.) significantly promoted grain and biomass yield[7]. Further, an even greater effect on yield was evident for the inoculated wheat grown on a drought-stressed field[7]. Thus, inoculation with PGPRs containing this gene could possibly be used to promote yield in agricultural areas with limited soil moisture, or in fields subjected to other types of abiotic stresses. An example was shown for wheat plants growing under salt-stressed conditions in pots and inoculated with PGPRs (*Pseudomonas* and *Serratia* spp.). These PGPRs contained the gene encoding for ACC-deaminase and the wheat plants significantly increased their shoot height, root length, grain yield, per 100-grain weight and straw yield[73].

Non-legume Oilseed crops

One of the main issues facing a canola or sunflower plant in an agricultural field is competition from neighbouring plants for sunlight. Thus, in crop fields crowded with plants, the competition strategy involves reallocation of growth resources from the expanding leaf (area) to elongation growth (etiolation) of the stem. The outcomes of this competition for sunlight are taller (and often slimmer) plants with arrested leaf area development, a situation which is likely to cause reduced seed yield (Fig. 6). For example, shading of sunflower plants was reported to cause a reduction in pericarp weight, fruit volume and total yield per plant[43]. The elongation phenomenon seen with shaded plants is regulated by multiple plant hormones in both sunflower and canola seedlings. Sunflower plants which etiolate while being subjected to light competition have higher levels of endogenous GAs and auxin (IAA), while their ethylene production is reduced[37,38,39]. This implies that the etiolation phenomenon may be preventable by application of ethephon, or by application of growth retardants that inhibit GA biosynthesis. Stem etiolation is a shoot

morphology similar to that seen when auxin or GA_3 is applied to young seedlings (Fig. 7). The importance of GAs in regulating sunflower shoot morphology is confirmed by the high accumulation of *GA20ox* transcripts during active shoot growth[17]. *GA20ox* is a gene family that encodes an important group of enzymes in GA biosynthesis. Additionally, there are dwarf phenotypes of GA-insensitive sunflower mutants that have impaired downstream GA signaling[56].

However, other sunflower mutants exhibiting dwarfism can be GA-sensitive, i.e. their phenotype can be rescued by application of a growth-active GA – these mutants have been shown to be impaired in GA biosynthesis[25].

Figure 6. Seven-day old seedlings of sunflower grown under simulated natural light (left) and various levels of reduced light irradiance (middle and right).

Figure 7. Top: 5-day-old seedlings of sunflower treated with GA_3 (left). Untreated controls (right). Bottom, left: 7-day-old sunflower seedlings treated with IAA (right). Untreated control (left). Bottom, right: 14-day-old sunflower seedlings treated with ethephon (right). Untreated control (left).

Canola (cv. Westar) plants also show similar responses to light competition. Surprisingly, though, ethylene production is increased in etiolating plants. Thus, transgenic canola plants carrying the bacterial gene that

encodes for the ACC deaminase enzyme (which reduces ethylene production), exhibit a more robust shoot morphology when subjected to light competition stress (Fig. 8)[40]. Hence, ethylene effects in crop plants can be species-specific
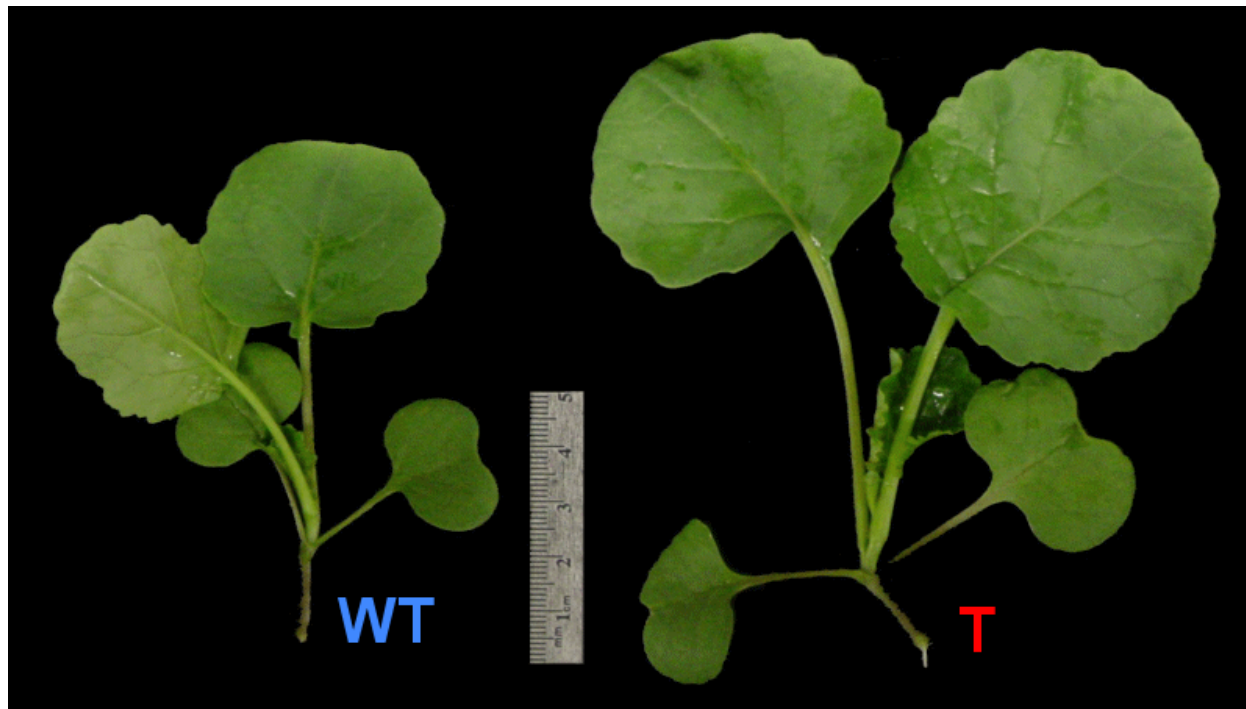

Figure 8. Canola 14-day-old wild type (WT, left) and ACC-deaminase transgenic (T, right) seedlings.

Inoculation of cv. Westar canola plants with bacteria that carry the gene that encodes for ACC deaminase was also successful in alleviating growth inhibitory effects caused by other abiotic stresses. Specifically, inoculation with *Pseudomonas tolaasii* ACC23 protected the plants against the toxic effects of cadmium (Cd^{2+}) and effectively maintained or promoted the growth of canola, relative to un-inoculated canola plants[20]. Other experiments[14] showed that inoculation with *Kluyvera ascorbata* SUD165 effectively maintained a normal phenotype for canola plants grown under high levels of nickel, lead and chromium (Ni^{2+}, Pb^{2+}, and CrO_4^{2-}. In a similar approach[65], inoculation with *Pseudomonas fluorescens* G10 and *Microbacterium* sp. G16 protected canola plants against the inhibitory effects of high concentrations of Pb^{2+}. In addition to alleviating abiotic stress responses, inoculation with bacteria that carry the gene encoding for ACC deaminase can also promote growth of un-stressed plants. For example, the priming of sunflower seeds with *Pseudomonas*

fluorescens UTPf76 resulted in a higher germination index, an increased germination percentage, increased germination rate and a higher vigor index, as well as more robust seedling growth. The latter included increases in stem and root elongation, number of lateral roots, and fresh and dry biomass accumulation[48].

In canola plants, the crop yield (both seed number and weight) is tightly regulated, not only by the availability of nutritional factors[68], but also by several plant hormones[11]. Seed number per silique is postulated to be dependent on endogenous IAA and growth-active GA1 and GA4 concentrations during the early stages of seed development[32,77]. Seed number per silique was also improved by applying a synthetic auxin, 4-chlorophenoxyacetic acid[49].

Ethylene also plays an important, dose-dependent role in early seed development and subsequent maturation in canola. An increase or decrease in ethylene production (relative to "optimum" ethylene production)

during ovary development causes a loss of yield via seed abortion[69]. Thus, transgenic canola plants carrying the gene encoding for ACC deaminase (which reduces ethylene production) exhibit significant decreases in all of silique length, seed number and seed weight, the latter being due to selective seed abortion (Figs. 9 and 10)[69]. Also of interest is the fact that these ACC deaminase transgenic plants had lower levels of both endogenous growth-active GAs and the auxin, IAA[69]. Hence, as for vegetative tissues, a certain "optimum" level of ethylene appears to be required for normal reproductive development.

Figure 9. 60-day-old siliques of wild type (WT), cv. Westar canola (the three siliques on the left) and of a transgenic canola which over expresses the gene that encodes for the ACC deaminase enzyme (T, three siliques on the right).
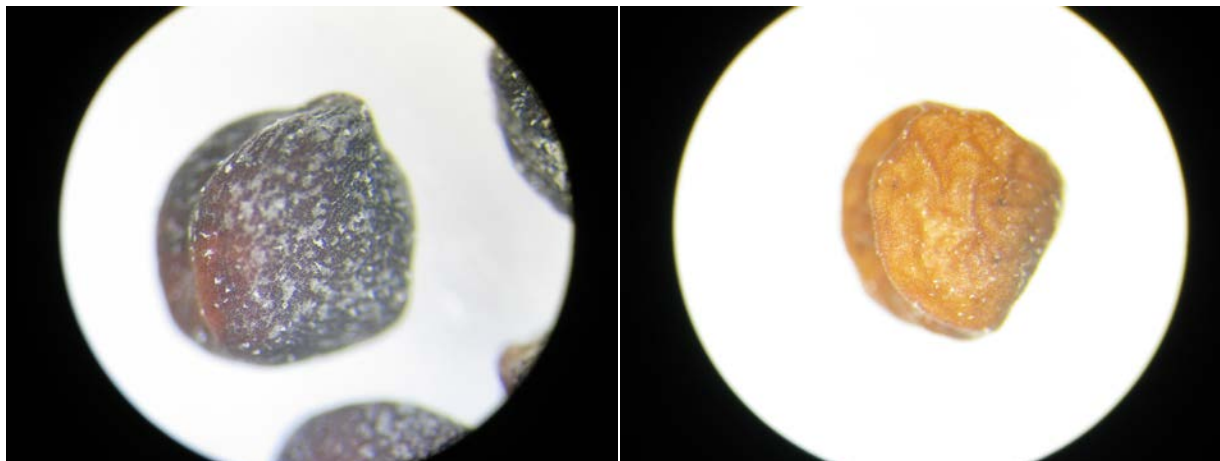
Figure 10. 50-day-old seeds of WT canola cv. Westar (left) and also for a transgenic canola which over expresses the gene that encodes for the ACC deaminase enzyme (T, right).

For sunflower plants, inoculation with various PGPRs, which can either produce hormones *per se*, or induce the plant to produce hormones, significantly increased the height, leaf area index, biological yield, 1000-seed weight, number of seeds, seed head diameter and harvest index[2]. However, the growth-stimulative effect of PGPR inoculation (as well as the effect of applied hormones) on plants is often temporary, as shown in the example of a *Bacillus subtilis* inoculation of sunflowers[44]. PGPRs appear to be naturally associated with sunflower plants – for example, 299 bacteria strains were recently isolated from sunflower plants in the field, with *Enterobacter* and *Burkholderia* being the predominant genera isolated from roots and the rhizosphere, respectively. The *Burkholderia* strains, at least, were correlated with the promotion of growth in the sunflower plants that was seen after innoculation[3].

Insect predation can also influence sunflower plant morphology by modulating endogenous auxin levels. For example, injecting the synthetic auxin, 2,4-D, simulates the phenotype seen when the sunflower midge (*Contarinia schulzi Gagne*) attacks the plant[12].

The use of a 2,4-D injection was even proposed as a screening test for sunflower genotype susceptibility to midge infestation[12].

Pulse and legume oil-seed crops
Application of GA_3 promotes cell elongation in pea stem internodes[13] and lentil epicotyls[51]. The field pea (*Pisum sativum* L.) cultivars that are typically grown in Western Canada have a semi-dwarf stem length. This

shorter stem length (as verified for the cultivar Carneval[9]) is a result of a mutation (*le-1*) that decreases the efficiency of the GA 3-oxidase enzyme that produces growth-active endogenous GA_1 (Fig. 11)[46]. The presence of the mutation results in lower levels of GA_1 within the internode, yielding a plant with shorter internodes and a reduced total stem length[60]. Foliar application of GA_3 markedly increases the length and number of internodes formed in semi-dwarf pea plants[66]. Finally, application of GA biosynthesis inhibitors can consistently reduce stem length in pea[10,55].

Figure 11. Field pea (*Pisum sativum* L.) containing the *LE* gene (right two plants) or the *le-1* (left three plants) mutation that inhibits GA1 biosynthesis and thereby results in shorter internodes.

The semi-leafless trait called *afila* or *af* (plants have stipule leaves that surround the main stem, but lack leaflets on the tendril petioles, Fig. 12) is also common in field pea (*Pisum sativum* L.) cultivars grown in Western Canada. This is a popular field trait, as the tendrils are more pronounced as the result of the *afila* mutation, and the intertwining of the tendrils between plants acts to keep the stems upright as they grow, facilitating machine harvesting of the crop. The development of pea leaves and tendrils is also tightly regulated by plant hormones. During the development of leaves and tendrils, which originate from the apical bud, leaflets form in locations with low auxin levels and tendrils form in regions of high auxin, both within the apical bud primordia. It is hypothesized that the *af* mutation affects the auxin gradient in the primordial apical bud tissue, such that tendrils are formed instead of leaflets[21].

Figure 12. Field pea (*Pisum sativum* L.) containing the *AF* gene (left) or the *af* mutation that affects the auxin gradient in the primordial apical bud tissue. Tendrils are thus formed instead of leaflets.

The ability of ethylene to control pea stem elongation is well elucidated[22]. For example, the physical resistance encountered by a growing seedling pushing against the soil surface crust as it attempts to emerge from the soil, promotes ethylene biosynthesis[28]. In pea seedlings, ethylene inhibits cell elongation and promotes cell lateral expansion in the stem tissue, making the stems shorter and thicker, thereby enabling the elongating shoot to more effectively push through the mechanically restrictive soil surface layer[16,28,50].

Ethylene production from a 20 square metre stand of soybean (*Glycine max*) plants was monitored during the vegetative and reproductive phases of development under non-stress conditions in an atmospherically closed growth chamber[70]. Ethylene production from the soybean stand gradually increased during the vegetative growth phase, then declined during pod fill. Ethylene can also be involved in abiotic stress responses in plants. However, ethylene's role in abiotic stress responses varies with the stress and how the stress is applied. In soybean plants, gradual development of drought stress did not promote

ethylene production or increase levels of ACC, an important precursor of ethylene. In contrast, a rapid development of drought stress increased levels of both compounds[71]. Dry air that promotes leaf membrane damage can also increase ethylene production and raise levels of ACC, the precursor of ethylene[71]. Drought stress is often accompanied by high temperatures, which can also promote ethylene production, within certain limits (up to ca. 35oC), after which ethylene production is inhibited[1].

The isolation of PGPR microorganisms from pulse crops (pea, lentil and chickpea) in Western Canada revealed that about 5% carried a gene encoding for the ACC-deaminase enzyme and 7% of these PGPRs were capable of synthesising "indoles" (probably IAA). However, only the PGPR strains carrying the gene encoding for ACC-deaminase (see above, in Cereals section, for a description of the gene product) were able to promote the root elongation (of canola) *in vitro*[31]. Co-inoculation of a field-grown lentil crop with PGPR strains carrying the gene encoding for

ACC-deaminase, together with N2-fixing *Rhizobium leguminosarum*, proved to be a very effective methodology for increasing N assimilation and crop yield of the lentils, i.e. the number of pods, nodules per plant, nodule dry weight, seed yield and dry plant biomass were all increased[75]. A similar approach was also effective in enhancing nodule number and total plant biomass for both lentil and pea grown under environmentally controlled conditions[47]. Also, application of ACC, an ethylene precursor, at very low doses promoted seed yield and number of pods per plant for field-grown lentils[76]. Thus, as was shown for the canola that were transgenic for ACC deaminase[69], an 'optimal' level of ethylene production is required for maximal seed yield, but deviations from that optimum can cause decreases in yield.

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