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The Control of Apical Bud Growth and Senescence by Auxin and Gibberellin in Genetic Lines of Peas[1,2]

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Pea (*Pisum sativum* L.) lines G2 (dwarf) and NGB1769 (tall) (*Sn Hr*) produce flowers and fruit under long (LD) or short (SD) days, but senesce only under LD. Endogenous gibberellin (GA) levels were inversely correlated with photoperiod (over 9–18 h) and senescence: GA_{20} was 3-fold and GA_1 was 10- to 11-fold higher in flowering SD G2 shoots, and the vegetative tissues within the SD apical bud contained 4-fold higher levels of GA_{20}, as compared with the LD tissues. Prefloral G2 plants under both photoperiods had GA_1 and GA_{20} levels similar to the flowering plants under LD. Levels of indole-3-acetic acid (IAA) were similar in G2 shoots in LD or SD; SD apical bud vegetative tissues had a slightly higher IAA content. Young floral buds from LD plants had twice as much IAA as under SD. In NGB1769 shoots GA_1 decreased after flower initiation only under LD, which correlated with the decreased growth potential. We suggest that the higher GA_1 content of G2 and NGB1769 plants under SD conditions is responsible for the extended vegetative growth and continued meristematic activity in the shoot apex. This and the increased IAA level of LD floral buds may play a role in the regulation of nutrient partitioning, since more photosynthate partitions to reproductive tissue under LD conditions, and the rate of reproductive development in LD peas is faster than under SD.

Under LD conditions fruit development in G2 pea plants results in apical senescence, but under SD conditions apical growth continues, despite the presence of a large number of fruit. This is dependent on the presence of dominant alleles at the *Sn* and *Hr* genetic loci. The photoperiodic response of G2 plants is nonphotosynthetic and quantitative, such that a brief exposure of light during the night does not reverse its SD growth habit (Proebsting et al., 1976). The growth rate of developing seeds on G2 plants is more rapid under LD than under SD conditions, which is indicative of a greater nutrient drain exerted on LD plants by their reproductive structures. However, when some of the flowers on LD-grown plants were removed so that the total increase in seed weight on LD-grown plants was the same as or even lower than SD-grown plants, LD G2 plants still senesced, whereas plants under SD conditions did not (Gianfagna and Davies, 1981). Thus, nutrient demand of seeds alone cannot account for the induction of senescence.

Exogenous GAs can delay apical senescence of pea plants (Davies et al., 1977; Proebsting et al., 1978). Among applied plant hormones, including BA, NAA, GA_3, GA_{20}, and GA_9, only GA_3 delayed the senescence of LD-grown G2 peas indefinitely, whereas GA_{20} had a moderate effect. When the application of GA_3 was deferred until the plants had produced a full complement of fruits and were nearly senescent, the plants resumed active growth. Application of GA_3 also caused extensive flower abortion as well as increased stem length. Proebsting et al. (1978), using the lettuce hypocotyl bioassay, found that SD-grown G2 pea shoots contained a relatively large amount of a polar GA (GA_E) when compared with those of LD-grown G2 plants. The appearance of this GA_E was correlated with the continued, vigorous growth of SD-grown G2 peas. In leaves of photoperiod-insensitive lines (I types), short photoperiods increased neither the GA_E content nor the growth potential to any appreciable extent. Since this GA_E co-chromatographed with authentic [^{3}H]GA_1 and was biologically active, they suggested that GA_E might be GA_1 and that the presence of an increased amount of this GA was responsible for the extended growth potential of SD-grown G2 pea. Lockhart and Gottschall (1961) also noted the fact that GA_3 delayed apical senescence in Alaska peas, but they concluded that the effect was indirect. Ecklund and Moore (1968) confirmed the senescence-delaying effect of GA_3 on the apical bud of pea plants and were able to show that AMO-1618, an inhibitor of GA biosynthesis, accelerated senescence in Alaska peas.

The involvement of IAA in the processes of monocarpic senescence has received less attention. Nonetheless, Nooden et al. (1979) found that foliar applications of NAA, together with BA, prevented seed-induced monocarpic senescence in soybeans. The auxin content of soybean leaves was found to decline with leaf age and was higher in depodded soybean plants, compared with intact ones (Hein et al., 1984). Woolhouse (1983) proposed that the auxin content of the plant decreases following the conversion of all of the vegetative apices to inflorescences, leading to a reduction of cytokinin synthesis in the root. To the contrary, Davies et al. (1986b) reported that there was no apparent decline in the levels of cytokinins that were measured at the commencement of

[1] This paper is dedicated to the memory of Dr. G.A. Marx of the New York State Agricultural Experiment Station at Geneva, NY, who originally provided us with the G2 seed and contributed much enthusiasm to these investigations. Dr. Marx died on November 13, 1988, while still active in pea genetics.

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Abbreviations: LD, long day(s); SD, short day(s).

flowering in G2 peas, despite the fact that root growth slowed down at that time. In addition, the levels of endogenous cytokinins from LD-grown G2 shoots were similar to those of SD-grown G2 shoots.

To further determine the role of hormones in the senescence of the apical bud of peas, we have examined the levels of GA_1, GA_{20}, and IAA in the shoots of G2 and NGB1769 peas under different photoperiodic conditions by GC-MS. These two genetic lines possess dominant *Sn* and *Hr* alleles, which render the plants photoperiodically sensitive with respect to senescence. They differ in that G2 is dwarf (*le*) whereas NGB1769 is tall (*Le*). The levels of GA_{20} and IAA were also determined separately in very young flower buds and vegetative tissue from within the apical bud of G2 plants. Since G2 peas can grow for a progressively longer time under photoperiods of decreasing length, we also examined the effect of varying photoperiods on the GA_1 levels.

MATERIALS AND METHODS

Labeled Compounds

[1,2-^{3}H]GA_1 (37.7 Ci/mmol) was purchased from Amersham. [1,2,3-^{3}H]GA_{20} (0.43 Ci/mmol) was a gift from Dr. J.B. Reid (University of Tasmania, Hobart, Australia) (and originated from Dr. J. MacMillan, University of Bristol, UK). [17-$^2H_2$]GA_1 and [17-$^2H_2$]GA_{20} were gifts from Dr. R.P. Pharis (University of Calgary, Alberta, Canada), which were synthesized by Dr. L. Mander (Canberra, Australia). [$^{13}C_6$]IAA (ring-labeled) was purchased from Cambridge Isotope Laboratories (Woburn, MA). [1-(side chain)-^{14}C]IAA (specific activity 55 mCi/mmol) was purchased from Amersham.

Plant Material

Both G2 (*Sn, Hr,* and *le*) (Marx, 1968; Proebsting et al., 1976) and NGB1769 (*Sn, Hr,* and *Le*) lines of pea (*Pisum sativum* L.) were grown in 4-L plastic pots in a peat/vermiculite medium and maintained at either 9-h (SD) or 18-h (LD) photoperiods (average light intensity 300 μmol m^{-2} s^{-1}) in growth chambers under F72 T12 cool-white fluorescent lamps (Sylvania) supplemented with light from incandescent lamps. Plants were watered and fertilized twice a day and lateral shoots were routinely trimmed. Temperatures in the growth chambers were maintained at 19°C during the light period and 17°C during the dark period. Prefloral stage peas (G2 or NGB1769) were plants that had been grown in either SD or LD for 3 weeks following germination and had developed to node 4 or 5 (counting the cotyledon as node 0). Postfloral stage G2 peas were plants with 4 to 5 reproductive nodes (15–16 total nodes) and were still growing vigorously at the time of harvest. Since NGB1769 plants usually produced more than 30 reproductive nodes before the onset of senescence under LD conditions, selected postfloral stage NGB1769 plants possessed 10 to 15 reproductive nodes.

The first reproductive node was the node at which a flower bud was initiated, regardless of whether it had aborted. GA_{20}, GA_1, and IAA were extracted from shoot tips, including the apical bud down to and including the first, fully expanded leaf, or from the tissues within the apical bud. The young flower buds and vegetative tissue from within the apical bud that were used in this experiment were dissected from unfolded apical buds (each weighing about 200 mg), which included two or three pairs of developing leaves, prior to visible symptoms of apical senescence. The flower buds were removed using forceps under a binocular microscope. A single LD apical bud contained only 3 to 4 flower buds, whereas the same size SD apical bud contained 7 to 8 flower buds, although the flower buds from LD-grown plants were usually 2 to 3 times bigger than the flower buds from SD-grown plants (Kelly and Davies, 1986). On average 50 to 60 flower buds from 15 LD plants weighed 1.1 to 1.3 g (approximately 22 mg/bud), whereas 100 to 120 flower buds collected from 15 SD plants weighed only about 0.9 to 1.1 g (approximately 9 mg/bud). The vegetative tissue (60–70 mg/apex) consisted of the remainder of the apical bud, from which flower buds had been removed, excluding the lowest pair of stipules enfolding the apical bud and also the internode above the enfolding stipules.

Application of Plant Hormones

GA_3 and NAA (both from Sigma) and GA_1 (a gift from G. Elson, ICI, Jeallotts Hill, UK) were separately dissolved in a small amount of ethanol, and these solutions were diluted to less than 1% ethanol with water containing 0.5% Tween 20. The final concentration of the solutions was 10 μg/mL. Either 100 μL (1 μg) or 10 μL (0.1 μg) was applied three times weekly directly to the shoot apex.

GA and IAA Extraction and Purification

GA extraction and purification, HPLC conditions, and GC-MS were as described by Zhu et al. (1988). Purification and HPLC were carried out using [^{3}H]GA_{20} and [^{3}H]GA_1 (added at the rate of 50 nCi [38 ng] and 0.3 μCi [2.8 ng], respectively), as radiomarkers. Radioactive GA_{20} was added to pea shoot tip extracts (20 g of tissue or more) but not to the extracts of young flower buds and vegetative tissue from within the apical buds (1–2 g of tissue) because of the relatively low specific activity of the [^{3}H]GA_{20}. Quantitation was achieved by adding [^{2}H]GA_{20} and [^{2}H]GA_1 to each sample at the rate of 25 and 5 ng/g fresh weight, respectively. Spectra of GAs, with two or sometimes three isotopes (^{1}H, ^{2}H, and ^{3}H), were recorded by GC-MS using the scan mode. The GAs were quantitated from the ratio of the areas of the ion current peak for the M^+ ions for GA_{20} (m/z 418 and 420) and GA_1 (m/z 506 and 508).

IAA was extracted and purified in the same way as for GAs, except that plant material was ground in liquid N_2 using a mortar and pestle and subsequent extraction was done in boiling 80% aqueous methanol to prevent IAA degradation. 1-[^{14}C]IAA (10 nCi, 17 ng) was added to each extraction as a tracer. [$^{13}C_6$]IAA (Cambridge Isotope Labs) was added as an internal standard for GC-MS quantitation (Cohen et al., 1986) at 60 ng/g tissue for flower buds and vegetative tissue from within the apical bud and at 20 ng/g tissue for whole-shoot extractions. Methylated IAA was

injected into the GC-MS using the same temperature program as for GAs. IAA was quantitated from the area ratio of the m/z peaks 130 and 136. The calculated amount of [^{12}C]IAA that was detected was reduced by the amount of [^{14}C]IAA that was added.

RESULTS

Photoperiod and Growth Response in G2 and NGB1769 Plants

The number of reproductive nodes produced by G2 peas before the senescence of their apices was determined largely by the photoperiod (Fig. 1). Under the photoperiodic regimes of 18, 15, or 12 h, G2 peas produced 10 to 13 reproductive nodes in about 40 to 45 d, 25 to 30 reproductive nodes in about 80 to 90 d, and 90 to 100 reproductive nodes in 8 to 9 months, respectively, before the cessation of growth and senescence of the apical bud. Apical growth was indefinite under a 9-h photoperiod. In all cases, the criteria for senescence of the shoot apex were a decrease in the size of the apical bud combined with the cessation of growth and a visible loss of the green color. Once the apex has died, further growth cannot occur, yet a resumption of growth may occur at any phase of senescence prior to the death of the apical bud if the photoperiod is reduced to 9 h or the fruit are removed (Proebsting et al., 1976).

NGB1769 plants displayed approximately the same growth response as G2 under either LD or SD conditions, except that this line of peas grows for a much longer time than G2 under LD conditions. They produce 30 to 35 reproductive nodes before the senescence of the apical bud under 18-h photoperiods.

The internodes of G2 peas grown under either LD or SD conditions reached a maximum length of 3 to 4 cm soon after anthesis, and a gradual reduction in internode length was seen only under LD conditions, 4 to 5 reproductive nodes after flower initiation (Fig. 2). The internodes of NGB1769 plants are usually 12 to 14 cm long in either LD or SD conditions at the time of anthesis of the first flowers. After forming 10 to 15 reproductive nodes the LD-grown plants began producing progressively shorter internodes (Fig. 2).

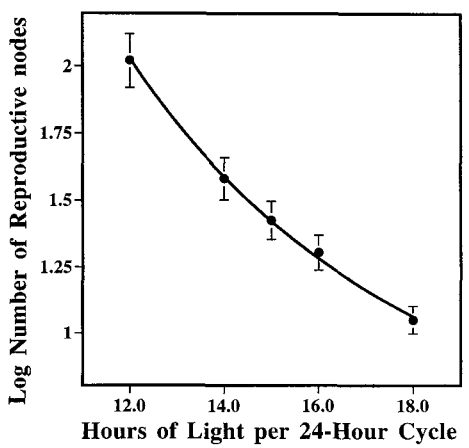

Figure 1. Photoperiod-dependent production of reproductive nodes by G2 peas before the senescence of the apex.

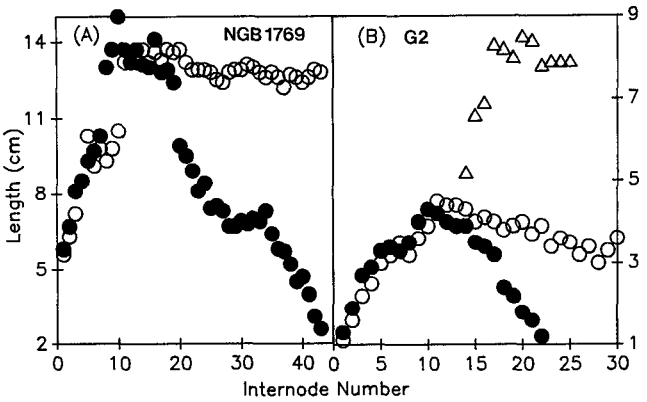

Figure 2. Internode length of pea plants. A, NGB1769 (n = 4, SE = ±0.3-0.7 cm); B, G2 (n = 4, SE = ±0.1-0.4 cm). ○, SD; ●, LD; △, LD G2 treated with 1 μg/plant GA_3 (twice weekly) starting at node 13.

Effects of Exogenous Hormones on G2 Peas

When GA_3 or GA_1 was applied directly to the apices of LD-grown G2 plants 2 weeks after flower initiation (1 μg/plant, twice a week), the internodes of these plants became substantially elongated (Fig. 2) and either 1 or 0.1 μg/plant substantially delayed the senescence of the plants (in excess of 40 reproductive nodes), whereas NAA had no significant effect. This confirms earlier results using only GA_3 (Proebsting et al., 1978). Moreover, when GA_3 and GA_1 were applied to G2 peas when they were nearly completely senescent, active growth resumed. GA_3 was slightly more effective in causing the resumption of vigorous vegetative growth and stem elongation than GA_1. Thus, the effects of GA_3 and GA_1 resembled the effect of SD growth conditions in terms of apical senescence of G2 peas, although the phenotype of an LD-grown G2 plant that was treated with GAs was different from an SD-grown G2 plant. The GA-treated, LD-grown plant lacked the robustness seen in SD-grown G2 plants and displayed extensive stem as well as peduncle elongation.

The Levels of GAs and IAA in Shoots and Apical Tissues in Relation to Apical Growth and Senescence

G2 shoots (including the apical bud down to the first fully expanded leaves) from SD growth conditions contained higher GA_{20} and GA_1 levels than those of LD-grown plant tissue (Tables I and II). The GA levels in LD shoots were analyzed while the plants were still vigorous and not displaying any visible signs of senescence. The level of GA_{20} in SD G2 shoots was 39.6 ng/g fresh weight, while it was only 13.5 ng/g fresh weight in LD shoots. The differences in the GA_{20} levels were significant only some time after the initiation of reproductive growth, since both LD- and SD-grown prefloral G2 seedlings contained very similar amounts of this GA (Table I). Because of the presence of the *le* locus, the GA_1 content of G2 seedlings was very low (0.3–0.4 ng/g fresh weight), regardless of the photoperiod (Table II). However, the level of GA_1 increased substan-

Table I. *GA_{20} content of shoot tips of G2 pea plants*

Shoot tips included tissues down to and including the first fully expanded leaf. Prefloral shoots were from plants 3 weeks after germination and postfloral plants were at the 4 to 5 reproductive node stage. Vegetative tissues and floral buds were dissected from within the apical buds of 15 postfloral stage pea plants; $n = 3$.

Growth Condition	Growth Stage	Shoot Tip	GA_{20} Content within the Apical Buds	
			Vegetative tissue	Floral buds
		ng/g fresh wt ± SE		
SD	Prefloral	15.2 ± 0.8	–[a]	–
LD	Prefloral	14.6 ± 0.6	–	–
SD	Postfloral	39.6 ± 1.7	41.3 ± 0.8	15.0 ± 1.2
LD	Postfloral	13.5 ± 0.6	11.1 ± 2.0	15.3 ± 0.9

[a] –, Not done.

tially after the initiation of reproductive growth. Two or three weeks following the opening of the first flower, the GA_1 content of SD-grown G2 plants increased more than 10-fold to 3.4 ng/g fresh weight, whereas the level in LD-grown plants at the same stage remained at the same level as in the G2 seedlings (Table II).

GA_1 levels in SD-grown postfloral shoots of NGB1769 peas were higher than those of LD-grown shoots at the same growth stage (9.2 versus 1.7 ng/g fresh weight, respectively; Table II). As in G2, the GA_1 content was similar in prefloral SD- or LD-grown NGB1769 plants (about 8 ng/g fresh weight), but, unlike G2, was similar to postfloral SD NGB1769 shoots. The level of GA_1 that was present in LD-grown shoots decreased very quickly following the onset of reproductive growth and reached 1.7 ng/g fresh weight after the production of 10 to 15 reproductive nodes, coincident with the start of the decrease in internode length and somewhat prior to the senescence of the apical bud (Table II; Fig. 2).

The IAA content of SD- or LD-grown G2 pea shoots at the above postfloral stage was very similar (about 14 ng/g fresh weight, Table III).

To further elucidate the hormonal levels and distribution patterns under either SD or LD growth conditions, flower buds and vegetative tissue from within the unfolded apical buds at the postfloral stage were analyzed separately. The levels of GA_{20} in floral buds did not vary between SD and LD conditions (15 ng/g fresh weight in both cases), whereas that of vegetative tissue from within the apical bud of SD-grown G2 plants contained a 3- to 4-fold higher level of GA_{20} than the same tissue from LD-grown plants (41.3 versus 11.1 ng/g fresh weight, respectively). In contrast, the vegetative tissue from within the apical bud of SD-grown G2 pea contained only a slightly higher amount of IAA when compared with that of LD-grown plant tissue (53.5 ng/g fresh weight in SD versus 42.7 ng/g fresh weight in LD; Table III). However, the IAA level of floral buds from LD-grown plants was twice that of SD floral buds (22.4 ng/g fresh weight in SD versus 43.3 ng/g fresh weight in LD; Table III). Because the flower buds in LD were more than twice the size of SD flower buds (22 versus 10 mg), the total amount of IAA per bud was at least 4-fold higher in LD conditions. The total concentration of IAA that was present in the apical tissue was severalfold higher than that of the whole shoots, whereas the concentrations of GA_{20} did not vary as much in different tissues (Tables I and III); SD-grown G2 vegetative tissue from within the apical bud contained 41.3 ng/g fresh weight GA_{20}, whereas whole shoots contained 39.6 ng/g fresh weight. Endogenous GA_1 levels in different parts of the unfolded apical bud were not determined because of the minute amount of tissue available and the fact that the levels of GA_1 in the whole shoots were very low under any environmental conditions.

The increased growth potential of G2 peas resulting from a gradually shortened photoperiod was reflected by their endogenous GA_1 content. Under an 18-h photoperiod, G2 pea shoots contained a very low amount of endogenous GA_1 (0.2–0.3 ng/g fresh weight) at all growth stages and became completely senescent after another 10 to 13 reproductive nodes (Figs. 1 and 3). Under a 15-h photoperiod the endogenous GA_1 level increased significantly to about 0.6 to 0.7 ng/g fresh weight upon the initiation of reproductive growth, and these plants did not senesce until 25 to 30 reproductive nodes were produced. Under a 9-h photoperiod the endogenous GA_1 level was 10 to 12 times that of 18-h-grown plants at a 4 or 5 reproductive node stage (Fig. 3).

The amount of IAA increased significantly from 14.1 ng/g fresh weight in LD shoots to 37.2 ng/g fresh weight in GA_3-treated LD shoots (Table III). The increase in the

Table II. *GA_1 content of G2 and NGB1769 pea shoot tips under long or short photoperiods*

Plant tissues were harvested at the same stage as in Table I, except postfloral NGB1769 plants were harvested at 10 to 15 reproductive nodes; $n = 3$ for G2 plants, $n = 2$ for NGB1769 plants.

Genetic Line	Growth Condition	Growth Stage	GA_1 Content
			ng/g fresh wt ± SE
G2	SD	Prefloral	0.4 ± 0.1
	LD	Prefloral	0.3 ± 0.1
	SD	Postfloral	3.4 ± 0.3
	LD	Postfloral	0.3 ± 0.1
NGB1769	SD	Prefloral	8.2 ± 0.3
	LD	Prefloral	7.6 ± 0.2
	SD	Postfloral	9.2 ± 0.4
	LD	Postfloral	1.7 ± 0.7

Table III. *IAA content of shoot tips of postfloral G2 pea plants*

Postfloral plants and apical bud tissues were used as per Table I; GA_3 as per Figure 2; $n = 4$.

Growth Condition	Shoot Tip	IAA Content within the Apical Buds		Amount per Floral Bud
		Vegetative tissue	Floral buds	
	ng/g fresh wt ± SE			*ng/bud*
SD	14.7 ± 0.5	53.5 ± 1.4	22.4 ± 0.5	0.20
LD	14.1 ± 0.5	42.7 ± 1.5	43.3 ± 1.1	0.95
LD + GA_3	37.2 ± 0.9	–[a]	–	–

[a] –, Not done.

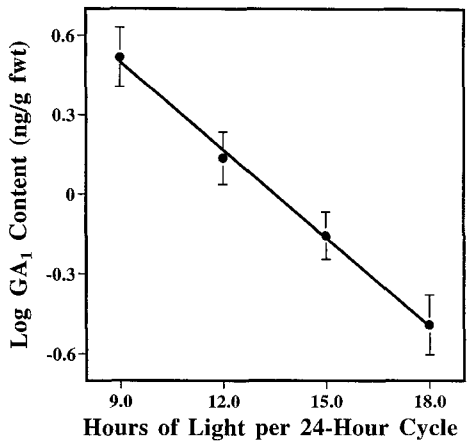

Figure 3. Photoperiod-dependent level of GA_1 in shoots of G2 peas. fwt, Fresh weight.

levels of IAA following GA_3 treatment correlated with an increase in the stem length (Fig. 2).

DISCUSSION

The maintenance of a higher concentration of GA_{20} and GA_1 in the shoots and of GA_{20} in the vegetative tissues of the apical bud correlated with the SD-induced prevention of senescence in G2 and NGB1769 peas. Prior to LD-induced senescence there was a decline of GAs in the vegetative tissue, combined with an increase in IAA in the more rapidly growing flower buds. We suggest that these hormonal changes directly or indirectly regulate the continued growth or senescence of the apical bud of the pea plant.

In the vegetative tissues within the apical bud we noted an inverse correlation between GA_{20} and apical senescence. Even though GA_1 is regarded as the prime GA growth regulator, Ross et al. (1992) noted a correlation between the level of GA_{20} and internode length within a genotype, although not across genotypes. This is most likely because the level of GA_1 is in part regulated by the activity of GA 20-oxidase (Hedden and Croker, 1992), which catalyzes three reactions that lead to the production of GA_{20}. Thus, the level of GA_1 may be linked to the level of its precursor, GA_{20}, when the level of GA_{20}-3β-hydroxylase remains constant. The level of GA_{20} in apical tissues of G2, therefore, probably reflects the level of GA_1 in these tissues.

Hormones and Senescence in Peas

The delay of apical senescence of G2 peas has been shown to be correlated with the presence of a graft-transmissible factor (Davies et al., 1977; Proebsting et al., 1977). Proebsting et al. (1977) suggested that this factor, produced in large quantities only under SD conditions and in the presence of both *Sn* and *Hr* loci, counteracted the effects of the senescence stimulus. Murfet and Reid (Murfet, 1971, 1973; Murfet and Reid, 1973) have also presented evidence that the *Sn* and *Hr* alleles result in the production of a hormone under SD conditions that favors vegetative growth in peas. Application to GA_3 or GA_1 to LD-grown G2 peas results in active vegetative growth as well as extensive flower abortion. Under SD, application of microgram quantities of GA_3 or GA_1 to G2 peas sporadically causes a reversion to the vegetative state and subsequent abortion of all of the flowers.

Proebsting et al. (1978) proposed that the prevention of senescence in G2 peas under SD growth conditions occurred because the level of a biologically active, polar GA (GA_E), regulated by the presence of the dominant alleles *Sn* and *Hr*, is sufficiently high to overcome the senescence stimulus that is associated with flower and fruit development. In the present study we have shown that the hormone regulating the prevention of apical senescence in both G2 and NGB1769 peas is probably GA_1, as was previously suggested by Proebsting et al. (1978). This is based on several observations. First, the decrease in the levels of GA_1 under LD preceded the onset of apical senescence, whereas the higher GA_1 level under SD conditions is associated with the enhanced growth potential of the apical bud in both lines of peas. For instance, under a 9-h photoperiod G2 pea shoots contained the highest amount of GA_1 (3.4 ng/g fresh weight) and grew very vigorously. The growth potential decreased in parallel with the levels of GA_1 as the latter decreased with an increased photoperiod (Figs. 1 and 3). Second, there is less GA_1 in LD G2 than in LD NGB1769, and senescence is more rapid in G2 than in NGB1769. By contrast, GA_1 in nonsenescing, postfloral SD G2 is higher than in postfloral LD NGB1769 (Table II), in which internode length declines as development proceeds toward senescence, even though NGB1769 is genetically tall (*Le*). Third, among the plant hormones tested so far, only GA_3 and GA_1 have the ability to prevent the senescence of LD-grown peas.

We have now shown not only the presence of GA_1 in G2 peas but a correlation of its levels with continued apical growth. Our previous failure (Davies et al., 1982) to identify GA_1 by GC-MS in G2 pea shoots can probably be attributed to the much lower sensitivity of the GC-MS we used in that study. The increased level of GA_{20} and GA_1 in SD-grown G2 plants is probably the result of an overall increase in the metabolic flux through the early 13-hydroxylase pathway under short photoperiods, since SD-grown G2 peas produced more [^{14}C]GA_{53}, GA_{44}, GA_{19}, and GA_{20} from applied [^{14}C]GA_{12}-aldehyde than LD-grown plants (Davies et al., 1986a; Halinska et al., 1989). Thus photoperiod possibly operates at one of the two metabolic steps between GA_{12}-aldehyde and GA_{53}, although modulation at other points of GA_1 biosynthesis is not excluded.

It is interesting that not only the appearance of increased endogenous GA_1 levels correlates well with the prolonged or nonsenescing growth response seen under SD conditions, but also that the time at which GA_1 reaches a certain low level under LD following the start of reproduction may account for the time of apical bud death in different lines of peas. For instance, NGB1769 has a high GA_1 content (8 ng/g fresh weight) during the vegetative growth stage (Table II) and this level goes down progressively only under LD conditions after the initiation of reproductive growth. It usually takes 3 or 4 months for the level of GA_1 to reach 0.3 to 0.4 ng/g fresh weight, and the growth of the

apical bud will come to a full arrest in approximately another month. In the case of G2 peas, the level of GA_1 is 0.2 to 0.5 ng/g fresh weight during the vegetative growth stage (Table II), and under LD conditions these peas will grow for only 1 month or 40 d after the start of flowering, before the senescence of the apex.

The Relationship among Hormonal Levels, Nutrient Partitioning, and Senescence in Peas

The delay of senescence in G2 peas correlates with a slower reproductive development under SD conditions (Kelly and Davies, 1986). Kelly and Davies (1988a) found that a higher percentage of leaf-exported carbon was transported to the apical bud and the leaf primordia within the apical bud and a lower percentage was transported to the young fruits and the flower buds within the apical bud under SD compared with LD conditions. They concluded that the relative strength of the young reproductive and vegetative sinks is mediated by photoperiod and genotype in peas. Relative sink strength, via control of assimilate allocation, regulates the rate of reproductive development relative to vegetative growth and senescence (Gifford and Evans, 1981; Kelly and Davies, 1988b; Sklensky and Davies, 1993).

The level or application of IAA and GA has been previously correlated with sink strength in other systems (Patrick, 1979; Patrick et al., 1979). One possible mechanism by which this may occur is by increased Suc hydrolysis. For example, in etiolated pea epicotyls GA application enhanced growth, invertase activity, and sugar accumulation (Miyamoto et al., 1993), whereas IAA has been shown to have similar effects in other situations (Morris and Arthur, 1984, 1986). We, therefore, propose that the levels of these hormones also regulate photosynthate partitioning within the apical bud of peas. The decreased IAA level (Table III) in SD G2 floral buds compared with that of LD floral buds may be viewed as an indication of weakened sink strength and could, therefore, account for the slower reproductive development seen in SD G2 peas. The increased GA_1 content in SD G2 shoots (Table II), on the other hand, may be responsible for maintaining the active growth of the vegetative apical bud relative to the floral buds. Under LD conditions the increase in IAA levels in the flower buds, combined with the decrease in GA levels in the vegetative tissue, would lead to an increased partitioning of nutrients to the reproductive structures and enhance the senescence of the apical buds. Fruits, or a senescence factor from them, are not central to senescence induction (Hamilton and Davies, 1988a, 1988b; Kelly and Davies, 1988b). Their ability to cause nutrient depletion of the apex is determined by their sink strength relative to that of the apex. Reid (1980) also suggested that senescence in peas was induced early in the reproductive phase and was only enhanced by the fruit. The current results are consistent with these conclusions.

Hormones, Stem Elongation, and Senescence

Internode length in peas has been correlated with the content of GA_1 in the expanding internode (Ross et al., 1992; Reid and Howell, 1995). In the current study, the internode length of SD-grown G2 peas did not increase relative to the LD-grown plants, despite the fact that the GA_1 content increased more than 10 times under SD conditions. This may be due to a reduced response of SD G2 to GA_1, since the overall GA_1 content in SD may not exceed a threshold level that is required to induce further stem elongation. Exogenous GA_1 or GA_3 did produce a similar amount of elongation growth in G2 shoots under SD or LD conditions, but applications were at the microgram level, more than 100 times the amount present in the growing part of a SD shoot.

The capacity to respond by increases in length may also decline with age. Using a normally senescing, tall line of peas, Ross et al. (1992) noted that even though exogenous GA_3 caused proportionally more elongation when growth had almost stopped prior to apical arrest compared with when the plants were growing at a maximal rate, GA_3 did not have the ability to restore internode length to anywhere near the values seen earlier in development. On the other hand, NGB1769, the tall genotype, displayed a positive correlation between the internode length and the GA_1 content during ontogeny, which is very similar to that noted in the different genotypes by Ross et al. (1992). There are other possible reasons why the increased level of GA_1 in G2 shoots had no effect on stem length. The elevated GA_1 may have been localized in cells or tissues associated with developing primordia and young leaves but not in the expanding internodes, or the increase in stem elongation may involve IAA in addition to GA_1. Law and Davies (1990) reported a good correlation between IAA level and the stem length of several different pea genotypes, including slender lines in which GA is lacking. More recently, we (Yang et al., 1993, 1995) demonstrated that applications of IAA enhance stem growth in intact pea plants, with the effect being more pronounced in dwarf plants containing lower auxin levels (Law and Davies, 1990). Therefore, it is clear that both GA and IAA are needed in stem elongation, so that when one of them is missing or present at low level the elongation response will be diminished. In the case of G2 peas, both GA_1 and IAA levels were low during the vegetative growth stage and displayed the dwarf phenotype. After flower initiation, the amount of GA_1 in SD-grown pea shoots increased, but the level of IAA remained similar in LD and SD. Higher levels of exogenous GA_3 or GA_1 promoted stem elongation as well as IAA accumulation. The IAA level was severalfold higher 3 d after GA application (Table III).

Conclusion: The Regulation of Senescence in Pea Plants

The senescence of the apical bud in peas, which is the first symptom of the coming demise of the whole plant, is preceded by a decrease of GA_1 in the shoot and GA_{20} in the vegetative tissues of the apical bud, and an increase of the IAA level in the young flower buds. This correlates with a shift in the partitioning of photosynthate from the vegetative to the developing reproductive structures within the apical bud. Although GA_1 was not measured within the apical bud, we suggest that short photoperiods result in the increased biosynthesis of 13-hydroxylated GAs in the vegetative tissue within the apical bud and that the resulting higher levels of GA_1 enhance the sink activity of these

tissues such that vigorous apical growth continues. Flower buds develop under these conditions but much more slowly than under senescence-promoting conditions. Under senescence-promoting (LD) conditions fewer 13-hydroxylated GAs are produced, and vegetative vigor declines at the same time that the IAA level of the buds increases. This leads to more photosynthate moving to the reproductive tissue so that the flower buds grow rapidly at the expense of further vegetative growth, leading to the senescence of the apical bud. Senescence is a consequence of nutrient diversion to the young, developing reproductive structures. Growth of seed enhances the process but is not the cause of the process.

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LITERATURE CITED

Cohen JD, Baldi BG, Slovin JP (1986) IAA labeled with carbon-13 in the 6 carbons of the benzene ring a new internal standard for quantitative mass spectral analysis of IAA in plants. Plant Physiol **80:** 14–19

Davies PJ, Birnberg PR, Maki SL, Brenner ML (1986a) Photoperiod modification of [^{14}C]gibberellin A_{12} aldehyde metabolism in shoots of pea, line G2. Plant Physiol **81:** 991–996

Davies PJ, Emshwiller E, Gianfagna TJ, Proebsting WM, Noma M, Pharis RP (1982) The endogenous gibberellins of vegetative and reproductive tissue of G2 peas. Planta **154:** 266–272

Davies PJ, Horgan R, Heald JK, McGaw BA (1986b) Endogenous cytokinins in vegetative shoots of peas. Plant Growth Regul **4:** 311–323

Davies PJ, Proebsting WM, Gianfagna TJ (1977) Hormonal relationships in whole plant senescence. *In* PE Pilet, ed, Plant Growth Regulation. Springer-Verlag, New York, pp 273–280

Ecklund PR, Moore TC (1968) Quantitative changes in gibberellin and RNA correlated with senescence of the shoot apex in the "Alaska" pea. Am J Bot **55:** 494–503

Gianfagna TJ, Davies PJ (1981) The relationship between fruit growth and apical senescence in the G2 line of peas. Planta **152:** 356–364

Gifford RM, Evans LT (1981) Photosynthesis, carbon partitioning and yield. Annu Rev Plant Physiol **32:** 485–509

Halinska A, Davies PJ, Lee JW, Zhu Y-X (1989) Further identification of endogenous gibberellins in the shoots of pea, line G2. Plant Physiol **91:** 1255–1258

Hamilton DA, Davies PJ (1988a) Export of organic materials from developing fruits of pea and its possible relation of apical senescence. Plant Physiol **86:** 951–955

Hamilton DA, Davies PJ (1988b) Sucrose and malic acid as the compounds exported to the apical bud of pea following $^{14}CO_2$ labeling of the fruit. No evidence for a senescence factor. Plant Physiol **88:** 466–472

Hedden P, Croker SJ (1992) Regulation of gibberellin biosynthesis in maize seedlings. *In* CM Karssen, LC Van Loon, D Vreugdenhil, eds, Progress in Plant Growth Regulation. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 534–544

Hein MB, Brenner ML, Brun WA (1984) Effects of pod removal on the transport and accumulation of abscisic acid and indole-3-acetic acid in soybean leaves. Plant Physiol **76:** 955–960

Kelly MO, Davies PJ (1986) Genetic and photoperiodic control of the relative rates of reproductive and vegetative development in peas. Ann Bot **58:** 13–21

Kelly MO, Davies PJ (1988a) Photoperiodic and genetic control of carbon partitioning in peas and its relationship to apical senescence. Plant Physiol **86:** 978–982

Kelly MO, Davies P J (1988b) The control of whole plant senescence. CRC Crit Rev Plant Sci **7:** 139–173

Law DM, Davies PJ (1990) Comparative indole-3-acetic acid levels in the slender pea and other phenotypes. Plant Physiol **93:** 1539–1543

Lockhart JA, Gottschall V (1961) Fruit-induced and apical senescence in *Pisum sativum* L. Plant Physiol **36:** 389–398

Marx GA (1968) Influence of genotype and environment on senescence in peas (*Pisum sativum* L.). Bioscience **18:** 505–506

Miyamoto K, Ueda J, Kamisaka S (1993) Gibberellin-enhanced sugar accumulation in growing subhooks of etiolated *Pisum sativum* seedlings. Effects of gibberellic acid, indoleacetic acid and cycloheximide on invertase activity, sugar accumulation and growth. Physiol Plant **88:** 301–306

Morris DA, Arthur ED (1984) Invertase activity in sinks undergoing expansion. Plant Growth Regul **2:** 327–337

Morris DA, Arthur ED (1986) Stimulation of invertase activity by indol-3yl-acetic acid in tissues undergoing cell expansion. Plant Growth Regul **4:** 259–271

Murfet IC (1971) Flowering in *Pisum*: reciprocal grafts between known genotypes. Aust J Biol Sci **24:** 1089–1101

Murfet IC (1973) Flowering in *Pisum: Hr*, a gene for high response to photoperiod. Heredity **31:** 157–164

Murfet IC, Reid JB (1973) Flowering in *Pisum*: evidence that gene *Sn* controls a graft-transmissible inhibitor. Aust J Biol Sci **26:** 675–677

Nooden LD, Kahanak GM, Okatan Y (1979) Prevention of monocarpic senescence in soybeans with auxin and cytokinin: an antidote for self-destruction. Science **206:** 841–843

Patrick JW (1979) Auxin-promoted transport of metabolites in stems of *Phaseolus vulgaris* L. Further studies on effects remote from the site of hormone application. J Exp Bot **30:** 1–13

Patrick JW, Johnstone GFS, Wareing PF (1979) Mobilizing ability of gibberellic acid and kinetin applied to mature decapitated stems of *Phaseolus vulgaris*. Ann Bot **44:** 517–519

Proebsting WM, Davies PJ, Marx GA (1976) Photoperiodic control of apical senescence in a genetic line of peas. Plant Physiol **58:** 800–802

Proebsting WM, Davies PJ, Marx GA (1977) Evidence for a graft transmissible substance which delays apical senescence in *Pisum sativum* L. Planta **135:** 93–94

Proebsting WM, Davies PJ, Marx GA (1978) Photoperiod-induced changes in gibberellin metabolism in relation to apical growth and senescence in a genetic line of peas (*Pisum sativum* L.). Planta **141:** 231–238

Reid JB (1980) Apical senescence in *Pisum*: a direct or indirect role for the flowering genes? Ann Bot **45:** 195–201

Reid JB, Howell SH (1995) Hormone mutants and plant development. *In* PJ Davies, ed, Plant Hormones: Physiology, Biochemistry and Molecular Biology. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 448–485

Ross JJ, Reid JB, Dungey HS (1992) Ontogenetic variation in the levels in gibberellin A_1 in *Pisum*. Implications for the control of stem elongation. Planta **186:** 166–171

Sklensky DE, Davies PJ (1993) Whole plant senescence: reproduction and nutrient partitioning. Hortic Rev **15:** 335–366

Woolhouse HW (1983) Hormonal control of senescence allied to reproduction in plants. *In* WJ Meudt, ed, Beltsville Symposia on Agricultural Research, VI, Strategies of Plant Reproduction. US Department of Agriculture, Washington, DC, pp 201–233

Yang T, Davies PJ, Reid JB (1995) Genetic dissection of the relative roles of auxin and gibberellin in the regulation of stem elongation in intact light-grown peas. Plant Physiol **110:** 1029–1034

Yang T, Law DM, Davies PJ (1993) Magnitude and kinetics of stem elongation induced by exogenous indole-3-acetic acid in intact light-grown pea seedlings. Plant Physiol **102:** 717–724

Zhu Y-X, Davies PJ, Halinska A (1988) Gibberellin A_{12} and gibberellin A_{12}-7-aldehyde as endogenous compounds in developing seeds of *Pisum sativum*. Phytochemistry **27:** 2549–2552