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Internode Length in Pisum. Mutants *lk*, *lka*, and *lkb* do not Accumulate Gibberellins

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Abstract. The levels of gibberellin A_1 (GA_1), GA_8, GA_{19}, GA_{20}, GA_{29}, and GA_{44} in the short *Pisum sativum* L. mutants *lk, lka,* and *lkb,* and comparable wild-type plants, were determined by gas chromatography-selected ion monitoring (GC-SIM) using ^2H or ^{13}C internal standards. The mutants possessed similar GA_1 levels to wild-type plants, consistent with their classification as GA-sensitivity rather than GA-synthesis mutants. However, these mutants differ from certain sensitivity mutants in other species, in which substantial accumulation of GA_1 occurs. The results suggest that if the proposed feedback model for the regulation of GA synthesis occurs in peas it is not the reduced growth per se that is the trigger for elevated levels of C_{19} GAs. The results are also consistent with the hypothesis that in those GA-sensitivity mutants which do not accumulate C_{19} GAs, the biochemical lesion may be well down the transduction pathway which leads from GA_1 reception to stem elongation.

A number of short mutants with reduced sensitivity (responsiveness) to applied gibberellin A_1 (GA_1) have been identified in garden peas (*Pisum sativum* L.: *lk, lka, lkb, lkc, lw,* and *lm;* Jolly et al. 1987, Reid 1990, Reid and Potts 1986, Reid and Ross 1989, Reid et al. 1991). Of these sensitivity mutants, *lkc* has been shown by gas chromatography-selected ion monitoring (GC-SIM) (Reid et al. 1991) to possess normal levels of GA_1. This contrasts with results from GA-sensitivity mutants in other species, such as wheat (*Rht3* and *Rht1;* Appleford and Lenton 1991), maize (*D8;* Fujioka et al. 1988), *Arabidopsis* (*gai;* Talon et al. 1990), barley (*gai;* Boother et al. 1991), *Phaseolus vulgaris* (Endo et al. 1989), and sweet pea (*lb;* Ross et al. 1990), which suggest that accumulation of C_{19} GAs, such as GA_1 is common in this category of mutant. For example, GA_1 levels are 60 times higher in *D8* plants than in comparable wild-type plants and 20 times greater in *Rht3* wheat than in the wild-type.

Recently, it has been suggested that this accumulation results from a feedback regulation of GA biosynthesis (Appleford and Lenton 1991, Croker et al. 1990, Scott 1990). It is postulated that in short GA-sensitivity mutants the ineffectiveness of GA_1 in promoting growth, or the reduced growth rate per se, results in an increase in GA_{19} oxidase activity. This in turn reduces the level of GA_{19} and increases the levels of C_{19} GAs, such as GA_{20}, GA_{29}, GA_1, and GA_8, compared with wild-type plants. In sensitivity mutants which accumulate C_{19} GAs the reception of the GA signal is thought to be altered (Scott 1990). On the other hand, in nonaccumulators the biochemical lesion may be well down the transduction pathway leading from GA reception to elongation. Genes *lka* and *lkb* are thought to fulfil this role, since in these mutants, cell wall-yielding properties are altered (Behringer et al. 1990). Consequently, it was considered of significance to determine whether these mutations affect the levels of GAs from the early 13-hydroxylation pathway—the major pathway for GA biosynthesis in shoots of this species (Graebe 1987). Previous bioassay studies (Reid and Potts 1986, Reid and Ross 1989) have suggested that the genotypes *lk, lka,* and *lkb* may have levels of GA-like substances similar to wild-type tall plants. However, more precise quantification is required and the levels of inactive GAs need also to be determined. Hence, the levels of GA_1, GA_{20}, GA_{29}, GA_8, GA_{19}, and GA_{44} were determined in the three mutants *lk, lka,* and *lkb* and essentially isogenic wild-type plants, using GC-SIM with either ^2H- or ^{13}C-labeled internal standards.

Table 1. Harvest details of plants grown for quantification of GA levels, and the quantity of internal standards added.

Genotype	Phenotype	Height from soil (cm)	Leaves expanded	Age of plants (days)	Tissue harvested above node	Number of plants harvested	Fresh weight (g)	Quantity of GA internal standard added (ng)						
								GA_1	GA_3	GA_8	GA_{19}	GA_{20}	GA_{29}	GA_{44}
Wild type	Tall	35.5 ± 0.8	8.6 ± 0.8	22	8	23	24.8	250	100	—	250	500	500	375
lk	Erectoides	4.5 ± 0.3	8.6 ± 0.1	22	8 or 9	39	10.8	110	100	—	110	220	220	160
Wild type	Tall	36.1 ± 0.9	8.1 ± 0.1	26	7 or 8	28	13.0	90	—	70	40	195	120	20
lka	Semi-erectoides	14.1 ± 0.3	8.1 ± 0.1	26	7 or 8	30	13.4	90	—	70	40	195	120	20
lkb	Semi-erectoides	18.3 ± 0.3	7.7 ± 0.2	26	7 or 8	25	11.9	90	—	70	40	195	120	20

Materials and Methods

Plant Material

The genotypes used during this study are held in the collection at Hobart. In experiment 1, *Lk*-(tall) and *lk lk* (erectoides) plants derived from an F_5 plant from a cross between cv. Torsdag (*Lk*) and line JI 1420 (*lk*) (Reid 1986) were grown. In experiment 2, the pure breeding lines used were the wild-type tall cv. Torsdag and the two single gene mutant lines derived from this cv., NGB 5865 (*lka*) and NGB 5862 (*lkb*) (Reid and Ross 1989).

Growing Conditions

All plants were grown at a density of 2 per 14 cm slimline pot in a 1:1 mixture of vermiculite and dolerite chips topped with 2–3 cm of potting mix and given nutrient solution twice a week. They were grown under an 18-h photoperiod in a heated glasshouse as described by Reid and Potts (1986).

GA Extraction and Purification

The apical portions of the plants were harvested and placed into cold methanol (−20°C). In the experiment with *lk* plants, the apical portion included the uppermost expanded leaf and (to ensure sufficient material) the internode below it, whereas in the experiment with mutants *lka* and *lkb* the internode below the uppermost expanded leaf was not included. Counting of nodes started from the cotyledons as 0, and only the main shoots were harvested. Further harvest details are shown in Table 1. The internal standards used were $[17\text{-}^{13}C, 17\text{-}^{3}H_2]GA_{29}$ (provided by Professor B. O. Phinney, Los Angeles, CA, USA) and $[17,17\text{-}^{2}H_2]GA_1$, $[17,17\text{-}^{2}H_2]GA_3$, $[17,17\text{-}^{2}H_2]GA_8$, $[17,17\text{-}^{2}H_2]GA_{19}$, $[17,17\text{-}^{2}H_2]GA_{20}$, and $[20\text{-}^{2}H_1]GA_{44}$, and were added prior to the commencement of extraction. Table 1 shows the quantities added. GAs were extracted as before (Reid et al. 1990) and purified for mass spectrometry using high-performance liquid chromatography (HPLC) as described previously (Reid et al. 1991). The HPLC zones collected were 8–20 min (GA_8, GA_{29}), 21–34 min (GA_1, GA_3), and 34–46 min (GA_{19}, GA_{20}, and GA_{44}).

Quantification of GAs by GC-SIM

The procedure followed that described by Reid et al. (1991). The ions pairs monitored for the quantification of the respective GAs were: 506/508 (GA_1), 504/506 (GA_3), 594/596 (GA_8), 434/436 (GA_{19}), 418/420 (GA_{20}), 506/507 (GA_{29}), and 432/433 (GA_{44}). Additional ions were monitored to confirm the identification of the GA under investigation. The peak area corresponding to the internal standard was corrected for the contribution by naturally occurring isotopes in the endogenous GA (using a computer program). In addition the peak area corresponding to the endogenous GA was corrected for the contribution by unlabeled material in the internal standard. This gave the corrected ratio of endogenous ion intensity to internal standard ion intensity. The product of this ratio and the amount of internal standard added was divided by the fresh weight of tissue to give the endogenous GA level.

Results and Discussion

The level of GA_1 in the expanding portion of the young shoot was similar in the mutants *lk*, *lka*, and *lkb* and their respective wild-type tall isolines (Table 2). This confirms previous bioassay results suggesting that the stature of these mutants is not attributable to a deficiency in GA_1 (Reid and Potts 1986, Reid and Ross 1989). In contrast, GA-biosynthesis mutants of similar stature to *lka* and *lkb* (e.g., *le*) possess less than 10% the level of GA_1 found in the wild-type (Ross et al. 1992). There is also no evidence of an accumulation of this biologically active GA as occurs in *D8* maize or *Rht3* wheat (Appleford and Lenton 1991, Fujioka et al. 1988). There are some differences in the levels of GA_{19}, GA_{20}, and GA_{29} between the genotypes but these differences do not appear to be of biological significance. The levels of all three GAs are elevated in the mutants and this could possibly be due to an increase in the ratio of leaf to stem tissue in these plants. Smith et al. (1992) have shown that leaf tissue has higher GA_{20} and GA_{29} levels than comparable stem tissue. Unlike *D8* maize plants (Fujioka et al. 1988) no evidence for the presence of GA_3 could be found in *lk* pea plants. In wild-type plants, GA_3 was either not present or its level was below 0.14 ng $(g\ FW)^{-1}$, consistent with previous results (Ross et al. 1992).

As far as comparisons between mutants and their

Table 2. Gibberellin levels in the mutants, *lk*, *lka*, and *lkb*, and comparable wild-type plants.

	Level [ng (g FW)$^{-1}$]					
Genotype	GA_1	GA_8	GA_{19}	GA_{20}	GA_{29}	GA_{44}
Wild type	12.1	NA	1.9	14.4	4.4	0.65
lk	8.2	NA	2.4	19.7	19.9	0.30
Wild type	10.0	31.8	2.6	16.8	10.2	1.24
lka	10.4	31.2	3.5	29.8	10.7	1.26
lkb	8.4	41.2	4.2	19.3	14.3	1.60

NA, not analyzed.

corresponding wild-types are concerned, these findings appear to break the nexus between a reduction in response to GA_1 and an accumulation of C_{19} GAs. Clearly, the marked reduction in growth (60–90%) observed in mutants *lk*, *lka*, and *lkb* does not appear to lead to an increase in GA_{19} oxidase activity, since substantial increases in GA_{20} and GA_1 levels were not observed and GA_{19} levels were not reduced. Therefore, if the feedback model of GA biosynthesis operates in peas, it is not reduced growth per se that triggers an increase in C_{19} GA production. Rather the trigger may be provided by an event somewhat closer to the initial perception of the GA_1 signal. This supports the proposal by Appleford and Lenton (1991) that in wheat, GA_1 accumulation is not regulated by growth rate per se, but instead by the degree of GA_1 action.

Overall, these results are consistent with the mutants *lk*, *lka*, and *lkb* being GA-sensitivity mutants, but not of the "accumulation" type. None of these mutants is a true phenocopy of GA-deficient dwarfs, and recent results have suggested that *lka* and *lkb* may alter the wall-yielding characteristics of the cell wall while not influencing other GA-mediated steps, such as solute transport (Behringer et al. 1990). The results are therefore consistent with the hypothesis that non-accumulating GA-sensitivity mutants, such as *lk*, *lka*, and *lkb*, may be affected well down the transduction pathway which leads from GA_1 reception to stem elongation.

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