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Branching Mutant *rms-2* in *Pisum sativum*[1]

Grafting Studies and Endogenous Indole-3-Acetic Acid Levels

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Isogenic lines of pea (*Pisum sativum* L.) were used to determine the physiological site of action of the *Rms-2* gene, which maintains apical dominance, and its effect on endogenous free indole-3-acetic acid (IAA) levels. In mutant *rms-2* scions, which normally produce lateral branches below node 3 and above node 7, apical dominance was almost fully restored by grafting to *Rms-2* (wild-type) stocks. In the reciprocal grafts, *rms-2* stocks did not promote branching in wild-type shoots. Together, these results suggest that the *Rms-2* gene inhibits branching in the shoot of pea by controlling the synthesis of a translocatable (hormone-like) substance that is produced in the roots and/or cotyledons and in the shoot. At all stages, including the stage at which aerial lateral buds commence outgrowth, the level of IAA in *rms-2* shoots was elevated (up to 5-fold) in comparison with that in wild-type shoots. The internode length of *rms-2* plants was 40% less than in wild-type plants, and the mutant plants allocated significantly more dry weight to the shoot than to the root in comparison with wild-type plants. Grafting to wild-type stocks did not normalize IAA levels or internode length in *rms-2* scions, even though it inhibited branching, suggesting that the involvement of *Rms-2* in the control of IAA level and internode length may be confined to processes in the shoot.

Recently, transgenic plants have enabled much progress to be made in determining the role of plant hormones in the control of apical dominance. Transgenic cytokinin-overproducing plants (e.g. *ipt*; Medford et al., 1989) and transgenic plants with reduced levels of IAA (e.g. *iaaL*; Romano et al., 1991) have been produced and reported to exhibit reduced apical dominance. This, together with the fact that transgenic plants with elevated levels of IAA (e.g. *iaaH* and *iaaM*; Sitbon et al., 1992; Romano et al., 1993) have increased apical dominance, provides good evidence for a role for IAA and cytokinins in the control of branching. In addition, transgenic plants that contain both cytokinin and auxin-overproducing genes have an intermediate phenotype (Klee and Estelle, 1991), supporting the theory that the ratio of cytokinin to auxin may be the crucial factor determining the propensity to branch. Furthermore, Romano et al. (1993) have shown that IAA influences branching in tobacco and *Arabidopsis* independently of the concentration of ethylene.

However, it is possible that the ratio of cytokinin to auxin is not the only regulatory factor that influences branching. For example, although Romano et al. (1991) reported a reduction in free IAA in juvenile 35S-*iaaL* tobacco plants of up to 19-fold in comparison with WT plants, they also reported that the growth of axillary buds was inhibited in both WT and 35S-*iaaL* plants until the vegetative-to-floral transition occurred. Indeed, the difference in apical dominance caused by the 35S-*iaaL* gene construct was related only to the subsequent growth of axillary buds after they were released rather than the initial promotion of release itself. In addition, transgenic cytokinin-overproducing tobacco plants (Medford et al., 1989) have a phenotype similar to 35S-*iaaL* plants (Romano et al., 1991). It is possible that in tobacco the axillary buds of juvenile transgenic plants are insensitive to a favorable ratio of hormones for release, or alternatively, some other factor may be involved. Tamas et al. (1992), based on studies with tobacco plants transformed with *rol* genes and *luxA* and *luxB* reporter genes, have suggested that a novel extractable substance exists in the shoot of tobacco that interacts with IAA in the inhibition of lateral shoot production.

In view of the uncertainties described above, nontransgenic branching mutants may be useful to confirm current theories on apical dominance or provide evidence for the involvement of as-yet unknown processes. There are no mutants in higher plants for which it has clearly been demonstrated that the normal endogenous levels of either IAA or cytokinin are altered as the primary action of a single gene mutation. However, some mutants have been isolated that have altered apical dominance, and grafting studies have been performed to characterize their action. From studies using mutants of tomato with increased apical dominance, it has been suggested that endogenous root factors do not play an essential role in the control of lateral branching (Craigella lateral suppressor, Tucker, 1979; *torosa-2*, Mapelli and Kinet, 1992). It is possible that the control of branching in these mutants involves shoot-derived cytokinins (Sossountzov et al., 1988; Mapelli and Kinet, 1992).

Other grafting studies using tomato mutants have indicated that the root system could not influence the release of axillary buds from apical dominance but could influence the subsequent growth of the buds following release (Brenner et al., 1987). In poinsettia, grafting studies involving different branching cultivars have provided evidence for a translocatable substance(s) produced in the roots that can promote the

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Abbreviations: MeOH, methanol; SIM, selected ion monitoring; WT, wild-type.

release of axillary buds in the scion from apical dominance (Stimart, 1983). Some evidence for graft-transmissible effects on branching has also come from sweet pea/garden pea intergeneric grafting studies involving different flowering genotypes (Ross and Murfet, 1985). However, it is suggested (Beveridge et al., 1992) that these effects may result primarily from effects of the flowering genes on assimilate partitioning.

A new recessive branching mutant, *rms-2*, recently isolated from the garden pea (Arumingtyas et al., 1992) shows increased outgrowth of basal and aerial laterals and reduced internode length. In the present study we used grafting techniques to investigate the physiological basis for the action of the *Rms-2* gene on apical dominance. Furthermore, endogenous IAA levels were determined by GC-MS-SIM in the shoot of mutant and WT plants and in various graft combinations. Pleiotropic effects of the *rms-2* mutation on dry weight and stem and root growth were also examined.

MATERIALS AND METHODS

Plant Materials

The *rms-2* mutant phenotype in pea (*Pisum sativum* L.) is conferred by a recessive allele of the *Rms-2* gene (Arumingtyas et al., 1992). The mutant line WL5951 was derived from cv Parvus (Hobart line 77) by Dr. S. Blixt; the mutant line K524 was derived from cv Torsdag (Hobart line 107) by Dr. K.K. Sidorova. The mutagenic agent in each case was ethyl methanesulfonate. Further details are given by Arumingtyas et al. (1992). All results reported were obtained with the Torsdag isolines, except where otherwise described.

Growing Conditions

Except where specified, plants were grown two per pot in 14-cm slim-line pots containing a 1:1 (v/v) mixture of vermiculite and 10-mm dolerite chips topped with 4 cm of pasteurized peat/sand potting mixture. For the determination of the dry weight of seedlings, 48 plants per genotype were grown in 14-L tote boxes. Grafts (1 plant per pot) were made epicotyl to epicotyl as described by Murfet (1971), except that the grafted plants were enclosed initially in plastic bags to increase humidity. The seedlings were grafted 6 to 7 d after sowing and there was no macroscopic sign of lateral bud release in either genotype at this time. Lateral buds that subsequently grew from the cotyledonary node of the stock were excised because they tend to weaken the growth of the grafted shoot (scion). Slow or weak grafts, usually less than 10% of the total, were excluded from the analysis. Lateral measurements were recorded after the plants had produced over 17 expanded leaves. Unless otherwise stated, all plants were grown under an 18-h photoperiod obtained by extending the natural light with a 1:1 mixture of fluorescent (40-W white) and incandescent (100 W) lights that provided an intensity of 25 to 30 μmol m^{-2} s^{-1} at the pot top. To reduce the variation in vigor of the main stem, basal laterals were removed, except in the case of grafts. The nodes of plants were numbered starting from the first scale leaf as node 1.

IAA Extraction and Purification

The harvested tissue was immediately placed into a volume (5 mL g^{-1} fresh weight) of cold MeOH (−20°C) containing 0.25 mg mL^{-1} butylated hydroxytoluene. Deuterated ($^2H_5$) IAA was added (100 ng g^{-1} fresh weight) and the portions were homogenized and stored at −20°C. IAA was extracted overnight with 1 M ammonium acetate buffer (pH 6.5) and distilled water (80% MeOH:10% buffer:10% water) at 0 to 4°C. The extract was filtered through Whatman No. 1 filter paper, reduced in volume to 0 to 5 mL under vacuum at 30°C, and washed into a beaker through a Millipore filter (pore size 0.45 μm) with 2 × 5 mL of a 50:50 (v/v) mixture of 1 M ammonium acetate buffer and water, followed by a 2-mL wash of the filter. The filtrate was adjusted to pH 3 using 1 M H_3PO_4 and partitioned three times against two-thirds volumes of diethyl ether containing 10 mg L^{-1} butylated hydroxytoluene. Following storage at −20°C, any ice was quickly filtered from the extract with Whatman No. 1 filter paper and the ether was evaporated under vacuum at 30°C. The dry residue was then washed with 2 × 5 mL of 1 mM HCl, which was passed through a preconditioned (5 mL of MeOH followed by 5 mL of 1 mM HCl) Sep-Pak C_{18} cartridge (Waters Associates, Melbourne, Australia). A further 10 mL of 1 mM HCl and 10 mL of 10% MeOH in distilled water were passed through the Sep-Pak. The IAA was then eluted with 40 mL of 30% MeOH in distilled water and dried under vacuum at 30°C.

GC-MS-SIM Analysis of IAA

The purified sample was methylated with ethereal diazomethane, dried, dissolved with 2 μL of dry pyridine, and silylated with 10 μL of *N,O*-bis-trimethylsilyltrifluoroacetamide at 60°C for 5 min. GC-MS-SIM analysis of the trimethylsilyl derivatives was performed with a Hewlett-Packard 5890 gas chromatograph linked via direct capillary inlet to a Hewlett-Packard 5970 mass selective detector. Samples of 1 μL were injected in the splitless mode at 260°C onto a 25-m × 0.32-mm internal diameter HP5 fused silica column with a 0.52-μm film thickness. The column was coupled to the mass-selective detector via an open split interface held at 290°C. Helium was used as a carrier gas with an initial flow rate of 2 mL min^{-1} under a pressure of 15 p.s.i. The initial temperature in the column oven of 60°C was increased to 150°C at 30°C min^{-1} followed by a 10°C min^{-1} gradient to 290°C. IAA eluted under these conditions at about 11 min, and peak areas were determined for ions of m/z 202, 207, 261, and 266. Free IAA levels were calculated by isotope dilution using the method described in Cohen et al. (1986). The IAA internal standard, indole-2,4,5,6,7-$^2H_5$-IAA, was synthesized by Merck and Co., Inc. (Rahway, NJ). The IAA extraction procedure produced clean traces for the ions monitored in the region of the elution of IAA during GC-SIM analysis (data not shown).

RESULTS

Grafting Studies

Branching in the scions of *rms-2*/*rms-2* self-grafts was profuse, with laterals being produced at nodes 1 and 2, and

from node 8 and above (i.e. nodes 3 to 7 inclusive were devoid of laterals; Figs. 1 and 2). This is similar to the pattern of branching observed in ungrafted *rms-2* plants (Arumingtyas et al., 1992). Branching in *rms-2* scions was almost entirely inhibited by grafting to WT stocks, suggesting that the *rms-2* mutation alters the level of a graft-transmissible substance that is exported from the stock. In contrast, branching in WT scions was not promoted by grafting to *rms-2* stocks, suggesting that the level of the graft-transmissible substance is also under genetic control in the shoot. In *rms-2*/WT grafts, very short laterals occurred at node 2 and above node 10 (Fig. 1). Thus, the stock appeared to control the overall propensity of the plant to branch rather than the pattern of branching along the stem.

Apart from its effect on branching, *rms-2* also causes a reduction in internode length (Arumingtyas et al., 1992). The stem length between nodes 1 and 12 (L_{1-12}) of *rms-2* scions remained 40% shorter than that of WT scions regardless of the stock genotype (Table I). Therefore, the effect of *rms-2* on internode length occurs independently of the presence of axillary shoots and appears to be controlled primarily by processes in the shoot. The *rms-2* mutation also results in thinner stems.

Dry Weight Measurements

Apart from promoting branching and reducing internode length, *rms-2* also reduced root growth (Fig. 3) and influenced dry matter partitioning (Fig. 4). The dry weights of the root and shoot of 10-d-old *rms-2* seedlings were significantly less (by 42 and 28%, respectively, $P < 0.001$) than for those of comparable WT seedlings, whereas the lower mean dry weight of *rms-2* cotyledons was not significantly different ($P > 0.05$) from the WT value (Fig. 4). The greater percentage reduction in the dry weight of the root compared with that of the shoot of *rms-2* seedlings resulted in the mutant having a significantly higher ($P < 0.001$) shoot-to-root dry weight ratio than that found in WT seedlings.

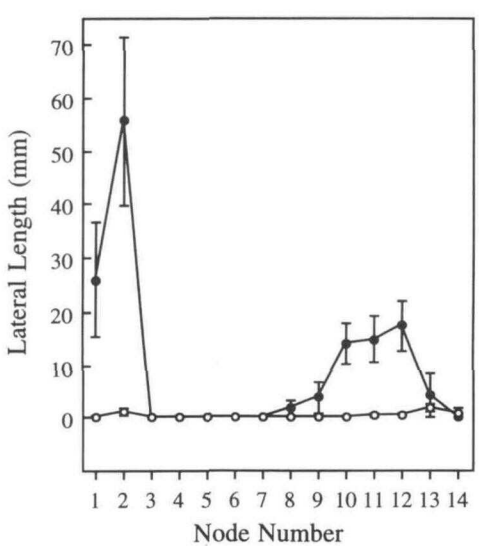

Figure 1. Lateral lengths at each vegetative node of *rms-2* scions either self-grafted (●) or grafted to WT stocks (○). Data are shown as means ± SE. Table I lists the number of plants per treatment. WT scions (grafts WT/WT and WT/*rms-2*) did not produce laterals. The isolines used were K524 (*rms-2*) and Torsdag (WT).

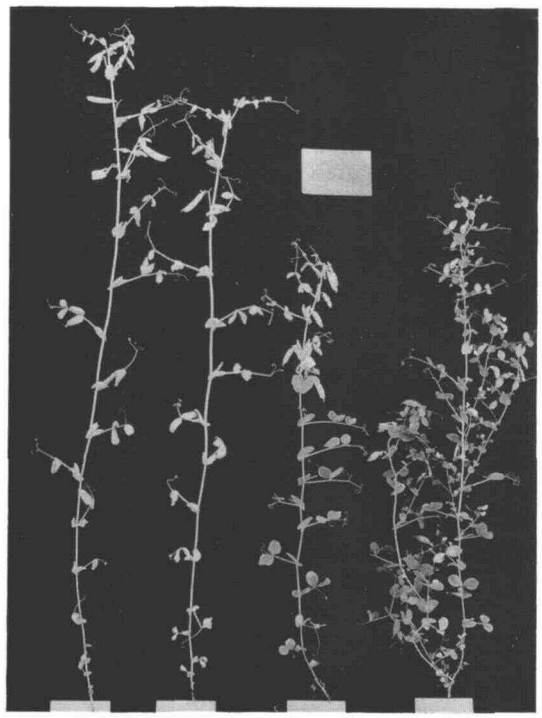

Figure 2. Reciprocal grafts of *rms-2* (K524) and WT (cv Torsdag) plants. Arranged left to right are grafts of WT/WT (scion/stock), WT/*rms-2*, *rms-2*/WT, and *rms-2*/*rms-2*.

Endogenous IAA Levels

In five separate experiments, free endogenous IAA levels were consistently higher in the mutant plants than in WT plants (Tables II, III, and other data not shown). For example, when the plants had 8 to 9 leaves expanded, the concentration of endogenous IAA in the nodal segment immediately below the highest expanded leaf of *rms-2* plants (node 7 or 8) was about 3-fold higher than that in comparable segments of WT plants (Table II). At this time, although the axillary buds at this node of *rms-2* plants were swollen in comparison with those of the WT, axillary buds on accompanying plants were still not released from apical dominance 7 d later, even though the plants had developed at least another 2 expanded

Table I. *Mean ± SE for length of the main stem between nodes 1 and 12 (L_{1-12}) for grafts of the rms-2 (K524) and WT (cv Torsdag) plants in Figure 1*

n, Number of plants per treatment.

	Graft Combination			
	WT/WT	WT/*rms-2*	*rms-2*/WT	*rms-2*/*rms-2*
L_{1-12} (cm)	68.6 ± 0.9	64.9 ± 1.9	43.3 ± 0.6	40.3 ± 1.1
n	9	8	10	10

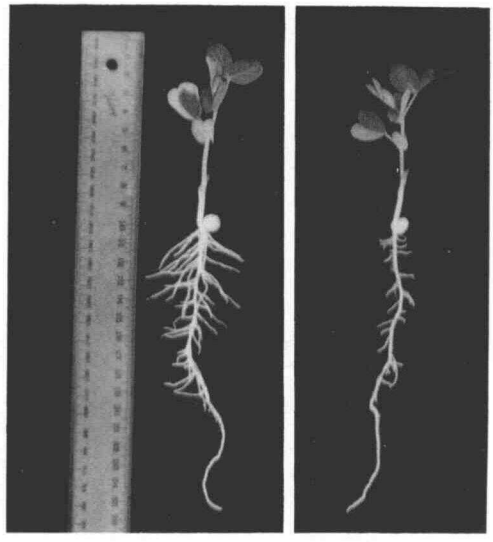

Figure 3. Seventeen-day-old WT (left; cv Torsdag) and *rms-2* (right; K524) seedlings.

leaves. When the plants had 10 to 12 leaves expanded, which is the stage in the ontogeny of *rms-2* plants when the release of aerial lateral buds from apical dominance occurs, a 3- and 5-fold accumulation of IAA was again observed at the node below the highest expanded leaf of *rms-2* plants (in comparison with those of WT plants; Tables II and III, respectively). An accumulation of free IAA was also apparent in the nodal segment at the highest expanded leaf and at the oldest unexpanded leaf and apical portions of *rms-2* plants (Table II). In addition, a 1.6-fold increase in free IAA level was observed in *rms-2* plants in the internode between the node at the highest expanded leaf and the node below it (data not shown).

The elevated concentration of IAA in *rms-2* plants was not due to the smaller fresh weight of *rms-2* portions, since the difference, although reduced, was also apparent when calculated on a ng/portion basis. Furthermore, since the accumulation of free IAA in *rms-2* plants was observed in two genetic backgrounds (Torsdag and Parvus; Table II), where the mutant was derived independently, it is unlikely to be the result of a change at a second gene. Moreover, the elevated level of IAA in *rms-2* plants was not simply due to high levels of IAA in the axillary buds. For example, when the plants had 12 leaves expanded, the relatively large bud (3–4 mm) at the node below the highest expanded leaf of *rms-2* plants contained only about 1% of the total IAA present in the nodal segment, even though the segment accumulated a concentration of IAA more than 5-fold greater than in WT plants (Table III). It should also be noted that the oldest unexpanded leaf (Table II) contained considerably less IAA/g than the apical portion, illustrating the importance of the relative quantity of leaf and internode present in the apical portion of comparable extracts.

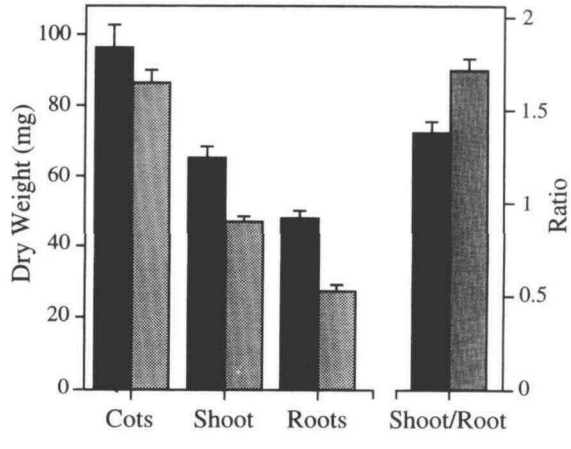

Figure 4. Dry weights and shoot-to-root dry weight ratio for 10-d-old WT (solid; cv Torsdag) and *rms-2* (shaded; K524) seedlings. Immediately after harvest, the portions were left to dry at 60°C for 32 d before weighing. Cots, Cotyledons.

Endogenous IAA Levels in Grafted Plants

The accumulation of free IAA in *rms-2* shoots in comparison with WT shoots was not substantially affected by grafting to *rms-2* or WT stocks (Table IV). The portions for analysis were harvested at about the time of axillary bud release in the self-grafted *rms-2* plants, and the effect of grafting on bud release was determined from the total lateral lengths of the decapitated grafts 13 d after the harvest. These total lateral lengths (Table IV) were consistent with the degree of branching of the intact grafts (Fig. 1). Free IAA levels were 1.5- to 3-fold higher in *rms-2* scions than in WT scions, which was similar to the results obtained from ungrafted *rms-2* and

Table II. *Level of free IAA in rms-2 and WT plants*

Portions harvested were the apical portion, which was that portion above the oldest unexpanded leaf (which included the stipules and petiole); the node at the highest expanded leaf; and the node below the highest expanded leaf. The nodal segments consisted of a 1-cm portion of the petiole and stem from each side of the node. The axillary buds in the mutant were swollen in comparison with those of the WT. The plants were 29 d old at the time of harvest. The Torsdag and Parvus isolines were grown in separate experiments. LE refers to the mean ± SE for the number of expanded leaves at harvest; *n* is the number of plants harvested.

	IAA			
	Torsdag isolines		Parvus isolines	
	WT	*rms-2*	WT	*rms-2*
	ng g⁻¹ fresh weight			
Apical portion	128	168	121	141
Oldest unexpanded leaf	52	84		
Node at the highest expanded leaf	111	299	156	241
Node below the highest expanded leaf	102	301	126	281
LE (nodes)	8.4 ± 0.1	8.3 ± 0.0	11.5 ± 0.2	10.3 ± 0.1
n	24	24	13	12

Table III. *Level of free IAA in nodal segments and axillary buds of rms-2 and WT plants*

Portions harvested were the nodal segment at the node below the highest expanded leaf and the axillary bud at that node. The buds were 3.4 ± 0.3 mm (about 6 mg) and <1 mm (about 0.05 mg) in length (and fresh weight) for *rms-2* and WT plants, respectively. The plants (Torsdag isolines) were 39 d old. LE refers to the mean ± SE for the number of expanded leaves at harvest. $n = 44$.

	IAA Level			
	WT		*rms-2*	
	IAA ng g^{-1} fresh weight	*ng portion^{-1}*	*IAA ng g^{-1} fresh weight*	*ng portion^{-1}*
Node below the highest expanded leaf	44	9.5	244	35.9
Axillary bud	<211	<0.03	77	0.4
LE (nodes)	12.7 ± 0.2		12.2 ± 0.2	

WT shoots (Table II). However, the levels in the grafted plants were lower than in the ungrafted plants. This may have been because the grafted plants were older and less vigorous than the ungrafted plants represented in Tables II and III or because they were grown during a colder part of the year.

IAA Levels in Decapitated Plants and in Plants at Different Ontogenetic Stages

Free IAA levels were determined at the uppermost node of decapitated WT plants 2 d after decapitation above the highest expanded leaf (Table V). At this stage, the buds were swollen and similar to those on the *rms-2* plants described in Table II. A 5-fold reduction in free IAA was observed in decapitated plants in comparison with the same node of intact plants.

In WT plants harvested at a range of ontogenetic stages, the concentration of IAA located in the segment at the node below the highest expanded leaf increased to a maximum at node 9 (d 36) and decreased thereafter (Table VI). The ontogenetic stage that contained the highest level of free IAA is similar to that where aerial lateral buds are released in *rms-2* plants. At all ontogenetic stages, the level of IAA in WT plants at nodes 4 or 5 was lower (2- to 5-fold) than the level at the node below the highest expanded leaf, even though nodes 4 and 5 rarely produce laterals in either *rms-2* or WT plants (Fig. 1; Arumingtyas et al., 1992). WT and *rms-2* plants both have a higher propensity to branch at nodes 1 and 2 than at nodes 4 or 5 (Fig. 1; Arumingtyas et al., 1992), although the level of IAA was similar in these two regions even early in the ontogeny of the plants (e.g. d 21) when basal lateral outgrowth is likely to occur (Husain and Linck, 1966). Consequently, axillary bud release does not correlate with reduced IAA levels in the WT.

DISCUSSION

This report characterizes the action of a recessive mutation, *rms-2*, which causes increased branching in pea. The simplest interpretation of our results is that *rms-2* plants are deficient in a substance inhibitory to branching that can be supplied across a graft union from WT plants. WT stocks inhibited branching almost entirely in grafted *rms-2* scions (Fig. 1). However, *rms-2* stocks did not promote branching in WT scions. Therefore, the *Rms-2* gene inhibits branching by acting in both the root and/or cotyledons and in the shoot. The mechanism controlling branching in cultivars of poinsettia

Table IV. *Level of free IAA in reciprocal grafts of rms-2 and WT plants*

Refer to Table II for a description of the portions harvested. Axillary buds at the node below the highest expanded leaf on *rms-2/rms-2* self-grafts were swollen and 2 to 4 mm in length, whereas those on *rms-2*/WT plants and WT scions were ≤2 mm and ≤1 mm in length, respectively. TLL (mean ± SE) refers to the total lateral length of decapitated grafted plants 13 d after the harvest of the portions for extraction. The grafted plants were 43 d old at harvest. LE (mean ± SE) and *n* refer to the number of expanded leaves at harvest and the number of plants, respectively.

	IAA			
	WT/WT	WT/*rms-2*	*rms-2*/WT	*rms-2/rms-2*
	ng g^{-1} fresh weight			
Apical portion	9.0	9.5	16.6	13.1
Node at the highest expanded leaf	12.7	11.2	30.5	22.9
Node below the highest expanded leaf	6.8	11.9	30.3	20.2
TLL (mm)	227 ± 12.8	248 ± 36.2	379 ± 25.5	768.5 ± 41.5
LE (nodes)	10.5 ± 0.15	10.9 ± 0.18	11.5 ± 0.21	11.1 ± 0.14
n	6	7	6	8

Table V. *Effect of decapitation on the level of IAA in the nodal segment at the node of the highest expanded leaf of WT (cv Torsdag) plants*

Half of the 49-d-old plants were decapitated 1 cm above the highest expanded leaf and segments at this node (usually node 18) were harvested for each treatment 2 d later. Axillary buds at this node were removed and weighed. Plants were grown in an 8-h (daylight) photoperiod. $n = 6$.

	Decapitated	Intact
IAA ng g^{-1} fresh weight	16.7	79.3
Fresh weight nodal segment (mg)	211.1 ± 11.5	232.3 ± 15.0
Fresh weight axillary bud (mg)	2.12 ± 0.37	0.60 ± 0.15

(Stimart, 1983) appears to be different than that in the *rms-2* mutant for at least two reasons. First, stocks of the nonbranching cultivar were not able to inhibit the initiation of bud growth in scions of branching cultivars, and second, the initiation of bud growth was promoted by grafting the scion of a nonbranching cultivar to the stock of branching cultivars.

These data from the *rms-2* mutant and poinsettia (Stimart, 1983) are consistent with the suggestion that the ratio of cytokinin to auxin is the factor that controls apical dominance (Klee and Estelle, 1991) if the root-derived substance(s) is (a) a cytokinin or (b) a precursor required for the synthesis of an auxin. For the *rms-2* mutant, however, the latter possibility is unlikely because not only is there little evidence for a root-derived precursor of auxin, but IAA levels were actually elevated in the mutant shoot (Tables II–IV). If the *rms-2* mutation promotes branching by causing an elevation in cytokinin levels, it may exert this effect by blocking the catabolism of active cytokinins. This possibility would also account for a number of observations including most, if not all, aspects of the mutant phenotype, including its elevated endogenous IAA levels. For example, transgenic *ipt* cytokinin-overproducing plants have reduced height and stem width, smaller roots, and smaller, darker leaves (Medford et al., 1989), similar to *rms-2* plants (Table I, Figs. 2 and 3, and observations not shown). Second, Smigocki and Owens (1989) found that the free IAA levels in 35S-*ipt*-transformed *Nicotiana plumbaginifolia* cytokinin-overproducing plants were elevated nearly 3-fold in comparison with those of control plants. Furthermore, exogenous cytokinin application has caused elevated endogenous IAA levels in nodal segments of pea (Li and Bangerth, 1992), soybean tissue cultures (Wyndaele et al., 1988), and in *Cuscuta* vines (Paliyath et al., 1989). Consequently, it is important that the cytokinin levels in the root and shoot of the *rms-2* mutant be determined.

The effect of *rms-2* on IAA level is consistent with the suggestion of Prasad et al. (1993) that, in *Ipomoea nil*, a reduction in free IAA level may not be necessary for the release of lateral buds from apical dominance. Indeed, in many instances, the elevated free IAA levels in pea appear to be more closely related to the promotion, rather than the inhibition, of axillary bud release. For example, the *rms-2* branching mutant has elevated levels of IAA both prior to and during axillary bud release. Second, the ontogenetic stage at which release of aerial axillary buds occurs in the mutant correlates with elevated IAA levels in the WT; and the stem region (nodes 4–5), where little or no branching occurs in either *rms-2* or WT plants, corresponds to the nodes where IAA levels are relatively low in WT plants. However, branching in *rms-2* scions was strongly inhibited by grafting to WT stocks, even though the accumulation of IAA (and reduced internode length) still occurred in the shoot (Tables I and IV). Furthermore, elevated IAA levels in pea do not necessarily result from a commitment to axillary bud outgrowth because decapitation caused a large drop in the level of free IAA in WT plants (Table V). Because quite convincing evidence exists that supports a role for IAA in the inhibition of bud growth (Romano et al., 1991, 1993; Sitbon et al., 1992), the elevated IAA levels in the *rms-2* mutant may result from the involvement of IAA in a mechanism that is part of the process of maintaining apical dominance. For example, they may arise as a consequence of a feedback mechanism or they may result from entering one of the several stages of axillary bud release

Table VI. *Level of free IAA in WT (cv Torsdag) plants at various ontogenetic stages*

Portions harvested were segments at the node below the highest expanded leaf, node 5, and nodes 1 and 2. The segments were as described in Table II, except for the node 1 and 2 segment, which consisted of a section of stem from 1 cm above to 1 cm below the scale leaves. LE (mean ± SE) refers to the number of leaves expanded on the day of harvest. n = number of plants. n.a., Not applicable; n.d., not done.

	IAA				
	10 d	21 d	36 d	53 d	64 d
	ng g^{-1} fresh weight				
Node below the highest expanded leaf	17[a]	104	196	81	86
Node 5	n.a.[a]	37[b]	37	37	23
Nodes 1 and 2	17[a]	43	40	n.d.	n.d.
LE (nodes)	<3	6.2 ± 0.1	10.6 ± 0.1	15.3 ± 0.1	18.6 ± 0.2
n	39	25	21	16	14

[a] Data from nodes 1 and 2. [b] Value represented is that determined for node 4; the node below data are for node 5 at age 21 d.

described by Stafstrom and Sussex (1988) and Stafstrom (1993).

We suggest that the shorter, thinner internodes of *rms-2* plants are a consequence of the elevated IAA level in these plants, as in the transgenic IAA-overproducing (*iaaH* and *iaaM*) tobacco plants (Sitbon et al., 1992). This suggestion is consistent with the internode length, branching habit, and IAA level in grafted *rms-2* and WT plants. First, the absence of lateral branches in *rms-2*/WT plants did not cause an increase in internode length, as might have been expected from compensatory growth (Jacobs and Bullwinkel, 1953; Beveridge et al., 1992). Second, elevated levels of IAA remained in *rms-2*/WT plants.

In conclusion, the *rms-2* mutation appears to promote branching in pea by altering the synthesis of a mobile substance that influences axillary bud outgrowth. The level of this substance, which is translocated to the shoot from the roots and/or cotyledons, also appears to be altered by gene action in the shoot. In addition, the *rms-2* mutation results in elevated levels of free IAA and reduced internode length in the shoot, regardless of axillary bud growth. This mutant should prove to be a valuable tool in the elucidation of the control of apical dominance in pea, and in particular may provide more insight into the role of auxin in the process. The fact that the *rms-2* mutant has elevated IAA levels may aid in studies on the regulation of endogenous IAA levels.

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