The rms1 Mutant of Pea Has Elevated Indole-3-Acetic Acid Levels and Reduced Root-Sap Zeatin Riboside Content but Increased Branching Controlled by Graft-Transmissible Signal(s)

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Rms1 is one of the series of five ramosus loci in pea (Pisum sativum L.) in which recessive mutant alleles confer increased branching at basal and aerial vegetative nodes. Shoots of the non-allelic rms1 and rms2 mutants are phenotypically similar in most respects. However, we found an up to 40-fold difference in root-sap zeatin riboside ([9R]Z) concentration between rms1 and rms2 plants. Compared with wild-type (WT) plants, the concentration of [9R]Z in rms1 root sap was very low and the concentration in rms2 root sap was slightly elevated. To our knowledge, the rms1 mutant is therefore the second ramosus mutant (rms4 being the first) to be characterized with low root-sap [9R]Z content. Like rms2, the apical bud and upper nodes of rms1 plants contain elevated indole-3-acetic acid levels compared with WT shoots. Therefore, the rms1 mutant demonstrates that high shoot auxin levels and low root-sap cytokinin levels are not necessarily correlated with increased apical dominance in pea. A graft-transmissible basis of action has been demonstrated for both mutants from reciprocal grafts between mutant and WT plants. Branching was also largely inhibited in rms1 shoots when grafted to rms2 rootstocks, but was not inhibited in rms2 shoots grafted to rms1 rootstocks. These grafting results are discussed, along with the conclusion that hormone-like signals other than auxin and cytokinin are also involved.

Apical dominance is usually defined as the mechanism whereby the apex reduces the extent of lateral branching or axillary bud release and growth. The precise factors that control whether a vegetative bud will form and subsequently grow out remain unclear (for review, see Stafstrom, 1993; Cline, 1994), and even the term “apical dominance” seems to underestimate the possibility that the roots play an important role in the control of branching. Most textbooks conclude that the ratio of (shoot-derived) auxin to (root-derived) cytokinin probably controls branching (e.g., Salisbury and Ross, 1992). However, studies providing evidence for secondary or novel signals (e.g., Morris, 1977) have frequently been overlooked in an attempt to provide a simplified model.

Although several branching mutants have been investigated recently (e.g., the axr1 mutants of Arabidopsis [Estelle and Somerville, 1987; Leyser et al., 1993]; amn1 from Arabidopsis [Chaudhury et al., 1993]; das1 from Petunia hybrida [Napoli, 1996]; and the ramosus mutants of pea [Pisum sativum L.] [Beveridge et al., 1994, 1996]), a genetic model has not been developed for the control of apical dominance. We are currently investigating five different ramosus genes that maintain apical dominance in the garden pea. Mutations at these loci cause increased branching at basal and aerial nodes (rms1, rms2, rms3, rms4, and rms5; Blixt, 1976; Apisitwanich et al., 1992; Arumngtyas et al., 1992).

The rms2, rms3, and rms4 mutants have been described by Beveridge et al. (1994, 1996). They differ from WT plants mainly in regard to increased lateral bud release and growth. The mutant shoots are not deficient in IAA. One of the mutations, rms2, controls the level of a graft-transmissible substance because WT rootstocks inhibit branching in rms2 shoots (Beveridge et al., 1994). The Rms2 gene also acts in the shoot because rms2 rootstocks do not promote branching in WT shoots. Mutant rms2 plants have elevated IAA levels (2- to 5-fold) in the shoot. The rms3–2 mutation causes a slight elevation in IAA level (up to 2-fold) in the shoot, and the length of lateral branches in rms3–2 shoots is reduced when grafted to WT rootstock. Grafting studies between rms2 and rms3–2 seedlings indicate that the Rms2 and Rms3 gene products are different (Beveridge et al., 1996). Considering that neither mutation promotes branching by controlling IAA levels, and that the mutations appear to affect the level of different graft-transmissible substances produced in the root and shoot, it is possible that a substance other than IAA and cytokinin may also be involved in the control of branching in pea.

Recent studies (Beveridge et al., 1997) have provided evidence for a different graft-transmissible signal that regulates cytokinin export from the roots. In this case the signal moves from shoot to root. Grafting studies showed
that the \textit{rms4} mutation promotes branching by a primary action in the shoot (Beveridge \textit{et al.}, 1996), but that \textit{rms4} shoots also cause a large down-regulation (to 40-fold less [9R]Z) in the export of cytokinins from both WT and \textit{rms4} roots (Beveridge \textit{et al.}, 1997). The ability of the shoot to regulate cytokinin levels in the roots was also shown by the fact that \textit{rms4} roots have normal (similar to WT) xylem sap cytokinin concentrations when grafted to WT shoots. IAA quantifications in \textit{rms4} and WT plants indicated that IAA is not the signal that controls the export of cytokinin from the roots in \textit{rms4} plants (Beveridge \textit{et al.}, 1997). It was suggested that the low cytokinin concentration in the root sap of \textit{rms4} plants may be a feedback consequence of the commitment to branching in \textit{rms4} shoots.

Currently, 11 mutant alleles have been assigned to the \textit{Rms1} locus (Arumingtyas \textit{et al.}, 1992; Rameau \textit{et al.}, 1997; Symons and Murfet, 1997). This paper concerns the physiological characterization of the \textit{Rms1} mutant phenotype. Most of the work presented involves experiments with the type-line \textit{rms1–1}, which was derived from cv Parvus (Blixt, 1976). The \textit{rms2–2} mutant was also derived from cv Parvus, enabling a direct comparison between \textit{rms1–1} and \textit{rms2–2} plants. We also present results from reciprocal grafts of \textit{rms1–1} or \textit{rms1–2} seedlings with various \textit{rms2}, \textit{rms4}, and WT seedlings, report on endogenous IAA levels, and characterize \textit{rms1} and \textit{rms2} plants in terms of the cytokinin concentration in the xylem sap of the roots. The possible interactions between the \textit{Rms1} and \textit{Rms2} genes and the importance of IAA and root-derived [9R]Z in the control of branching are discussed.

**MATERIALS AND METHODS**

The mutant lines used in this study were derived from tall, photoperiodic, WT pea (\textit{Pisum sativum} L.) cultivars by S. Blixt or K.K. Sidorova using radiography or ethyl methanesulfonate. The WT cultivars have a late-flowering, quantitative, long-day habit. Further details are given by Arumingtyas \textit{et al.} (1992). The \textit{rms1}, \textit{rms2}, and \textit{rms4} mutants are all recessive and are characterized by a fairly similar pattern of increased branching. The mutant lines used were WL5237 (\textit{rms1–1}) and WL5951 (\textit{rms2–2}) from cv Parvus, WL5147 (\textit{rms1–2}) from cv Weitor, and K524 (\textit{rms2–1}) and K164 (\textit{rms4–1}) from cv Torsdag. The double-mutant line HL252, which has the genotype \textit{rms1–1 rms2–2–}, was derived from the cross WL5237 $\times$ WL 5951. The genotype of HL252 was confirmed by backcrossing with both parents.

The plants were grown as described previously (Beveridge \textit{et al.}, 1994, 1997) in heated greenhouses with a 16- or 18-h photoperiod. Nodes were numbered acropetally from the first scale leaf as node 1, and lateral lengths were measured from the base of the lateral (in the leaf axil) to the apex of the lateral shoot. Unless specified otherwise, lateral branches were removed from nodes 1 to 3 over the first 2 to 3 weeks after planting to encourage uniform growth of the main shoot.

**Grafting Technique**

Grafts were performed prior to any macroscopic sign of bud release, as described by Beveridge \textit{et al.} (1994). The freshly harvested tissue was weighed and placed directly into MeOH/BHT at $-20^\circ$C with the deuterated internal standard (indole-[2, 4, 5, 6, 7-$^2$H$_4$]-IAA; synthesized by Merck and Co., Rahway, NJ). The purification procedure included a Sep-Pak C$_8$ cartridge step prior to methylation with ethereal diazomethane. The dry IAA methyl ester was dissolved in 200 $\mu$l of distilled water and partitioned three times against ether. The ether fraction was dried under a stream of $N_2$ before being dissolved in 3 $\mu$L of dry pyridine and trimethylsilylated with 10 $\mu$L of $N,O$-bis(trimethylsilyl)acetamide. After GC-MS-SIM analysis, the endogenous IAA levels were calculated by comparison with the internal standard using the isotope-dilution method described in Cohen \textit{et al.} (1986).

**Root-Xylem Sap [9R]Z Analysis**

The xylem sap was harvested from the roots by the syringe-suction method during a 1-h period immediately after decapitation below node 1, as described by Beveridge \textit{et al.} (1997). Deuterated ($^2$H$_4$) [9R]Z was added (2 ng mL$^{-1}$ or 5.7 pmol mL$^{-1}$) to the crude sap and the samples were partially purified by using a Sep-Pak C$_8$ cartridge and eluting with 80% methanol. [9R]Z was quantified by GC-electron impact MS-SIM as described by Beveridge \textit{et al.} (1997).

**RESULTS**

**Mutant Phenotype**

In most respects the phenotype of \textit{rms1–1} plants is similar to that of \textit{rms2–2} plants (Fig. 1). Plants of both genotypes branch vigorously at nodes 1 and 2 and, depending on environmental conditions, tend not to branch from nodes 3 and 4 but recommence branching at about node 5 and above (Fig. 2). Bud release and outgrowth in wholly vegetative mutant plants appears to be linked to the development of leaves and internodes in the plastochron and to the overall vigor of the plant, rather than to any developmental stage or signal. However, the rate and vigor of lateral bud release and growth appears to increase in all genotypes with the onset of flowering and, like Stafstrom (1993), we found that even cv Parvus plants may branch at

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the uppermost vegetative nodes just below the flowering node.

Branching tends to be more vigorous in rms1-1 plants than in rms2-2 plants, demonstrated mainly from the length of lateral branches at and above node 5 (Fig. 2). This was partly due to longer internodes and partly to a greater number of expanded leaves on the lateral branches of rms1-1 plants. Under 18-h conditions the internodes on the main stem of rms1-1 plants were 10 to 30% shorter than those of comparable WT plants, and were longer than rms2-2 plants at nodes 12 to 15, just below the flowering node (Fig. 3). In most genetic backgrounds and during most developmental stages, the rate of leaf expansion of rms1 plants tended to be slightly less than that of WT plants but higher than that of rms2 plants. The rms1 plants have a normal flowering response to photoperiod (Arumingtyas et al., 1992) and only occasionally have a reduction in the node of flower initiation in comparison with WT plants, possibly related to differences in the rate of leaf expansion (Table I).

Mutant rms1-1 plants have thinner stems (Table I; Blixt, 1976) and shorter leaflet lengths (Table I) than those of cv Parvus plants. However, the reduction in leaflet area in rms1 plants is not as great as in rms2 plants (Table I; Beveridge et al., 1996). The total dry weight of rms1-2 seedlings was about 15% less than that of comparable WT seedlings, but the ratio of shoot-to-root dry weight was not significantly altered (Table II).

Phenotype of rms1 rms2 Double-Mutant Plants

The shoot of rms1-1 rms2-2 double-mutant plants showed increased branching compared with rms1-1 and rms2-2 plants (Fig. 1). In a 12-h natural photoperiod, rms2-2 plants branched vigorously from the basal nodes (3-5 branches from nodes 1-3) and rms1-1 plants produced a single branch at each vegetative node. Figure 2 shows the lateral lengths at each vegetative node of cv Parvus, rms1-1, and rms2-2 plants. Figure 3 shows the stem lengths between nodes 1 to 6, 6 to 9, 9 to 12, and 12 to 15 of the main stem of the 64-d-old cv Parvus, rms1-1, and rms2-2 plants.
double-mutant plants had a total of 5 to 7 branches at nodes 1 to 3, and also produced a branch at each subsequent vegetative node. Double-mutant rms1–1 rms2–2 plants also developed second-order lateral branches, confirming the observations of Stafstrom (1993) based on putative double mutants between lines WL5237 and WL5951.

### IAA Level

The endogenous IAA level was determined from an apical portion and nodal segments at the axil of each of the two uppermost expanded leaves of cv Parvus, rms1–1, and rms2–2 plants when the plants had 7 to 8 leaves expanded (Fig. 4). No evidence was obtained to suggest that rms1–1 plants might have been deficient in IAA. Rather, the endogenous IAA level in rms1–1 plants was significantly higher than in WT plants (Fig. 4). The difference between rms1–1 and WT plants was greatest (up to 2-fold) for the nodal segments. Similar results were obtained when rms1–2 plants were compared with their initial line, cv Weitor (data not shown). The IAA level in rms2–2 plants was higher than in rms1–1 plants and about 3-fold greater than in WT plants. This is consistent with previous results for rms2–1 (Beveridge et al., 1994).

### Xylem Sap Cytokinin Levels

The concentration of [9R]Z was determined in the xylem sap of 28-d-old cv Parvus, rms1–1, and rms2–2 plants and rms2–2 double-mutant plants by GC-electron impact MS-SIM analysis (Fig. 5). The cytokinin concentration in the xylem sap of rms1–1 was decreased (down to 15-fold) in comparison with the concentration in the sap of WT plants. In contrast, the concentration in rms2–2 plants was increased to about 2- to 3-fold. Consequently, the difference in xylem sap [9R]Z concentration between rms1 and rms2 plants was about 40-fold. The cytokinin concentration in the rms1–1 rms2–2 double-mutant plants was intermediate between that of WT and rms2–2 plants, and therefore considerably greater than in rms1 plants.

The unlikely possibility that other cytokinins in the root sap of rms1 plants may be elevated while the [9R]Z content is reduced was not supported by ELISA quantifications (data not shown). ELISA analysis (Beveridge et al., 1997) with an anti-[9R]Z antibody with cross-reactivity against [9R]Z, 5′-monophosphate of [9R]Z, zeatin, and dihydrozeatin (Besse et al., 1992) confirmed the above trends for individual plants (n = 8–10). Similar differences exist in the crude root sap from individual rms1–10 (M3T-884) and WT (cv Térèse) plants (data not shown).

The cytokinins in rms2 root sap require further investigation to determine if the flux of cytokinins to the shoot is significantly altered. The high concentration of [9R]Z in the root sap (2.5-fold) is partly due to the smaller root size (Beveridge et al., 1994), and the physiological importance

### Table I. Stem width, leaflet length, number of leaves expanded, and node of flower initiation of cv Parvus, rms1–1, and rms2–2 plants

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Stem Width</th>
<th>Leaflet Length</th>
<th>Leaflet Breadth</th>
<th>No. of Leaves Expanded</th>
<th>Node of Flower Initiation</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>cv Parvus</td>
<td>3.53 ± 0.07a</td>
<td>33.2 ± 0.9a</td>
<td>22.9 ± 0.6a</td>
<td>21.8 ± 0.2a</td>
<td>17.8 ± 0.2a</td>
<td>8</td>
</tr>
<tr>
<td>rms1–1</td>
<td>2.91 ± 0.07b</td>
<td>27.4 ± 0.8b</td>
<td>23.1 ± 1.0a</td>
<td>20.6 ± 0.2b</td>
<td>16.3 ± 0.2b</td>
<td>7</td>
</tr>
<tr>
<td>rms2–2</td>
<td>2.90 ± 0.06b</td>
<td>23.8 ± 0.4c</td>
<td>19.5 ± 0.2b</td>
<td>20.1 ± 0.1b</td>
<td>17.0 ± 0.2c</td>
<td>7</td>
</tr>
</tbody>
</table>

### Table II. Total dry weight and shoot-to-root dry weight ratio of cv Weitor and rms1–2 seedlings

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Total Dry Weight</th>
<th>Shoot:Root Ratio</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>cv Weitor</td>
<td>174.0 ± 6.8a</td>
<td>1.38 ± 0.08a</td>
<td>15</td>
</tr>
<tr>
<td>rms1–2</td>
<td>147.3 ± 4.5b</td>
<td>1.09 ± 0.24a</td>
<td>16</td>
</tr>
</tbody>
</table>

### Figure 4. IAA level in cv Parvus, rms1–1, and rms2–2 shoots. Portions harvested were the apical portion (Apical), which was that portion above the oldest unexpanded leaf; the node at the highest expanded leaf (Node A); and the node below the highest expanded leaf (Node B). The nodal segments consisted of a 1-cm portion of the petiole and stem each side of the node. The plants had seven to eight leaves expanded and were 23 d old at the time of harvest. Data represented are the mean of three different pools of either seven or eight plants.
of the elevated concentrations is lessened in view of the fact that the quantity and flow rate of the xylem sap is slightly reduced in rms2 plants (see Jackson, 1997). In contrast, the up to 15-fold drop in cytokinin concentration in the sap of rms1 plants may be an underestimation (rather than an overestimation) of the total reduction in cytokinin export from the roots, as the xylem sap flow rate is also slightly reduced in rms1 plants. Nevertheless, there was no significant change in the root/shoot dry weight ratio of rms1 plants compared with WT plants (Table II).

**Figure 5.** \[9R\]Z concentration in the pooled root-xylem sap of cv Parvus, rms1-1, rms2-2, and rms1-1 rms2-2 (double-mutant) plants. The plants had about nine leaves expanded and were 28 d old at the time of harvest; \(n = 8\) to 10.

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**Grafting Studies and the Site of Gene Action**

cv Parvus rootstocks caused a substantial inhibition of branching (bud release and/or growth) at nodes up to and including node 12 of rms1-1 scions, whereas rms1-1 rootstocks had little or no effect on branching in cv Parvus scions (Fig. 6). In the cv Weitor background, where WT plants have very strong apical dominance, branching throughout the main stem axis was almost completely inhibited in rms1-2 scions by grafting to WT rootstocks (data not shown; see Fig. 7 for rms1-2 scions on cv Torsdag rootstocks). As reported previously for rms2-1 and cv Torsdag (Beveridge et al., 1994), branching in rms2-2 shoots was also substantially reduced when grafted to cv Parvus rootstocks, and rms2-2 rootstocks did not promote branching in WT scions. The total lateral lengths of rms1 or rms2 shoots on WT rootstocks were significantly greater than for WT shoots on mutant rootstocks, indicating that Rms1 and Rms2 gene activity in the shoot was more effective at inhibiting branching than when present only in the roots (Fig. 8). Because of differences in growth at the upper nodes (Fig. 6), the total lateral length of these inhibited rms2-2 shoots (graft treatment, rms2-2/cv Parvus) was significantly greater than for inhibited rms1-1 scions grafted to cv Parvus stocks (Fig. 8).

In plants grown from reciprocal grafts between rms1-1 and rms2-2 seedlings, rms2-2 rootstocks significantly reduced the total lateral shoot length and inhibited bud release at several nodes along the stem of rms1-1 scions.
Mutant \textit{rms1} plants have slightly elevated IAA levels in the shoot (Fig. 4) and a very reduced root-sap [9R]Z content (Fig. 5). Therefore, in the four \textit{ramosus} mutants studied to date, there is an increase in shoot IAA levels (\textit{rms2} [Beveridge et al., 1994] and \textit{rms3–2} [Beveridge et al., 1996]), a decrease in root-sap [9R]Z levels (\textit{rms4} [Beveridge et al., 1997]), or both (\textit{rms1}). Superficially, these changes are opposite to those predicted if auxin inhibits and cytokinin promotes axillary bud outgrowth. Nevertheless, the changes do not preclude a role for these hormones in the regulation of branching. Rather, it is suggested that the altered root-sap [9R]Z and shoot IAA content in mutant plants may be part of feedback mechanism(s) involved in down-regulating branching (Beveridge et al., 1994, 1997).

However, we suggest that the auxin/cytokinin ratio model (e.g. Sachs and Thimann, 1967) is an oversimplification of the control system, at least in pea. Studies with \textit{rms1} (described below) and \textit{rms2–1} and \textit{rms3–2} (Beveridge et al., 1996) indicate that at least one other graft-transmissible signal is involved. Branching was extensively inhibited in \textit{rms1} shoots grafted to WT rootstocks and in WT shoots grafted to \textit{rms1} stocks, indicating that the \textit{Rms1} gene can inhibit branching whether it is present in the rootstock or shoot (Fig. 8). \textit{Rms1} gene activity therefore occurs in the roots and the shoots. An end product of this gene activity is graft transmissible and causes an inhibition of branching. Although WT roots can inhibit branching in \textit{rms1} shoots, WT shoots do not require import of the \textit{Rms1} product from the roots because branching was completely inhibited in the WT/\textit{rms1} combination (scion/stock; Fig. 8). \textit{Rms1} gene activity was more effective at inhibiting branching when expressed in the shoot because branching was slightly greater in \textit{rms1}/WT plants than in WT/\textit{rms1} plants (Fig. 8).

The ability of WT roots to inhibit branching in \textit{rms1} mutant shoots indicates that \textit{rms1} and WT roots probably differ in the quantity or quality of the signal that is transmitted from root to shoot. This signal is not likely to be [9R]Z because low root-sap [9R]Z content (such as in \textit{rms1}; Fig. 5) is usually associated with increased rather than decreased apical dominance (e.g. Sachs and Thimann, 1967; Medford et al., 1989; Upadhyaya et al., 1991). The graft-transmissible basis of \textit{Rms1} action also indicates that the primary action of the \textit{Rms1} gene is more likely to control hormone supply to the bud rather than hormone reception and/or subsequent signal transduction within the bud itself. This does not preclude the possibility that the mutation causes an altered response to IAA and/or cytokinin.

Further studies may investigate the auxin and cytokinin responsiveness of the \textit{ramosus} mutants, but the interpretation of such results should not lose sight of the presence of altered levels of graft-transmissible signals (e.g. in \textit{rms1} and \textit{rms2}), which may modify the action of other hormones. The basipetal transport of \textsuperscript{3}H-IAA applied to the apical bud of \textit{rms1–1} plants occurs at a similar rate to that in comparable WT plants (G.M. Symons and C.A. Beveridge, unpublished results), indicating that \textit{Rms1} does not control the transport of IAA from the shoot apex.

\begin{figure}
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\includegraphics[width=\textwidth]{figure8}
\caption{Total lateral length (a) and main stem length (b) at d 38 of the reciprocally grafted cv Parvus, \textit{rms1–1}, and \textit{rms2–2} seedlings represented in Figure 6. Columns represent cv Parvus (black), \textit{rms1–1} (striped), and \textit{rms2–2} (shaded) rootstocks. Data are means ± st.}
\end{figure}
The graft-transmissible basis of Rms1 action, combined with the very low root-sap [9R]Z concentration and the high shoot IAA, indicates that a third signal may be involved in the control of apical dominance in pea. The possibility that cytokinins and IAA control the growth of buds after the first developmental stage of bud release (see Stafstrom, 1993) or in combination with other signals is supported by the phenotype of juvenile transgenic 35S-cytokinin-overproducing (ipt) and IAA-deficient (ial/l) tobacco (Nicotiana tabacum L.) plants. Transgenic ipt tobacco plants with the constitutive 35S promoter do not exhibit increased branching until the onset of flowering, even though they contain elevated cytokinin levels at the juvenile stage (Medford et al., 1989). Romano et al. (1991) stated that the difference in branching in 35S-ial/l tobacco plants, which have a similar phenotype to 35S-ipt plants, was the result of an increase in bud growth, rather than a promotion of bud release.

Mutant rms1 and rms2 plants differ greatly in the concentration of root-sap [9R]Z, but both mutations cause increased branching by altering the level of graft-transmissible signals (Fig. 8; Beveridge et al., 1994). In both cases, branching is inhibited in reciprocal graft combinations between mutant and WT seedlings (rms2/WT, WT/rms2). A relationship between the graft-transmissible signals controlled by the Rms1 and Rms2 genes was revealed by reciprocal grafting among rms1, rms2, and WT seedlings (Figs. 6–8; summarized in Fig. 9). Shoots of the combination rms1/rms2 had a nearly WT phenotype, whereas those of the reciprocal combination, rms2/rms1, had a ramosus phenotype. The expression of the ramosus phenotype in the rms2/rms1 graft combination is highly significant because it is the only combination in which an rms1 or rms2 shoot is not inhibited by grafting to any WT or mutant (rms1, rms2, rms3, or rms4) rootstock (except for the self-grafts). The inhibition of branching in rms1 scions by grafting to rms2 rootstocks implies that the Rms1 gene remains functional in the rms2 rootstock and that the Rms2 gene also remains functional in the rms1 shoot. However, the ramosus phenotype of rms2/rms1 plants indicates that the Rms1 and Rms2 genes do not act independently.

The additive branched phenotype of rms1−1 rms2−2 double-mutant plants compared with the single mutants is indicative of genes that control different signals (Millar et al., 1994). Alternatively, if the mutations acted on the same biochemical pathway and were leaky, an additive phenotype would also be obtained (Millar et al., 1994). Nevertheless, the explanation for the branching phenotype of rms2 shoots on rms1 rootstocks may be that activity of one of the Rms1 and Rms2 genes requires a signal (or precursor) controlled by the other gene. Furthermore, the Rms1 and Rms2 gene products do not appear to be transported in both directions between root and shoot. Indeed, branching in pea may include both a shoot-to-root signal (Beveridge et al., 1997) and a root-to-shoot signal (Fig. 8; Beveridge et al., 1994).

Unlike rms1 and rms2, the rms4 mutation appears to promote branching by an action confined to the shoot and may therefore act by controlling processes within the buds themselves (Beveridge et al., 1996, 1997). Like rms1, the rms4 mutation also causes reduced root-sap cytokinin concentrations (Beveridge et al., 1997). Using the rms4 mutant, we have shown that the shoot transmits a signal to the roots to regulate cytokinin export and have suggested that this signal forms part of a mechanism intended to regulate branching. The possibility that the low [9R]Z content (Fig. 5) in the root sap of rms1 plants is also a consequence of this process, whereas rms2 plants (which do not have reduced root-sap [9R]Z) are blocked in a part of the shoot-to-root cytokinin regulatory system, is the subject of future investigations. The high root-sap [9R]Z content in intact rms1−1 rms2−2 double-mutant plants compared with rms1−1 plants is consistent with this possibility, as it indicates that the rms2 mutation may inhibit the down-regulation of root-sap cytokinin content, which occurs in rms1 plants.

The 40-fold difference in the concentration of the most abundant pea xylem sap cytokinin ([9R]Z (Beveridge et al., 1997 and references therein)) in rms1 compared with rms2 root sap is not correlated with any major differences in shoot phenotype. Similarly, apart from the increased branching, rms4 plants have a normal phenotype, despite containing low root-sap cytokinin concentrations. Cytokinin homeostasis studies on whole plants are needed to determine the importance of root-derivived cytokinins and the extent of the shoot's ability to control its own cytokinin levels. Even with the present incomplete data on cytokinin content in pea (see also Davies et al., 1986) it is worth considering that for many developmental processes, the supply of cytokinins from the root to the shoot may not be as important as biosynthetic (Chen et al., 1985) and/or metabolic processes within the shoots themselves (Palni et al., 1988).

In summary, branching in plants appears to be the outcome of a highly regulated process. IAA influences the metabolism of cytokinins in the shoot (Palni et al., 1988; Zhang et al., 1995) and high doses of applied IAA influence the transport of cytokinins from the root to the shoot of
decapitated plants (Bangerth, 1994). IAA levels are also altered in cytokinin-overproducing \( ipt \) plants (Smigocki and Owens, 1989). Consequently, there is a high degree of interaction between cytokinin and auxin. However, the situation is probably even more complex because there is considerable evidence from the \( rms4 \) mutant of pea for the presence of a third (non-IAA) signal, which is exported from the shoot and down-regulates cytokinin export from the roots (Beveridge et al., 1997). Furthermore, the present study indicates that \( rms1 \) plants have altered levels of yet another signal that is produced throughout the plant and can move from root to shoot and influence branching.

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**LITERATURE CITED**


Beveridge CA, Ross JJ, Murfet IC (1994) Branching mutant \( rms-2 \) in \( P. sativum \). Grafting studies and endogenous indole-3-acetic acid levels. Plant Physiol 104: 953–959


Blik S (1976) Linkage studies in \( P. sativum \). XV. Establishing the \( Rms \) gene and linkage of \( rms \) and \( fas \) in chromosome 3. Agric Hortique Genetica 34: 83–87


Cohen JD, Baldi BG, Slovin JP (1986) \( ^{15} \text{C}_2-[\text{benzene ring}]-\text{indole-3-acetic acid}. A \text{new internal standard for quantitative mass spectrometric analysis of indole-3-acetic acid in plants. Plant Physiol} \) 80: 14–19


Rameau C, Bodelin C, Cadier D, Grandjean O, Miard F, Murfet IC, (1997) New \( rms \) mutants at loci \( Rms1 \), \( Rms3 \), and \( Rms4 \) resulting from the mutation breeding program at Versailles. Pisum Genet 29 (in press)


Upadhyaya NM, Rao JVDA, Dari FP, Letham DS, Kumar-Rao J (1991) Leaf curl syndrome of pigeonpea (\( Cajanus cajan \) Millsp.) is a systemic response to effective nodulation by the \( Rhizobium \) strain IC3342. Physiol Mol Plant Path 36: 357–373