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Pea *rms6* mutants exhibit increased basal branching

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Our studies on two branching mutants of pea (*Pisum sativum* L.) have identified a further *Ramosus* locus, *Rms6*, with two recessive or partially recessive mutant alleles: *rms6-1* (type line S2-271) and *rms6-2* (type line K586). Mutants *rms6-1* and *rms6-2* were derived from dwarf and tall cultivars, Solara and Torsdag, respectively. The *rms6* mutants are characterized by increased branching from basal nodes. In contrast, mutants *rms1* through *rms5* have increased branching from both basal and aerial (upper stem) nodes. Buds at the cotyledonary node of wild-type (WT) plants remain dormant but in *rms6* plants these buds were usually released from dormancy. Their growth was either subsequently inhibited, sometimes even prior to emergence above ground, or they grew into secondary stems. The mutant phenotype was strongest for *rms6-1* on the dwarf background. Although *rms6-2* had a weak single-mutant phenotype, the *rms3-1 rms6-2* double mutant showed clear transgression and an additive branching phenotype, with a total lateral length almost 2-fold greater than *rms3-1* and nearly 5-fold greater than *rms6-2*. Grafting studies between WT and *rms6-1* plants demonstrated the primary action of *Rms6* may be confined to the shoot. Young WT and *rms6-1* shoots had similar auxin levels, and decapitated plants had a similar magnitude of response to applied auxin. Abscisic acid levels were elevated 2-fold at node 2 of young *rms6-1* plants. The *Rms6* locus mapped to the *R* to *Gp* segment of linkage group V (chromosome 3). The *rms6* mutants will be useful for basic research and also have possible agronomical value.

Introduction

Five *Rms* (*Ramosus*) loci have been reported in pea (*Pisum sativum* L.) (Blixt 1976, Apisitwanich et al. 1992, Arumingtyas et al. 1992), with between two and 11 mutant alleles assigned to each locus (Rameau et al. 1997, Symons and Murfet 1997). Mutants at these five loci have reduced apical dominance and branch extensively from both the basal and aerial nodes. In some cases, lateral shoots are produced at every vegetative node starting from node 1 (Arumingtyas et al. 1992).

The *rms* pea mutants offer an excellent opportunity to explore the physiological control of branching. A model for branching in pea has recently been proposed based on studies with mutants *rms1* through *rms5* (Beveridge 2000, Morris et al. 2001). This model attributes roles for two novel graft-transmissible signals. Grafting studies demonstrate that a wild-type (WT) rootstock can inhibit bud release in *rms1*, *rms2* and *rms5* shoots, indicating that *Rms1*, *Rms2* and *Rms5* regulate the level of graft-transmissible substance(s) (Beveridge et al. 1994, 1996, 1997b, Morris et al. 2001). Grafting a small WT epicotyl interstock between an *rms1* scion and rootstock also inhibits branching (Foo et al. 2001). These grafting studies, together with endogenous cytokinin and auxin analyses, have demonstrated that *Rms1* and *Rms5* have similar physiological actions and control the level or transport of the same novel branching signal that moves in a root-to-shoot direction (Beveridge et al. 1997b, Morris et al. 2001). Like mutants *rms3* and *rms4*, *rms1* and *rms5* have reduced xylem sap cytokinin concentrations (Beveridge et al. 1997a, 1997b, Beveridge 2000, Morris et al. 2001). Furthermore, all five *rms* mutants do not appear auxin deficient (Beveridge et al. 1994, 1996, 1997a, 1997b, Morris et al. 2001), but the graft-transmissible signals regulated by *Rms1* and *Rms2* do

Abbreviations – EMS, ethyl methane sulphonate; WT, wild type.

affect the response of decapitated plants to exogenous auxin (Beveridge et al. 2000).

Unlike *Rms1*, *Rms2* and *Rms5*, genes *Rms3* and *Rms4* appear to act mostly in the shoot as branching is not inhibited in *rms3* and *rms4* scions when grafted to WT rootstocks (Beveridge et al. 1996, 1997a). Reciprocal grafts between *rms3, rms4* and WT plants show that the level of cytokinin exported by the root is determined by the shoot, as an *rms3* or *rms4* rootstock grafted to a WT shoot exports normal levels of zeatin riboside while the level of zeatin riboside in the xylem sap of WT roots is depressed 10- to 40-fold by grafting to mutant *rms4* shoots (Beveridge et al. 1997a, Beveridge 2000). Mutant *rms1* scions grafted to WT rootstocks do not cause depleted xylem sap cytokinin levels but in this case the mutant shoots have a reduced branching phenotype compared with mutant self-grafts (Beveridge 2000). These results suggest that a shoot-to-root messenger imposes feedback control over root cytokinin export. This messenger may not be auxin as *rms1*, *rms3*, *rms4* and *rms5* shoots possess essentially normal levels of indole-3-acetic acid (IAA) and have normal rates of basipetal IAA-transport (Beveridge et al. 2000, Morris et al. 2001, S. Morris, C. Turnbull and C. Beveridge unpublished data). Identifying the nature of possible novel compounds involved in the control of apical dominance represents a current challenge.

The dominant pea mutant *bsh* (*bushy*) branches profusely from the basal nodes but unlike the *rms* mutants, it has a strongly pleiotropic phenotype with short thin stems, tiny leaves, and reduced lateral root development (Symons et al. 1999). In contrast to mutants *rms1* through *rms5*, the *bsh* mutant is deficient in auxin and has elevated levels of cytokinin (Symons 2000).

In pea, branching phenotype is also influenced by gibberellin status and photoperiod. Mutant plants deficient in gibberellin, such as the widely used *le* dwarf cultivars, have a greater tendency to produce lateral shoots from the basal nodes than tall WT counterparts (Floyd and Murfet 1986, Murfet and Reid 1993, Murfet and Symons 2000a). Likewise, in plants with the WT, late flowering, quantitative long-day habit, production of basal lateral shoots is enhanced under short-day conditions (Floyd and Murfet, 1986, Arumingtyas et al. 1992, Napoli et al. 1999).

Here we report on a new *Rms* locus in pea, *Rms6*. In contrast to mutants *rms1* through *rms5*, *rms6* mutants show enhanced branching only at the basal nodes.

Materials and methods

Pea lines and mapping

Mutant line S2-271 (*rms6-1*) was selected at Versailles following ethyl methane sulphonate (EMS) treatment of cv. Solara. Mutant line K586 (*rms6-2*) was selected at Novosibirsk, Russia, following EMS treatment of cv. Torsdag and kindly provided by Dr K. Sidorova. Cv. Borek, multiple marker lines HL111 and HL243, and the *rms* type lines WL5237 (*rms1-1*), K524 (*rms2-1*), K487 (*rms3-1*), WL6042 (*rms3-3*), K164 (*rms4-1*) and Wt15244 (*rms5-1*) are held in the Hobart pea collection. Lines HL292 and HL293 were selected in the course of the current study from crosses K586 × K487 and S2-271 × HL111, respectively. All lines have the flowering genotype *Sn Dne Ppd hr* and behave as quantitative long-day plants. Lines Solara, S2-271, HL111, HL243, HL293, WL6042 and Wt15244 are dwarf (*le*) and Torsdag, K164, K487, K524, K586, HL292 and WL5237 are tall (*Le*). Solara and S2-271 have leaves with branched tendrils in the place of leaflets (*af*), and HL111 and HL293 have leaflets in place of tendrils (*tl*). Further details on the plant material are given by Arumingtyas et al. (1992) and Symons and Murfet (1997).

Mapping of the *Rms6* locus with conventional markers was performed using crosses with multiple marker lines HL111, HL243 and HL293 (Murfet and Rameau 2000). Mapping with RAPD markers was performed using 118 F_2 plants derived from the cross S2-271 × Torsdag; all F_2 plants were genotyped by growing 20 F_3 plants. Bulk segregant analysis was used to identify which RAPD markers, already mapped by Laucou et al. (1998), were linked to *Rms6*. Ten *rms6/rms6* and 10 *Rms6/Rms6* F_2 plants were selected for each DNA sample. DNA extraction, RAPD procedure and linkage analyses were done as described by Laucou et al. (1998) and Rameau et al. (1998).

Growing conditions and scoring methods

The growing conditions for the work in Table 1 were as described by Beveridge et al. (1997a); briefly: a 16-h photoperiod, one plant per 2-l pot filled with peat and supplied regularly with liquid nutrient (INRA, Versailles, France). Conditions for the work in Tables 2, 3 and 5 and Fig. 1 were as described by Symons et al. (1999); briefly: an 18-h photoperiod, one plant per 14-cm pot containing a 1:1 (v:v) mixture of 10-mm dolerite chips and vermiculite supplied weekly with liquid nutrient (Aquasol; Hortico Ltd, Melbourne, Australia). Conditions for the root study (Table 4) were similar except an 8-h photoperiod was used (8 h in daylight, 16 h in a dark chamber) and Aquasol was provided twice weekly. Growing conditions for the plants in Figs 3, 5 and 6 were as described by Morris et al. (2001). A natural photoperiod of approximately 13 h was used for Fig. 3. In Figs 5 and 6, the natural photoperiod was extended to 14 and 18 h, respectively, by weak incandescent light from 60-W globes. Plants were grown either 2 per 15-cm pot (Figs 3 and 5) or 4 per pot (Fig. 6). The growth medium used was a 1:1 (v:v) mixture of pasteurized sand/peat with nutrient provided by incorporation of Osmocote (2 g per pot; Scotts, Baulkam Hills, Australia).

Node counts commenced from the first scale leaf as node 1. The cotyledonary node is referred to as node 0, and day 0 as the day of planting. Nodes 0–3 are referred to as basal nodes (e.g. Table 3) whereas upper stem nodes are referred to as aerial nodes. First order lateral shoots

arise from nodes on the main stem and second order laterals from a node on a first order lateral. More than one lateral shoot may arise from one node. Lateral shoot lengths were measured from the base of the lateral to the lateral apex. Basal lateral shoots that grew strongly as rivals of the main stem were referred to as secondary stems. Further details of branching terminology in pea are given by Murfet and Symons (2000a).

Grafting studies

Grafts were made epicotyl to epicotyl between 7-day-old seedlings as described by Beveridge et al. (1994). This grafting procedure requires that lateral buds at the cotyledonary node are removed to enable formation of new vascular connections and growth of the scion without competition with cotyledonary shoots.

Table 1. Phenotype of homozygous *Rms6*, heterozygous and homozygous *rms6-1* plants in the F_2 of the cross between mutant line S2-271 (*rms6-1*) and initial line Solara (*Rms6*). F_2 plants were genotyped by growing F_3. Means with the same letter are not significantly different at $P = 0.05$. Photoperiod 16 h. *Only includes laterals that grew into above ground shoots.

	F_2 genotype		
Trait	*Rms6/Rms6*	*Rms6/rms6-1*	*rms6-1/rms6-1*
Number of plants	15	23	11
No. of plants with lateral shoots at cotyledonary node*	0	0	4
Height of main shoot (cm)	108a	102a	98a
No. of lateral shoots at the cotyledonary node*	0b	0b	0.5a
No. of lateral shoots >1 cm at nodes 1 and/or 2	0.5b	0.8b	1.5a
Total lateral length (cm)	34c	64b	136a
No. of pods on main stem	11.2a	7.7b	7.4b
No. of pods on lateral shoots	1.9c	4.3b	7.5a
No. of seeds on main stem	36.4a	25.4b	22.7b
No. of seeds on lateral shoots	6.1c	14.4b	23.2a
No. of seeds per pod	3.3a	3.3a	3.1a
Total seed weight (g)	16.1a	15.1a	17.4a
Individual seed weight (mg)	379a	381a	375a
Whole shoot DW (g)	7.1a	7.8a	8.5a

Table 2. Flowering data for WT (*Rms6/-*) and mutant (*rms6-1/rms6-1*) F_2 plants of cross Borek (*Rms6*) × HL293 (*rms6-1*). Photoperiod 18 h.

	Node of flower initiation		Flowering time (d)		
F_2 phenotype	Mean	SE	Mean	SE	Number of plants
Rms6	18.45	0.13	40.18	0.35	44
rms6	18.10	0.27	40.50	0.63	20

Table 3. Branching phenotype of double-mutant line HL292 (*rms3-1 rms6-2*), single mutant lines K487 (*rms 3-1*) and K586 (*rms6-2*), and their initial line cv. Torsdag. The data were obtained from mature plants grown under an 18-h photoperiod.

		Branching pattern:% of total lateral length					
Line	Total lateral length(cm)	Basal lateral shoots	Aerial lateral shoots	First order laterals	Second order laterals	Number of laterals >1 cm	n
Torsdag (WT)	0	0	0	0	0	0	3
K487 (*rms3-1*)	235	53	47	97	3	4.3	3
K586 (*rms6-2*)	93	100	0	100	0	1.0	4
HL292 (*rms3-1 rms6-2*)	445	77	23	97	3	15.7	3

Table 4. Root measurements for 41-day-old plants of K586 (*rms6-2*) and cv. Torsdag (WT) plants. Data are shown as mean ± SE for primary lateral roots, n = 6. Photoperiod 8 h.

	Number of lateral roots		Mean length of lateral roots (cm)		Length of main root (cm)	
Line	Mean	SE	Mean	SE	Mean	SE
Torsdag (WT)	59.2	4.8	17.3	0.6	30.6	2.9
K586 (*rms6-2*)	41.0	5.6	16.1	0.8	33.1	1.0

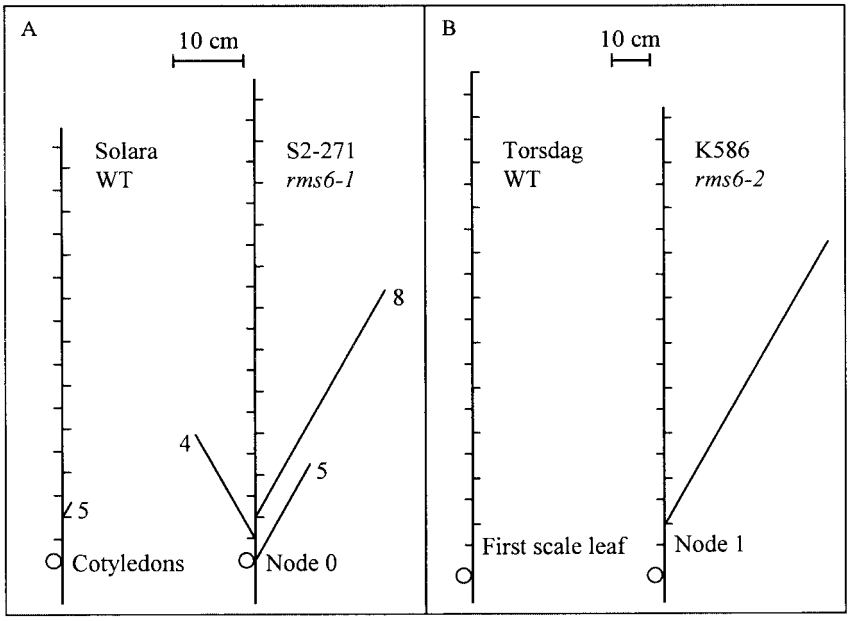

Fig. 1. A, scale diagram based on mean data for mutant S2-271 (*rms6-1*, n = 10) and its initial line, dwarf cv. Solara (WT, n = 10). Numbers beside lateral shoots indicate the number of plants out of 10 with a lateral shoot ≥1 cm at that node. B, scale diagram for mutant K586 (*rms6-2*, n = 4) and its initial line, tall cv. Torsdag (WT, n = 3); all mutant plants produced a lateral shoot at node 2. Photoperiod 18 h. Main stem and lateral shoot length (mean of all plants) is drawn to scale; individual internodes are not to scale.

Exogenous auxin studies

The response of decapitated plants to exogenous auxin was tested in S2-271 and cv. Solara plants (Fig. 5). Soil was removed from 9-day-old seedlings to reveal the cotyledons. As bud outgrowth had already commenced in mutant plants at day 9, the largest bud at each node was removed from plants of both genotypes immediately before decapitation as described by Beveridge et al. (2000) leaving secondary buds ≤2 mm. Plants were either left intact or decapitated above node 2. Decapitated plants were treated with 10 µl of lanolin containing 0, 1 or 5 g l^{-1} IAA to the decapitated stump. Auxin was applied daily after removal of the previous auxin supply.

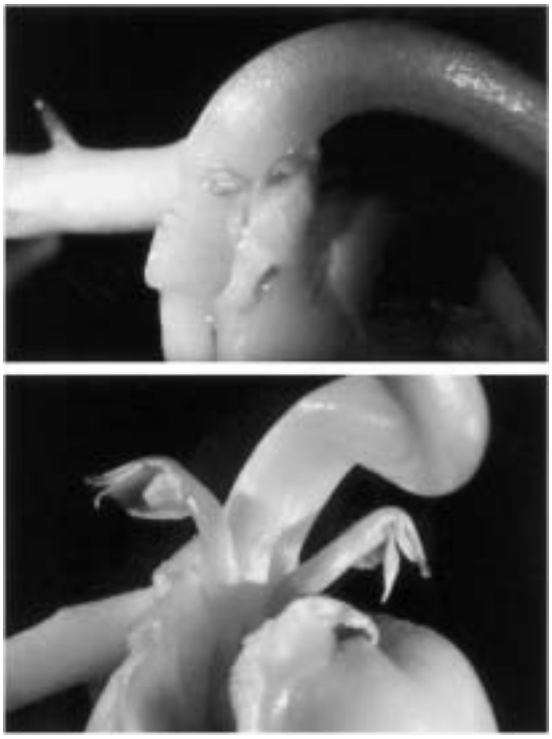

Fig. 2. Photographs centred on the cotyledonary node of 8-day-old seedlings of cv. Solara (top, WT) and S2-271 (bottom, *rms6-1* mutant) showing actively growing lateral buds in the mutant and dormant buds in the WT. The cotyledons of the WT were excised to allow a clear view of the dormant buds.

Hormone quantification

Indole-3-acetic acid (IAA) and abscisic acid (ABA) levels were examined in the shoots of 9-day-old cv. Solara and S2-271 seedlings (Fig. 6). Shoot tissue was harvested as described by Morris et al. (2001). Node 1 and node 2 portions consisted of stem tissue approximately 5 mm each side of the node 1 and node 2 leaf axils, respectively. The shoot tip consisted of all tissue above and including node 3.

Frozen tissue (1.5–4.5 g) was extracted as described by Morris et al. (2001), and 80–120 ng [$^{13}C_6$]-IAA (Cambridge Isotope Laboratories, UK) and 40–50 ng [$^{2}H_3$]-ABA (provided by Dr B Loveys, CSIRO Plant Industry, Adelaide, Australia) internal standard were added per sample.

Purified samples were methylated and trimethylsilylated (at 80°C for 20 min), followed by GC-MS-SIM analysis. IAA was quantified as described by Ross (1998). For ABA analyses, a SGE 25 m × 0.3 mm i.d. × 0.5 µm film thickness BP10 column was used, with the oven temperature programmed from 60°C to 200°C at 30°C min^{-1}, and then at 10°C min^{-1}. For quantification of endogenous IAA, the peak area ratios for the ion pairs

Table 5. Branching phenotype for reciprocal and self-grafted plants of line S2-271 (*rms6-1*, Mutant) and its initial line Solara (*Rms6*, WT). Data were recorded 44 days after grafting. Photoperiod 18 h.

	Total lateral length (cm)			
Graft combination Scion/Rootstock	Mean	SE	n	Proportion of plants with lateral >10 cm at nodes 1, 2 and/or 3
Mutant/Mutant	20.2	4.9	11	7/11
Mutant/WT	32.7	2.6	12	12/12
WT/Mutant	0.2	0.1	10	0/10
WT/WT	0.8	0.2	11	0/11

202/208 and 261/267 were measured, whereas the peak area ratio for endogenous ABA was calculated using the ion pairs 162/165 and 190/193. Calculations were performed as described by McKay et al. (1994) and Batge et al. (1999) following correction for isotopic impurity in the ABA internal standard.

Results

Phenotype and inheritance of mutant S2-271 (*rms6-1*)

The S2-271 mutant is characterized by increased branching from basal nodes and a tendency for buds at the cotyledonary node to grow into lateral shoots (Figs 1, 2 and 3). Buds at the cotyledonary node of WT plants remained dormant unless the main shoot was damaged during seedling emergence (Fig. 2). The phenotypic difference between WT and mutant was almost qualitative under an 18-h photoperiod, where the initial line Solara showed little tendency to branch while mutant S2-271 produced a total of 1–3 lateral shoots from nodes 0 (cotyledonary node), 1 and/or 2 (Fig. 1). The phenotypic difference became quantitative under a short-day, 13-h photoperiod where Solara and S2-271 both produced basal lateral shoots (Fig. 3). However, the total lateral length of S2-271 was twice that of Solara and, in contrast to the mutant, Solara never branched from the cotyledonary node. The small shoots released at the cotyledonary node of mutant plants did not always emerge above the ground or grow into secondary stems. Only half the S2-271 plants represented in Fig. 1 produced secondary stems at node 0. Lateral buds at aerial nodes remained small in both genotypes and length data gave no indication that the mutation stimulated growth of aerial buds (Fig. 3).

The S2-271 mutant showed single gene inheritance with partial dominance of the WT allele in the F_2 of cross S2-271 × Solara grown under a 16-h photoperiod (Table 1). Separation between WT and mutant plants was not fully distinct. Hence all F_2 plants were genotyped by growing 20 F_3 progeny. Using this procedure, we identified among 49 F_2 plants, 15 pure WT, 23 heterozygous and 11 pure mutant individuals (Table 1). These numbers are in good accordance with a monohybrid 1:2:1 ratio ($P > 0.5$).

To test for allelism with known *rms* mutants, line S2-271 was crossed with type lines WL5237 (*rms1-1*), K524 (*rms2-1*), WL6042 (*rms3-3*), K164 (*rms4-1*) and Wt15244 (*rms5-1*). Under the 18-h photoperiod used for the allelism tests, all F_1 plants had a WT phenotype with zero or minimal branching except for one of the four plants from the cross between dwarf lines S2-271 and WL6042, which produced a major basal branch. We conclude S2-271 carries a mutation at a new *Rms* locus, which we symbolized *Rms6* with S2-271 as the type line for allele *rms6-1*. The absence of basal branching from all but one of the F_1 plants from the allelism tests compared with production of basal branches by the majority of F_1 plants from cross Solara × S2-271 (Table 1) we attribute largely to the somewhat longer photoperiod used (18 versus 16 h) and the fact that the majority of F_1 plants from the allelism tests were tall (Solara and S2-271 are

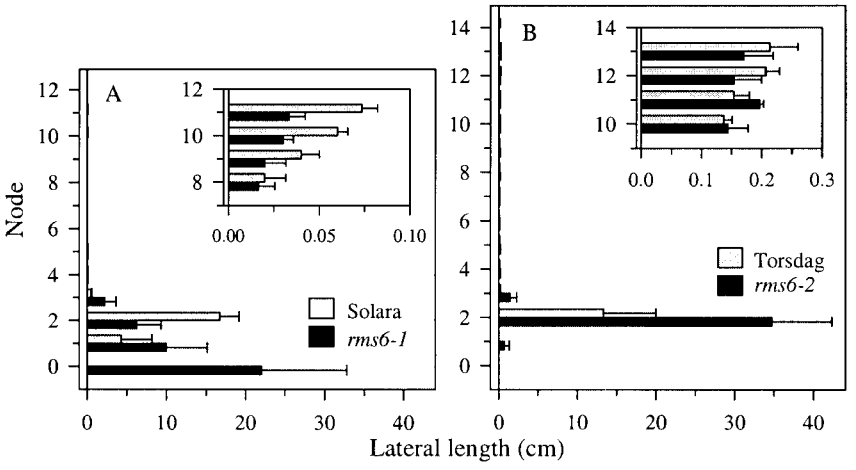

Fig. 3. Branching phenotype of S2-271 (*rms6-1* mutant) and cv. Solara (WT) plants (A) and K586 (*rms6-2* mutant) and cv. Torsdag (WT) plants (B). The inset shows lateral length data for four aerial nodes at an amplified scale. Lateral shoots at nodes 0–3 were measured with a ruler. Lateral buds at nodes 4–14 remained small and were measured under a dissecting microscope. Data are plotted for each node as mean ± SE, n = 3. Photoperiod 13 h.

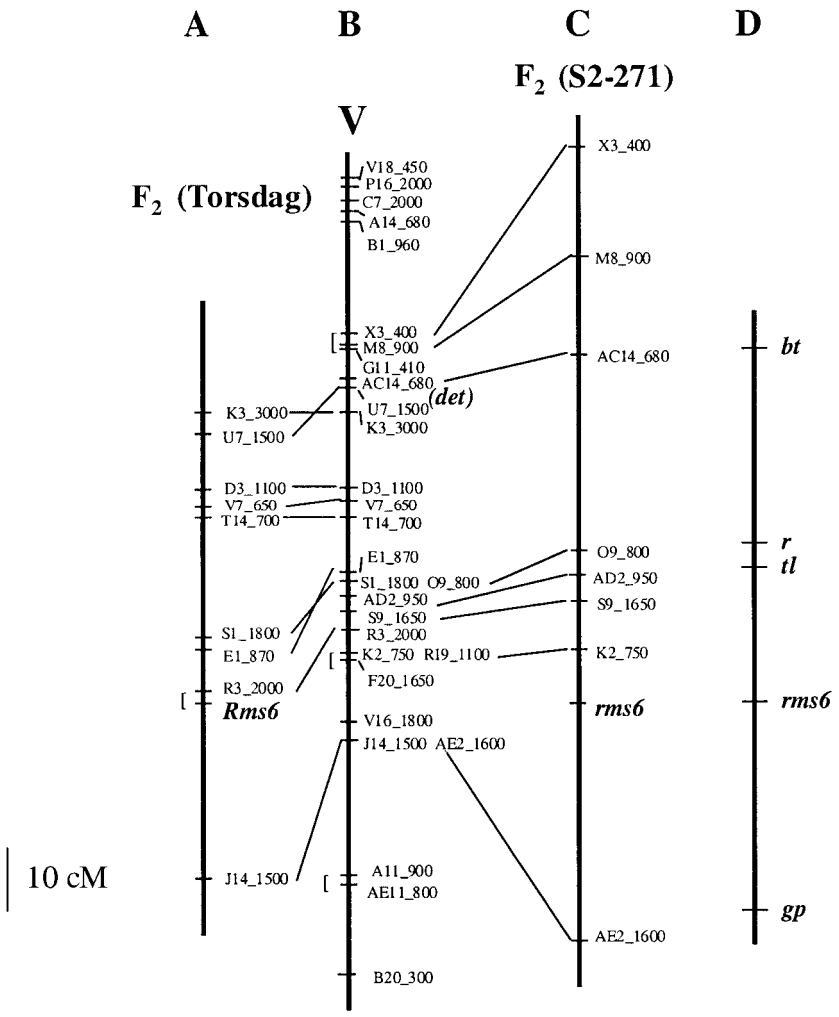

Fig. 4. A, C: RAPD linkage maps of the region of linkage group V surrounding the *Rms6* locus derived from analysis of the F_2 population of cross S2-271 × Torsdag; A, markers specific to Torsdag, and C, markers specific to S2-271; n = 118. B, map of linkage group V derived from analysis of the RIL population (from Laucou et al. 1998). D, conventional linkage map of the region surrounding the *Rms6* locus derived from analysis of F_2 populations of coupling phase crosses Torsdag × HL293 and Borek × HL293; n = 128 (from Murfet and Rameau 2000). Groups of markers with a square bracket to the left cannot be ordered.

dwarf). Short photoperiods and GA deficiency (as in *le* dwarfs) enhance any tendency for basal branching (Murfet and Reid 1993).

As stated, each F_2 plant from the cross Solara × S2-271 was genotyped by growing 20 F_3 progeny. This enabled comparison among F_2 plants with genotypes *Rms6/Rms6*, *Rms6/rms6-1* and *rms6-1/rms6-1* for a range of quantitative traits (Table 1). The degree and direction of dominance varied according to the trait examined. The WT allele *Rms6* was partially dominant over mutant allele *rms6-1* for the key branching traits total lateral length and number of lateral branches with a degree of dominance (see Falconer 1964) of 0.41 and 0.66, respectively. Number of lateral branches was almost fully effective for distinguishing WT and mutant segregants. All 38 F_2 plants carrying the WT allele produced no more than one basal lateral shoot. In contrast, all but one of the 11 homozygous *rms6-1* plants produced two or more basal lateral shoots. Production of lateral shoots at the cotyledonary node was confined to homozygous *rms6-1* plants; heterozygotes did not express this trait (Table 1). For the two traits, number of pods and seeds on the main stem, dominance was reversed with the mutant allele showing partial dominance of 0.6–0.8 over the WT allele. The presence of just one dose of the mutant allele was sufficient to significantly reduce the number of pods and seeds on the main shoot and significantly increase the number of pods and seeds borne on lateral shoots (Table 1). For the trait number of seeds on lateral shoots, there was essentially no dominance with heterozygotes almost exactly midway between the two homozygotes (Table 1). The *rms6-1* mutation had no significant effect on plant height, node of flower initiation, time to first open flower, shoot DW, number of seeds per pod, individual seed weight and total seed weight (Tables 1 and 2).

Mutant K586 (*rms6-2*)

Mutant K586 showed enhanced basal branching with a phenotype similar to, but weaker than, S2-271 (Figs 1 and 3). Under an 18-h photoperiod, initial line cv. Torsdag did not branch while mutant K586 generally, but not invariably, produced a basal lateral shoot (Fig. 1, Arumingtyas et al. 1992). Under a short-day, 13-h photoperiod, Torsdag and K586 both produced basal lateral shoots but the total lateral length of the mutant was more than twice

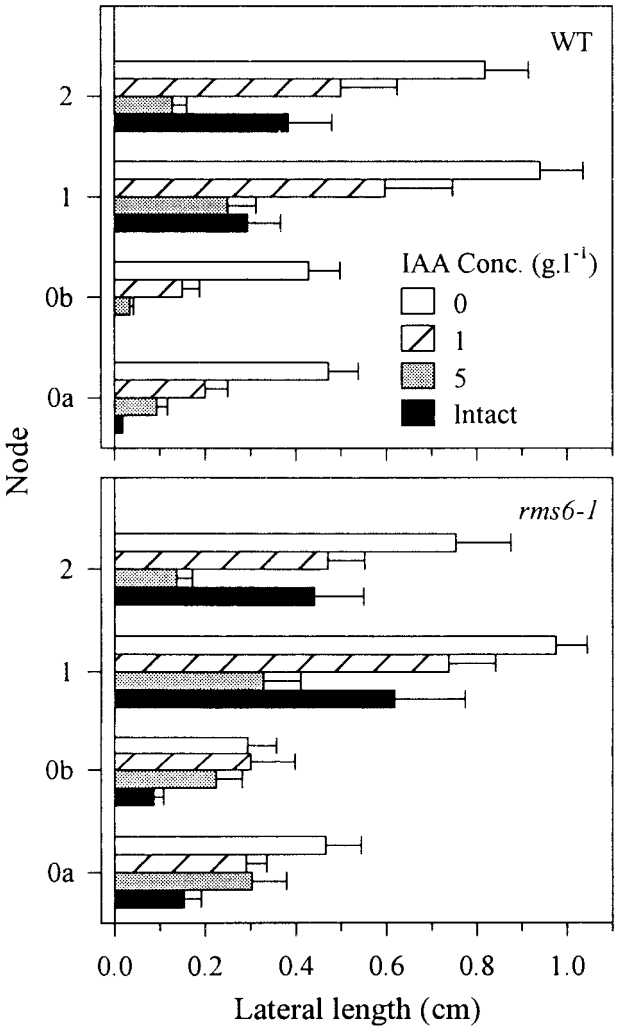

Fig. 5. Effect of exogenous auxin on decapitation-induced branching in cv. Solara (WT) and S2-271 (*rms6-1*) plants. Soil was removed from the pots to reveal the cotyledons (node 0). As two lateral shoots emerge at the cotyledonary node, these are shown as 0a and 0b. The plants were decapitated at age 9 days and data recorded at age 13 days. Data are plotted for each node as mean ± SE, n = 15-16. Photoperiod 14 h.

that of the WT (Fig. 3). As with S2-271, there was no indication that the K586 mutation stimulated growth of aerial lateral shoots (Fig. 3). The buds at the cotyledonary node of K586 plants did show some limited growth but, in contrast to S2-271, we did not observe any instance where they gave rise to a secondary stem.

Arumingtyas et al. (1992) found mutant K586 was not allelic with *rms1* through *rms5*, but refrained from assigning a gene symbol because they could not demonstrate clear single-gene inheritance. Nevertheless, the results for the F_2 of the cross between K586 and its initial line cv. Torsdag were consistent with single gene, partially recessive inheritance. In this F_2, the trait 'total number of leaves present on lateral shoots on day 27' was distributed in a continuous manner (data not shown; photoperiod 14 h). The F_1 plants occupied a position intermediate between the two parents. Arbitrarily cutting the F_2 distribution into three parts corresponding to Torsdag, intermediate, and K586, gave observed numbers of 12, 38 and 15 in good accordance with the single-gene 1:2:1 ratio ($P > 0.3$). Subsequently, K586 was crossed with *rms6-1* lines S2-271 and HL293. The F_1 plants had a mutant basal-branching phenotype like the parents. We conclude K586 carries a mutation at the *Rms6* locus and believe the evidence is now sufficient to designate K586 as the type line for allele *rms6-2*.

Map position of the *Rms6* locus

Rms6 was mapped relative to classical morphological markers and to RAPD molecular markers. Both analyses mapped the *Rms6* locus near the middle of linkage group V (Fig. 4).

To map the *Rms6* locus using conventional markers, line S2-271 was crossed with multiple marker lines HL111 and HL243. Significant linkage was observed in both crosses between *rms6* and linkage group V markers *r* and *tl* (data not shown). No recombinants between *rms6* and *r* or *tl* were obtained in the F_2 of these repulsion phase crosses. However, a line, HL293, homozygous for *rms6* and group V markers *bt*, *r*, *tl* and *gp* was subsequently obtained from the cross S2-271 (*rms6 Bt R Tl Gp*) × HL111

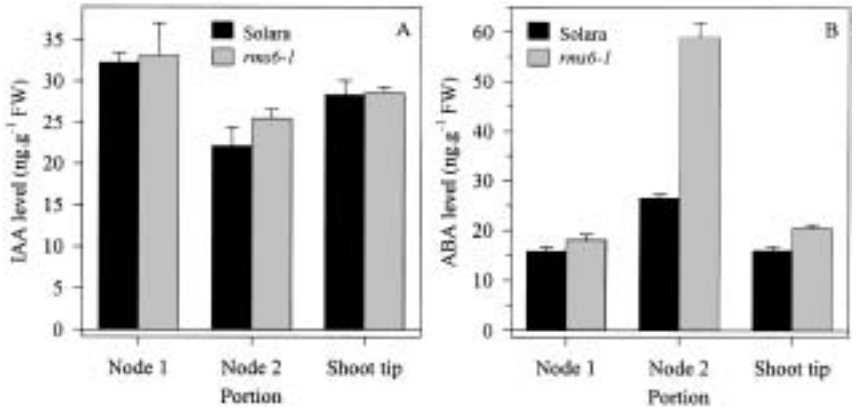

Fig. 6. Hormone levels in node 1, node 2 and the shoot tip of 9-day-old cv. Solara and *rms6-1* plants. A, indole-3-acetic acid (IAA) levels and B, abscisic acid (ABA) levels. Data are mean ± SE for three pools of 18–20 plants.

(*Rms6 bt r tl gp*). HL293 was then crossed with WT cvs. Torsdag and Borek. The coupling phase data (see Murfet and Rameau 2000) indicated that the *Rms6* locus is in linkage group V between *Tl* and *Gp*. The map sequence is shown in Fig. 4. Mapping using RAPD markers also showed the *Rms6* locus is in this segment of group V. In the bulk segregant analysis, the 4 RAPD markers E1_870, O9_800, AD2_950 and K2_750 on linkage group V were found to be polymorphic between two bulks and were confirmed to be linked to *Rms6* by segregation analysis. Other RAPD markers located on this group (Laucou et al. 1998) were then analysed on the 118 individuals of the F_2 population locating *Rms6* to a position about 3 cM from marker R3_ 2000 (Fig. 4).

Transgressive phenotype of the *rms3-1 rms6-2* double mutant

Three out of 64 F_2 plants of cross K487 (*rms3-1*) × K586 (*rms6-2*) showed a clear and extreme branching phenotype, which suggested they may represent the double-mutant class. We grew F_3 from these three plants and they bred true, consistent with this hypothesis. Line HL292 (*rms3-1 rms6-2*) was selected from one of these F_3 plants and the double-mutant genotype confirmed by backcrossing with K487 and K586. All three lines have a Torsdag background thus allowing direct phenotypic comparison (Table 3). Double mutant HL292 showed a clearly transgressive (additive) phenotype with a total lateral length some 1.9-fold and 4.8-fold longer than single mutants K487 and K586, respectively. Likewise, comparison of number of lateral shoots indicated a clear additive effect of the *rms3* and *rms6* mutations. Partitioning total lateral length into subcomponents to reflect branching pattern showed that, like the *rms3* single mutant, the double mutant produced both basal and aerial lateral shoots, and both first and second order lateral shoots. In contrast, branching in *rms6-2* was confined to first-order, basal lateral shoots. The basal branching effect of the *rms6-2* mutation was apparent in the double mutant as basal lateral shoots, by their increased number, contributed a greater proportion of the total lateral length than in the *rms3-1* single mutant.

Although the buds at the cotyledonary node of *rms6-2* plants all showed limited outgrowth in the seedling stage, none emerged above ground to produce a secondary stem. In contrast, all double-mutant plants produced a large lateral shoot from the cotyledonary node. Thus the *rms3-1* mutation had a synergistic effect on this action of the *rms6-2* mutation, even though the buds at the cotyledonary node of the *rms3-1* single mutant appeared to remain dormant.

Root morphology

The number of primary lateral roots was significantly reduced in the *rms6-2* mutant compared with WT whether grown in a dolerite/vermiculite growth medium (Table 4) or a peat/sand growth medium (data not shown). In contrast, the mean length of primary lateral roots was not significantly diminished in the mutant, and the length of the main root was very similar in both genotypes (Table 4 and data not shown).

Grafts of *rms6* and wild type

The results of reciprocal grafts between mutant line S2-271 (*rms6-1*) and its WT initial line Solara show that the *rms6* mutation acts primarily in the shoot to promote branching at nodes 1 and 2 (Table 5). Mutant *rms6-1* scions exhibited a mutant phenotype whether self-grafted or grafted to a WT rootstock. Likewise, *rms6-1* rootstocks did not stimulate outgrowth of basal lateral buds in WT scions. In contrast, the total lateral length of mutant scions was significantly ($P < 0.05$) greater in the combination mutant/WT (notation scion/rootstock) compared with the mutant self-grafts. Grafting studies with K586 (*rms6-2*) and its initial line Torsdag gave similar results (data not shown) to those obtained with *rms6-1*, thus confirming that *Rms6* regulates branching at nodes 1 and 2 primarily through action in the shoot. As cotyledonary lateral buds below the graft union were excised during the first 7–10 days after grafting, our results cannot reveal any affect of scion genotype on the release and growth of buds in the cotyledonary axils of the rootstock.

Exogenous auxin studies

Decapitation above node 2 following removal of the primary bud at each node, promoted bud outgrowth of the secondary bud in the axils at all remaining nodes of both WT and *rms6-1* plants (Fig. 5). Axillary bud lengths, and growth rates (data not shown), of lanolin treated (0 g l^{-1} IAA) decapitated *rms6-1* plants were generally similar to those of comparable decapitated WT plants. Under the 14-h photoperiod conditions, outgrowth of secondary axillary buds following removal of the primary buds also occurred at nodes 1 and 2 of WT plants and nodes 0, 1 and 2 of *rms6-1* plants that were not decapitated (control plants). However, particularly at nodes 0 and 1, bud outgrowth was greater in these *rms6-1* control plants than in WT control plants.

Exogenous auxin applied to the stump of decapitated plants reduced lateral bud outgrowth at nodes 1 and 2 of plants of both genotypes (Fig. 5). Auxin application decreased lateral bud lengths at nodes 1 and 2 of plants of both genotypes. The 5 g l^{-1} IAA treatment reduced bud lengths to at least the level of control plants. For plants of either genotype, the exogenous auxin treatments were not able to inhibit bud outgrowth at the cotyledonary node to the same extent as that observed in plants with intact shoots. Nevertheless, auxin treatments appeared more effective at inhibiting bud outgrowth at the cotyledonary node of WT plants than *rms6-1* plants. Both auxin treatments caused swelling of the stem at the site of auxin application in plants of both genotypes.

Similar results for application of exogenous auxin

were obtained for decapitated Torsdag and *rms6-2* plants except that in this case, bud removal prior to decapitation was not necessary as neither genotype had commenced bud release (data not shown).

Endogenous IAA and ABA levels

Endogenous IAA levels were similar in the nodes and shoot tip of WT and *rms6-1* seedlings (Fig. 6A). In contrast, ABA levels were elevated in young *rms6-1* shoots, especially at node 2 where they were increased 2-fold compared with WT seedlings (Fig. 6B).

Discussion

Our study identifies a new pea *Ramosus* locus, *Rms6*, located in the *R* to *Gp* segment of linkage group V (Fig. 4), which corresponds to chromosome 3 according to the most recent pea map (Weeden et al. 1998). Allelism tests were not made between either of the *rms6* mutants and two other pea mutants with increased branching, *ram* (Monti and Scarascia Mugnozza 1967) and *bsh* (Symons et al. 1999). However, *Ram* and *Bsh* are not located in the same chromosomal region as *Rms6*. The *Ram* locus is in the *Oh* region of linkage group VII (Monti 1970) and, unlike *rms6*, *bsh* showed no linkage with group V markers *r* and *gp* (Symons et al. 1999). Furthermore, the phenotypes of *ram* and *bsh* plants are clearly very different from the non-pleiotropic *rms6* phenotype (Monti 1970, Symons et al. 1999).

The phenotype of *rms6* (Figs 1 and 3) differs from the other five *rms* mutants in that branching is only promoted at the basal nodes. Mutants *rms1* through *rms5* show increased branching from both basal and aerial nodes (Blixt 1976, Apisitwanich et al. 1992, Arumingtyas et al. 1992, Stafstrom 1993, 1995). The growth of lateral shoots from the cotyledonary node is not unique to *rms6*; this trait has also been observed occasionally in *rms1*, *rms4* and *rms5* plants (Murfet and Symons 2000a, S. Morris, C. Turnbull and C. Beveridge, unpublished data). In contrast, bud outgrowth at the cotyledonary node has not been identified as part of the phenotype of *rms2*, *rms3* or *bsh*, even though *bsh* branches very extensively from other basal nodes (Symons et al. 1999). Like *rms2-1* and *bsh*, the *rms6-2* mutation decreased the number of lateral roots (Table 4, Beveridge et al., 1994, Symons et al. 1999, and data not shown).

Grafting results (Table 5) show the *rms6* mutation acts primarily in the shoot. This feature is shared with *rms3*, *rms4* and *bsh* where the primary action is also confined to the shoot (Beveridge et al. 1996, Symons et al. 1999). Interestingly, *rms4* and *rms6* plants both have slightly, but significantly, greater total lateral lengths when grafted to WT rootstocks compared with self-grafts. This was clear for both *rms6-1* (Table 5) and *rms6-2* (data not shown). Further studies may elucidate whether this is due to increased vigour of the WT rootstocks compared with *rms4* or *rms6* rootstocks. Ungrafted WT plants have more lateral roots than ungrafted *rms6* plants (Table 4) but we did not examine lateral root production in grafted plants.

Endogenous IAA levels appear similar in young shoots of WT and *rms6-1* plants (Fig. 6A) and exogenous auxin studies have indicated that the lesion in the shoot caused by *rms6* is not due to altered auxin response (Fig. 4). Particularly at nodes 1 and 2, different concentrations of exogenous auxin applied to the decapitated internode above node 2 caused a similar inhibition of lateral bud outgrowth in *rms6* and WT plants. A reduced response to exogenous auxin was apparent at the cotyledonary node of *rms6* plants. However, it is unresolved whether a similar quantity of auxin applied above node 2 would have reached the cotyledonary node in both genotypes, nor whether the action of another compound may have interfered with the auxin response at the cotyledonary node of *rms6* plants.

The physiological significance of increased ABA levels in young *rms6-1* shoots, especially around node 2 (Fig. 6B), is not clear at this time. We did not observe any difference in germination rate or susceptibility to wilt between WT and *rms6* plants. Analysis of ABA levels in other *rms* mutants may indicate whether increased ABA levels are common during bud outgrowth in *rms* mutants. Several other *rms* mutants, particularly *rms2*, have increased IAA levels at nodes of the uppermost expanded leaves. As argued previously for IAA, perhaps the altered ABA levels are part of a feedback mechanism intended to limit bud outgrowth. Alternative explanations include stress-related responses and diversion of metabolites from other pathways toward ABA.

The *rms3-1 rms6-2* double mutant showed strongly enhanced branching and a clearly transgressive phenotype (Table 3). An additive phenotype may be explained if, for example, one mutation blocked a feedback inhibition mechanism and the other directly promoted bud outgrowth. Three other *rms* double mutants have also been reported to express an additive phenotype: *rms1 rms2* (Stafstrom 1993, Beveridge et al. 1997b), *rms2 rms4* (Murfet and Symons 2000b) and *rms2 rms5* (Murfet and Symons 2000a). The transgression is particularly strong in the *rms2 rms5* double where branching is increased 3- to 4-fold compared with either single mutant. In these three cases, the individual mutants branch at similar nodes, and the combined effect of the two mutations is a further enhancement of branching at the same nodes, e.g. additional first order lateral shoots are produced at basal nodes in the *rms2 rms5* combination. In contrast to *rms6-2*, buds at the cotyledonary node remain dormant in *rms3-1*. Nevertheless, growth of lateral shoots from the cotyledonary node was strongly enhanced in the double mutant, indicating a synergistic effect of *rms3-1* on expression of *rms6-2* at this node.

Mutants at the first five *Rms* loci demonstrate classical Mendelian inheritance in that the WT allele shows full dominance, and the phenotypic difference between mutant and WT is clear in a diversity of circumstances. In contrast, for the two *rms6* mutants, the WT allele is incompletely dominant, and in segregating progenies, the

phenotypic difference between *Rms6/-* and *rms6/rms6* plants may be sufficiently unclear as to require progeny tests for unequivocal identification (Table 1). In general, the *rms6-1* mutation appears to be more severe than the *rms6-2* mutation (Fig. 1). However, the two mutations are derived from different initial lines with differences in genetic background. In particular, Torsdag is tall whereas Solara is an *le* dwarf. The *le* mutation results in gibberellin deficiency (Potts et al. 1982, Lester et al. 1997) and this situation, like the *rms6* mutations, enhances basal branching (Floyd and Murfet 1986, Murfet and Reid 1993, Murfet and Symons 2000a). In the absence of comparison in the same genetic background, we cannot be certain *rms6-1* is more severe than *rms6-2*.

Identification of a further branching mutant, *rms6*, adds to the diversity of pea mutants available for study of shoot architecture. In addition to their potential value for basic research, the *rms6* mutants may also have agronomic value for the pea crop. Fertility is not affected and overall yield for *rms6-1* in glasshouse conditions was slightly, but not significantly, greater than WT (Table 1). The main agronomical value of the increased basal branching in the *rms6* plants may prove to be the reduced indeterminacy of the main stem. By decreasing the number of pods per stem, competition for assimilates between pods may be reduced. Moreover, the increased number of secondary stems may allow a lower sowing density in the field.

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