The basal-branching pea mutant *rms*7-1

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Introduction

The *rmsl (ramosus1*) through *rms5* mutations increase both basal and aerial branching in pea (1,2, 5). In contrast, the *rms6* mutation increases basal branching only (12). Between two and eleven mutant alleles have been reported for each of these six RMS loci (12, 16). The *ram* mutant (9) and the auxin-deficient *bsh* mutant (17) were also named for their increased branching but their full phenotypic profile distances them somewhat from the *rms* series of mutants.

Branching habit in pea is also influenced by the background for length and flowering genes: basal branching is enhanced in a dwarf *(le)* background and under short to intermediate photoperiods in plants with **a** long-day flowering response (background *Sn Dne Ppd)* (6). Branching in lupin shows similar trends in response to environmental conditions (8).

Pea mutants *rmsl* through *rms5* have enabled significant progress in our understanding of the control of branching in plants. These *rms* mutants are the only increased branching mutants to be well characterized for involvement of long-distance signals, known and unknown, in branching control. This characterization was achieved by investigating grafting responses with WT (wild-type) plants, shoot auxin level and transport, auxin responses and root xylem sap cytokinin concentrations. These analyses indicated that two novel graft-transmissible signals are involved in branching control: a feedback signal controlled by RMS2 and a branching inhibitor controlled by RMS1 and RMS5 (4, 7, 10). The hypothesis for branching control has therefore been expanded to incorporate these novel long-distance signals together with the classical phytohormones auxin and cytokinin (3, 10). The recent cloning of RMSJ using the *Arabidopsis* MAX4 homolog from *Medicago truncatula* (15) will enable further testing of this hypothesis.

Mutations that specifically enhance basal branching under all photoperiods may be of agronomic value if they increase the number of secondary stems that match the main shoot in vigour and flowering time. Mutants *rmsl* through *rms5* do not fulfil these criteria as both basal and aerial branching is enhanced; their increased aerial branching in comparison with WT is often particularly evident under long-day conditions (2). In contrast, the *rms6* mutant plants show increased basal branching under both short and long photoperiods and any tendency to aerial branching is, if anything, diminished in the *rms6* mutant compared with WT (11, 12). In this paper we report on a recessive mutant at a new *ramosus* locus, RMS7, which, like the *rms6* mutant, has enhanced branching at the basal nodes only.

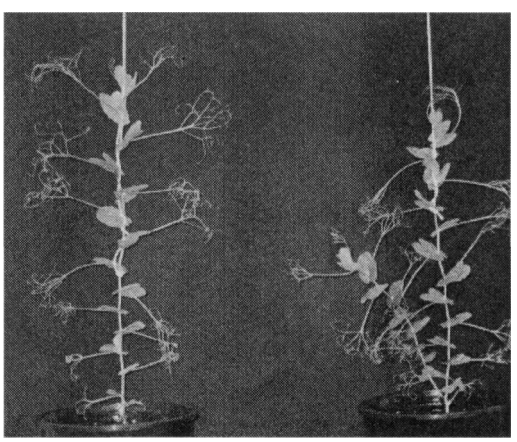

Fig. 1. Branching phenotype of cv. Terese (left) andM3T-475 (rms7-1; right) plants.

The *rms7-1* mutant

A mutant (M3T-475) with enhanced basal branching (Fig. 1) and recessive inheritance (Fig. 2) was selected at Versailles following EMS mutagenesis of the dwarf, semi-leafless *(af),* combining cultivar Terese. The M3T-475 mutant

had a branching phenotype very similar to the S2-271 (rms6-J) mutant described earlier (12) with increased basal branching but no sign of increased lateral growth from aerial (upper stem) nodes (Fig. 3). However, a cross between M3T-475 and S2-271 showed the two mutants were not allelic. M3T-475 also proved to be non-allelic with *rms1* through *rms5*. Accordingly, we assigned the new mutant to a further *ramosus* locus, RMS7, with M3T-475 as the type line for allele *rms7-1*. Basal lateral branches arose principally from nodes 1 and 2 of *rms7* plants (Fig. 3) but, as found for *rms6-1*, were also observed to grow from the cotyledonary node (node 0) in some cases, but to a lesser extent than for *rms6-1*. In contrast, basal branches arose principally from node 2 of cv. Terese (Fig. 3). For the control plants in Fig. 2, all M3T-475 plants had strong lateral outgrowth from node 1 (representing, on average, 42% of total lateral length) where as no Terese plant had a lateral at node 1. Thus the *rms7* mutation extended downwards the zone of basal nodes showing active lateral outgrowth to include node 1 and at times node 0. However, in a segregating population, we consider the ratio of lateral to main-stem length to be the more robust variable for separating WT and mutant plants than the absence/presence of a lateral at node 1. Five out of 22 F_2 plants in Fig. 2 with a low branching index indicative of WT plant had a lateral at node 1, and one of the ten F_2 plants with a high branching index indicative of a mutant plant had no lateral at node 1.

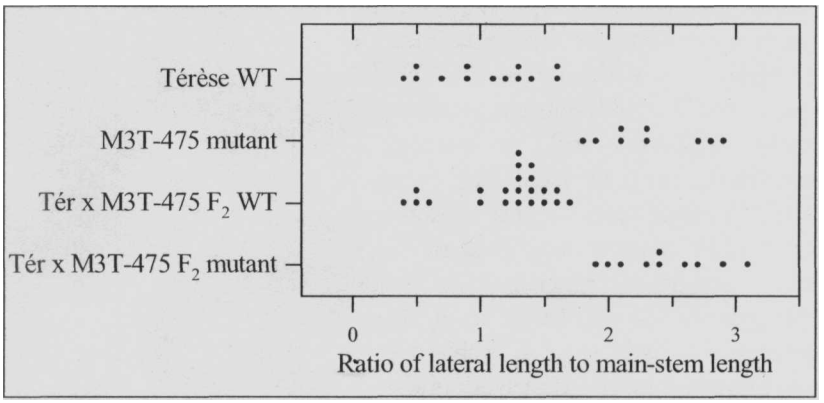

Fig. 2. Distribution of the branching index 'ratio of lateral to main-stem length! for initial line cv. Terese, mutant line M3T-475, and the F_2 population of cross M3T-475 x Terese. Data are from mature dry plants grown under a 16-h photoperiod. The observed F_2 numbers fit a 3:1 ratio (P> 0.4).

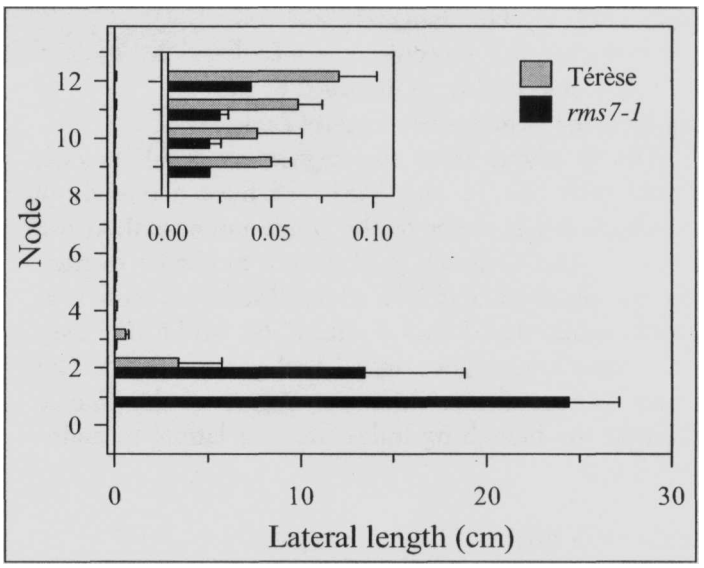

Fig. 3. *Branching phenotype of 30-day-old plants of cv. Terese and mutant line M3T-475* (rms7-l). *The inset shows the lateral length data for nodes 9 to 12 at an amplified scale (xl7). Laterals were measured by a ruler at nodes 0 to 3, with laterals at the higher nodes measured under a dissecting microscope.*

We have not yet succeeded in mapping RMS7. Our efforts have been hindered by difficulty in obtaining clear and unambiguous segregations in a non-fixed genetic background. The mutant phenotype of *rms7* lacks the clarity of expression of mutants *rmsl* through *rms5* and the quite systematic release of buds from cotyledonary node of *rms6-1* plants, which was used as a qualitative character for the mapping of RMS6.

The *rms6-1 rms7-1* double mutant

A plant with substantially more profuse basal branching than either single mutant was identified in the F_2 of the cross between M3T-475 and S2-271 as a candidate double mutant and a pure-breeding line selected. The double-mutant genotype was confirmed by backcrossing to both parents: all backcross F_1 plants displayed

a single-mutant phenotype (Fig. 4). The proven *nms6-1 rms7-1* line was added to the Hobart pea collection as HL300. The rmss*6-1 rms7-1* double-mutant phenotype is clearly additive, and a hierarchical quantum increase in basal lateral growth from WT initial lines, to single mutants, to the double mutant was evident under diverse conditions ranging from a long daylength of 24 or 18 h to a shorter daylength of 14 h (Fig. 4, Table 1). All lines branched more extensively under the 14-h photoperiod (Table 1) as expected for lines with a photoperiod-responsive, late-flowering background. Under the 14-h conditions, the lateral length of the WT lines Solara and Terese was approximately equal to main-stem length. However, the single and double mutants still out-branched their WT progenitors by a factor of 2 and 4, respectively, as measured by the ratio of lateral to main-stem length (Table 1).

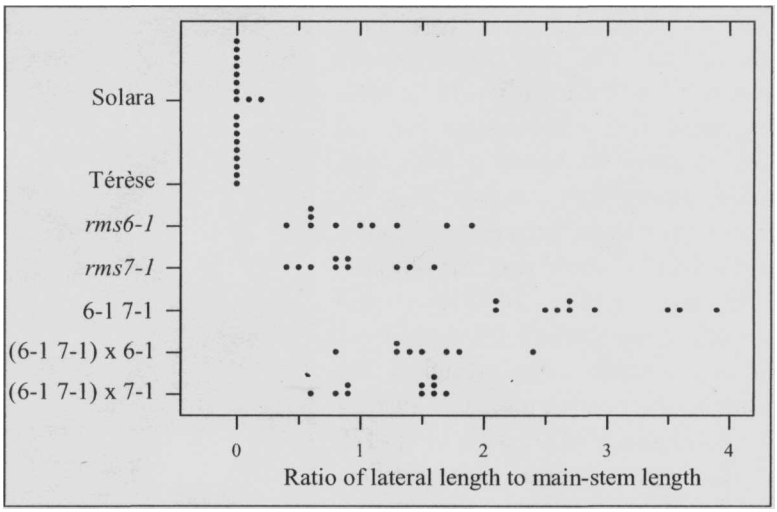

Fig. 4. Distribution of the ratio of lateral length to main-stem length for cv. Solara, cv. Terese, *single mutant S2-271* (rms6-l, *ex Solara), single mutant M3T-475* (rms7-l, *ex Terese), double mutant HL300* (rms6-l rms7-l), *and backcross F_1s of HL300 with S2-271 and M3T-475. Data are from mature plants; photoperiod 18 h.*

The F_1 plants from the crosses of double-mutant HL300 with S2-271 and M3T-475 have a branching phenotype much closer to the single mutants than the double mutant (Fig. 4), indicating a moderate to high degree of dominance of WT allele RMS6 over *rms6-1* on a homozygous *rms7-1* background (0.64) and RMS7 over rms7-1 on a homozygous *rms6-1* background (0.44). The figures in parentheses show the degree of dominance based on the branching index 'ratio of lateral to main-stem length'.

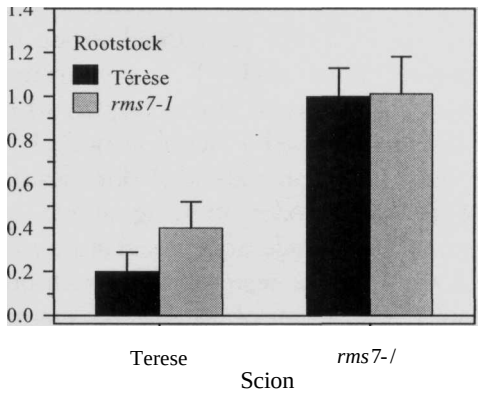

Fig. 5. *Branching phenotype of self- and reciprocally-grafted cv. Terese and M3T-475* (rms7-l) *plants aged 50 days. The grafts were made epicotyl to epicotyl using 7-day-old seedlings as previously described (12). Photoperiod 18 h;* n = *11-12.*

Grafts with *rms7*

The results of reciprocally grafting cv. Terese and *rms7-1* seedlings showed that the shoot branching phenotype was determined by the shoot genotype (Fig. 5). A WT rootstock did not reduce branching of an *rms7* scion and an *rms7* rootstock did not significantly

Table 1. Ratio of lateral length to main-stem length for 34-day-old plants of cv. Solara, *rms6-1* (ex Solara), cv. Terese, *rms7-1* (ex Terese), and double-mutant *rms6-1 rms7-1* raised under 14- or 24-h photoperiods. Data are shown as mean ± SE; n = 9-10. Means in the same row with the same superscript letter are not significantly different at $P < 0.05$.

	Ratio of lateral length to main-stem length				
Photoperiod	cv. Solara	*rms6-1*	cv. Térèse	*rms7-1*	*rms6-1 rms7-1*
24 h	0.16 ± 0.03^b	1.02 ± 0.13^a	0.09 ± 0.02^b	1.11 ± 0.07^a	2.66 ± 0.19
14 h	1.00 ± 0.13^b	1.92 ± 0.20^a	0.95 ± 0.12^b	2.18 ± 0.12^a	4.32 ± 0.22

increase branching in a WT scion. Reciprocal grafts were also performed between rms6-1 and *rms7-1* seedlings. Grafting an *rms7* scion to an *rms6* rootstock or an *rms6* scion onto an *rms7* rootstock did not enhance branching beyond the phenotype of the respective self-grafted controls (data not shown).

IAA levels and response

Endogenous IAA levels were similar in the nodes and shoot tip of cv. Terese and M3T-475 *(rms7-1)* seedlings (Fig. 6). The extraction procedure and measurements of IAA level by GC-MS-SIM were done as previously described (12).

The response to decapitation and application of exogenous auxin was observed for Terese (WT) and M3T-475 *(rms7-1)* plants by measurement of lateral growth at nodes 1 and 2 (Fig. 7). IAA was applied to the cut tip in lanolin as previously described (12). Branching was promoted in both WT and *rms7-1* plants by removal of the shoot tip. IAA treatment at a concentration of either 1 or 10 g/L reduced bud outgrowth in decapitated WT plants to the level of intact WT plants. In contrast, only the stronger 10 g/L treatment inhibited branching in decapitated *rms7-1* plants to the level of intact mutant plants. The weaker 1 g L^{-1} treatment had little effect on bud outgrowth in decapitated *rms7-1* plants.

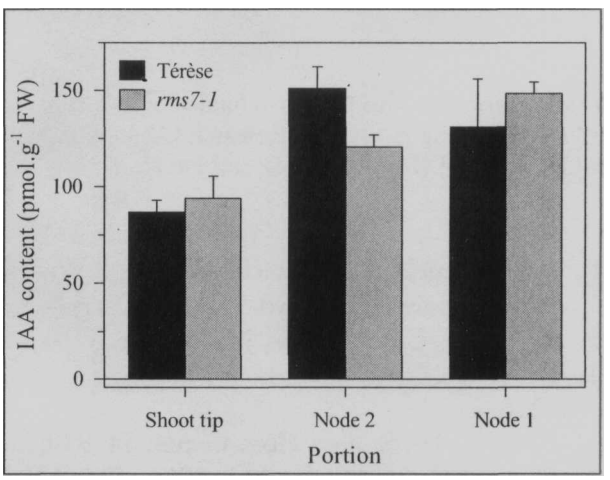

Fig. 6. IAA *levels in the shoot tip, node 2 and node 1 of 8-day-old cv. Terese and M3T-475* (rms7-l) *plants. The means and SEs are based on three pools of 18-20 plants.*

Discussion

The *rms7-1* mutant showed increased basal branching and recessive inheritance (Figs 1, 2 and 3). The shoot phenotype was determined by the shoot genotype in reciprocal grafts of the *rms7* mutant with initial line cv. Terese (Fig. 5). Auxin levels were similar in *rms7* and Terese plants (Fig. 6) and decapitated *rms7* seedlings were responsive to the application of exogenous auxin although somewhat higher levels of auxin were required for *rms7* than WT plants to restore bud outgrowth to the levels observed in intact plants (Fig. 7).

The graft results suggest that unlike RMS1, RMS2 and RMS5 (4, 7, 10), RMS7 is probably not concerned with production of a mobile signal but may be concerned with signal reception. Indeed the decapitation/auxin application results indicate the *rms7* mutation may result in impaired auxin reception as more auxin had to be applied to mutant than WT plants to achieve a comparable level of suppression of basal lateral outgrowth.

The *rms7* mutant shares many features with *rms6* (12). These two basal branching mutants showed an additive phenotype in the *rms6 rms7* double mutant (Fig. 4). This result may mean the two genes operate in different pathways but mutations at two loci in

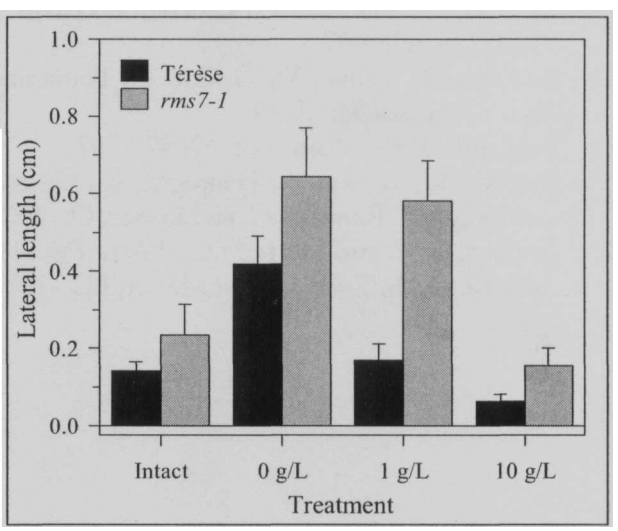

Fig. 7. *Effect of auxin (IAA) application on decapitation-induced branching in cv. Terese and* M3T-475 (rms7-l) *plants. Plants were decapitated after 10 days and scored after a further* 3 *days of treatment. Any growth from the cotyledonary node was removed daily. Photoperiod 14 h; n= 16.*

the same pathway can result in an additive double-mutant phenotype, e.g. pea gibberellin synthesis mutants (14).

With seven RMS loci now identified and up to 11 mutant alleles known at each locus, we may be approaching saturation for recognition of pea genes that result in increased branching when mutated. The search for genes that result in reduced branching when mutated has now begun, e. g. mutations in *SAX1* and *SAX2* result in suppressed axillary meristem initiation at certain nodes (13). The *rms7* mutant provides a further vehicle for research toward understanding the control of branching, and the ability of the *rms7* plants to produce an increased number of secondary stems may prove of agronomic significance.

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