Double mutant *rms2 rms5* expresses a transgressive, profuse branching phenotype

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Six loci have now been identified in the *rms* (*ramosus*) series in pea with up to 11 mutant alleles known at some loci (1, 2, 9, 11, 13, 14, 17). All mutants are recessive and express increased branching at basal and aerial nodes (*rms1* to *rms5*), or at basal nodes only (*rms6*). The *rms* mutants are valuable tools for studies aimed at understanding the control of lateral bud outgrowth. These studies have already yielded some valuable insights (4-8, 14-16) and a tentative model now exists for the control of branching in pea by the *rms* genes (3, 12). This model invokes a role for two unknown and possibly novel messengers.

Determination of the phenotype of *rms* double mutants can provide additional clues concerning the action and relationships of the *rms* genes, although the results may not allow unequivocal interpretation. To date, only a small number of *rms* double-mutant phenotypes have been reported. Two double mutants, *rms1 rms2* and *rms3 rms6*, are known to express a transgressive phenotype (7, 14, 15). In this paper, we report on another double, *rms2 rms5*, with a transgressive phenotype and several other double combinations where there is no indication of transgression.

Materials and methods

All lines used in the study are held in the Hobart pea collection and details of their origin and nature are given in previous papers (1, 2, 17). Mutant line K524 (*rms2-1*) was derived from tall (*Le*) cv. Torsdag and mutant lines Wt10852 (*rms5-2*) and Wt15241 (*rms5-3*) were derived from dwarf (*le*) cv. Paloma. All plants were grown one per 14-cm pot in the glasshouse at Hobart under an 18-h photoperiod obtained by extending the natural day before dawn and after dusk with light from a mixed fluorescent (40 W cool white tubes)/ incandescent (100 W globes) source providing about 25 μmols $m^{-2}s^{-1}$ at pot top. Nutrient was provided by application of Aquasol (Hortico Ltd, Melbourne) once per week. Details of the traits measured, and branching terminology used, are given in Fig. 1 and legend.

Results

The rms2 rms5 *double mutant shows major transgression*

The two crosses K524 (*rms2-1*) x Wt10852 (*rms5-2*) and K524 x Wt15241 (*rms5-3*) gave similar results and combined data have been used in Fig. 2 and Tables 1 and 2. Four phenotypic classes were easily recognisable among tall F_2 plants. WT (wild type) plants showed little or no branching and the double-mutant plants showed profuse branching (Fig. 2). The two single mutants displayed an intermediate level of

Table 1. Mean ± SE is shown for six traits of plants belonging to the wild-type, two single-mutant, and double-mutant branching classes in the F_2 of crosses K524 (*Le rms2-1 Rms5*) x Wt10852 (*le Rms2 rms5-2*) and K524 x Wt15241 (*le Rms2 rms5-3*). The means are for the combined data for all tall (*Le*) segregants in both crosses. n is shown in brackets.

	F_2 phenotype							
Trait	**Wild type (41)**		***rms5* (20)**		***rms2* (19)**		***rms2 rms5* (8)**	
Stem length from nodes 1-12 (cm)	**80.1**	**1.3**	**68.1**	**1.3**	**59.1**	**1.6**	**50.4**	**1.2**
Stem width at internode 12 (mm)	**3.92**	**0.05**	**3.17**	**0.08**	**2.43**	**0.05**	**1.76**	**0.08**
Days from sowing to harvest[1]	**76.5**	**0.5**	**80.2**	**0.7**	**87.3**	**0.9**	**87.8**	**0.6**
Number of pods	**8.2**	**0.2**	**12.1**	**0.7**	**19.5**	**1.2**	**24.1**	**1.9**
Number of seeds	**32.8**	**1.3**	**35.1**	**2.1**	**50.0**	**2.9**	**50.3**	**2.9**
Number of seeds per pod	**4.0**	**0.1**	**2.9**	**0.1**	**2.6**	**0.1**	**2.1**	**0.1**

[1]Plants were harvested when all seeds were dry.

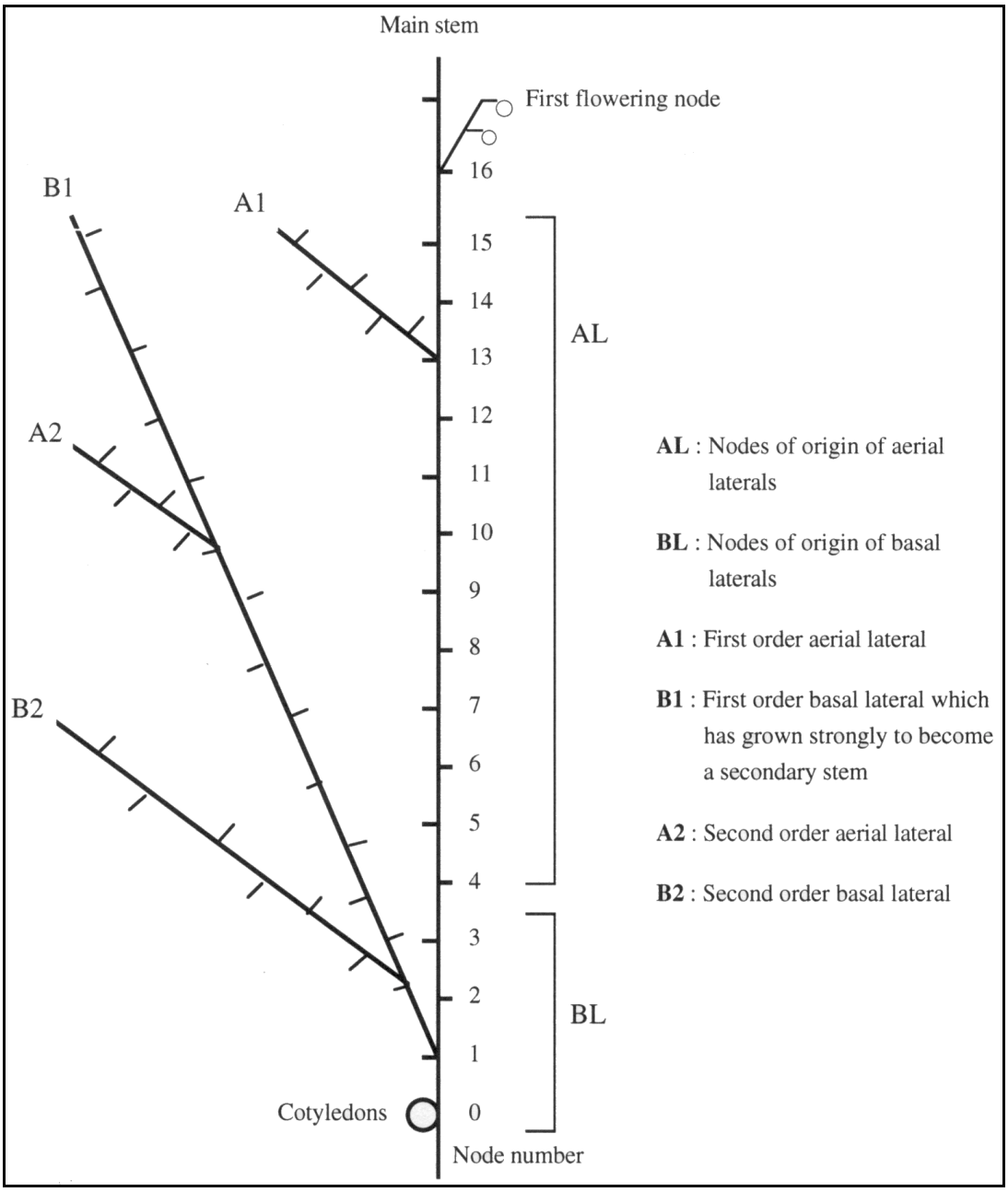

Fig. 1. Diagram of a pea plant illustrating the branching terminology used. Node counts started from the first scale leaf as node 1. Basal laterals were defined as arising from nodes 0 (cotyledonary node), 1, 2 or 3, and aerial laterals from 4 and above. Laterals arising at nodes 1, 2 or 3 on secondary stems (see below) were also considered basal. Laterals did occasionally arise from the cotyledonary node in rms5 and rms2 rms5 plants. First order lateral branches arise directly from a node on the main stem. More than one first order lateral may arise from one basal node, especially in rms2 and rms2 rms5 plants. Basal laterals that grew strongly enough to rival the main (primary) stem were termed secondary stems (e.g. B1 in Fig.1). Second order laterals arise directly from nodes on a first order lateral. Third order laterals occurred in some rms2 rms5 plants. In pea, lateral branches normally do not occur at reproductive nodes but one exceptional rms5 plant produced both an inflorescence and a lateral branch at the first reproductive node. Total lateral length (TLL) is the total length of all laterals longer than 1 cm. Sub-sets of TLL include total length of basal laterals (TLBL), aerial laterals (TLAL), first order laterals (TLFOL) and second order laterals (TLSOL).

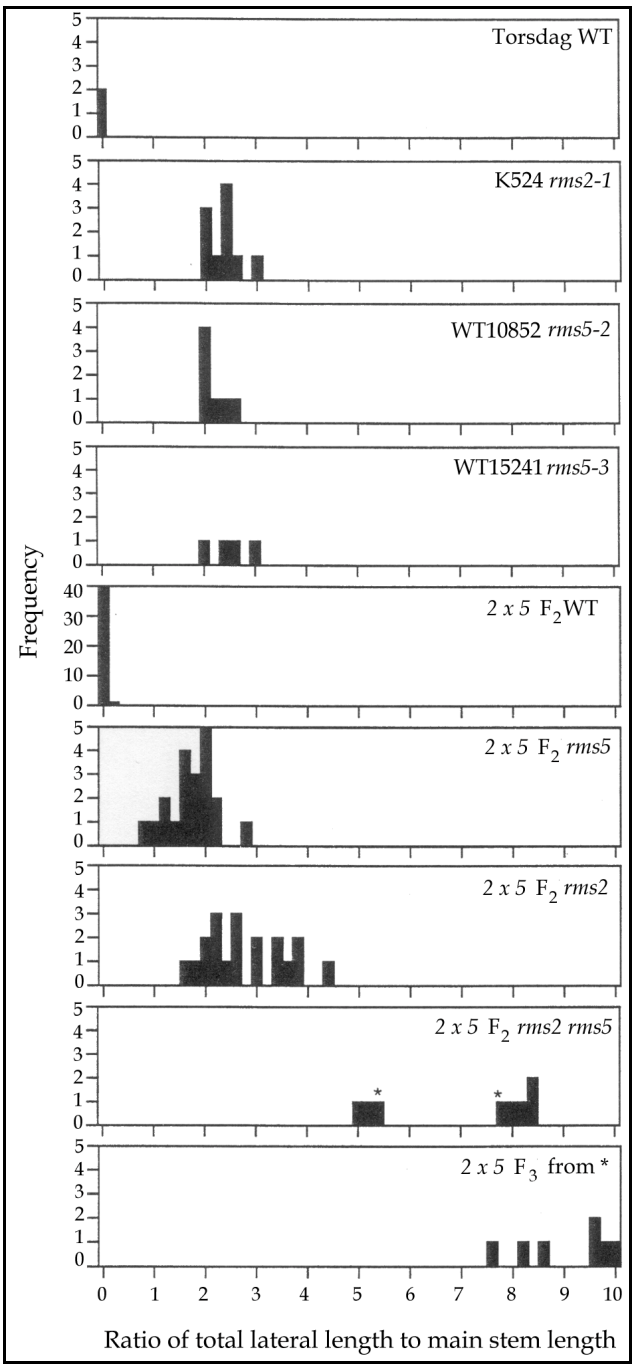

Fig. 2. The distribution of the branching index 'ratio of total lateral length to main-stem length' is shown here for parental and control lines cv. Torsdag (wild type), K524 (Le rms2-1 Rms5; ex Torsdag), Wt10852 (le Rms2 rms5-2; ex Paloma), and Wt15241 (le Rms2 rms5-3; ex Paloma); tall F_2 plants from crosses K524 x Wt10852 and K524 x Wt15241; and F_3 progeny from two double-mutant rms2 rms5 F_2 plants. Both crosses gave similar results and the F_2 data have been combined. Measurements were taken from mature plants grown under an 18-h photoperiod.

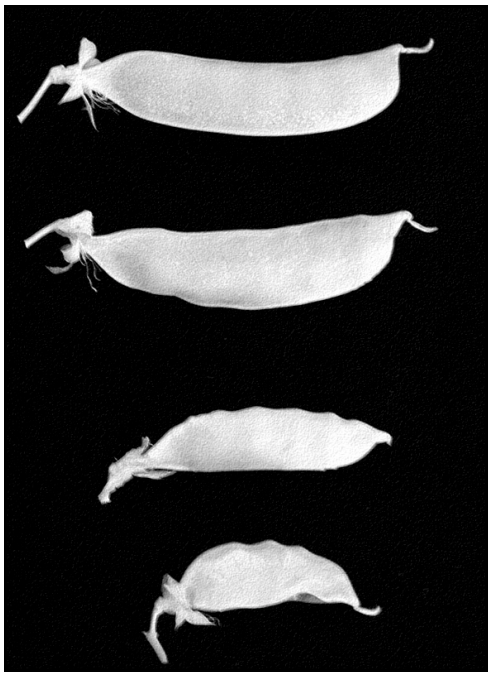

Fig. 3. Top: pod of cv. Torsdag (initial line for mutant line K524); upper middle: pod from a tall rms5-3 plant; lower middle: convexly curved pod of branching mutant K524 (rms2-1); bottom: convexly curved pod from a tall double-mutant rms2-1 rms5-3 plant, which has split open along the abaxial suture during seed fill.

branching (Fig. 2). However, *rms2* single mutants were distinguishable from *rms5* plants by a combination of traits, including shorter, thinner stems (Table 1), a tendency to wilt in sunny conditions and, in particular, *rms5* single mutants were generally straight like those of the *rms5* parental lines although a few pods on some plants displayed a convexly curved phenotype. The initial line for K524, cv Torsdag, has straight or slightly concave pods (Fig. 3). It is not clear at this time whether the bent pods of K524 are the result of a recessive mutation at a separate locus very close to *rms2*, or a pleiotropic effect of the *rms2-1* mutation itself. To date, we have never obtained a proven recombinant for allele *rms2-1* and the bent-pod trait. The bent pods sometimes split open along the abaxial suture as the seeds filled, especially on double-mutant *rms2 rms5* plants (Fig. 3).

Four branching phenotypes were also distinguishable among dwarf F_2 plants but in this case the separation between the WT and single-mutant

phenotypes was less obvious than for tall plants because, like the dwarf WT control, cv. Paloma (initial line for Wt10852 and Wt15241), dwarf WT segregants generally produced some basal and/or aerial laterals.

Segregation at the *Rms2* and *Rms5* loci was in good accordance with a dihybrid 9:3:3:1 ratio (Table 1, $P > 0.2$). Seven out of 8 tall putative double-mutant plants were tested by growing F_3 (4 plants per progeny) and backcrossing to both single-mutant parents (5-7 seeds of each backcross). All tests gave affirmative results. All backcross plants exhibited a mutant *rms* phenotype and the F_2 plants bred true in F_3. The double-mutant F_3 plants all branched extensively with the ratio of total lateral length (TLL) to main-stem length in the range of 8-10 (Fig. 2). This ratio is around 3.5-fold greater than for either single-mutant parent thus confirming a strongly transgressive phenotype for the *rms2 rms5* double mutant. The two double-mutant F_2 plants with TLL to main-stem length ratios of 5.5 and 7.9 (marked with asterisks in Fig. 2) gave rise to F_3 progeny with means of 9.0 and 9.2, respectively. Thus the F_2 difference appears to reflect the weak early growth of some F_2 seedlings (the seed was somewhat old) rather than a difference in genetic potential.

Branching pattern of the F_2 plants was influenced by the *le* mutation for dwarf stature in addition to the *rms2* and *rms5* mutations. The ability of the *le* mutation to increase basal branching has been reported before (10) and is clearly seen here in the data for the two single-mutant *rms* classes (Table 2). The percentage contribution of the basal laterals to total lateral length rose from 47% in tall (*Le*) *rms5* plants to 79% in dwarf (*le*) *rms5* plants ($P < 0.01$) and the number of basal laterals increased from 1.1 to 2.7 ($P < 0.001$). A similar effect of *le* is apparent in the *rms2* plants. Among tall plants, the *rms2* mutation was slightly more conducive than *rms5* to both basal and second order branching (Table 2). All *rms* plants produced both basal and aerial laterals with the exception of two tall *rms5* plants. In general, a gap pattern (see 2) occurred with no laterals arising from nodes immediately above the basal nodes (nodes 0-3). The gap was larger and more clearly defined in *rms5* than *rms2* plants. The profuse branching of the *rms2 rms5* double-mutant plants was reflected in their high level of basal and second order branching (Table 2). Some double-mutant plants produced third order laterals or branched from every main-stem node.

Table 2. Branching pattern for tall (*Le*) and dwarf (*le*) *rms* segregants in the F_2 of crosses K524 (*Le rms2-1 Rms5*) x Wt10852 (*le Rms2 rms5-2*) and K524 x Wt15241 (*le Rms2 rms5-3*). The means are for combined data from the two crosses. Basal laterals are defined as arising from the cotyledonary node (node 0) or nodes 1 to 3 on the main and secondary stems. Photoperiod 18 h.

	Basal laterals as a percentage of total lateral length (%)		Number of basal laterals longer than 10 cm		Second order laterals as a percentage of total lateral length (%)		
Phenotype	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**n**
Tall *rms5*	**47**	**6**	**1.1**	**0.2**	**3**	**1**	**20**
Dwarf *rms5*	**79**	**9**	**2.7**	**0.3**	**9**	**3**	**3**
Tall *rms2*	**58**	**3**	**2.3**	**0.3**	**18**	**2**	**19**
Dwarf *rms2*	**76**	**4**	**2.9**	**0.3**	**16**	**4**	**11**
Tall *rms2 rms5*	**84**	**4**	**9.4**	**1.1**	**23**[1]	**2**	**8**
Dwarf *rms2 rms5*	**83**		**7.0**		**42**		**1**

[1]Two double-mutant plants produced some small third order laterals that are included in this figure.

Among the four F_2 phenotypic branching classes, stem length and width declined in the sequence WT > *rms5* > *rms2* > *rms2 rms5* (Table 1, all differences significant at $P < 0.001$). For tall double mutants, the total length of the main stem was just under half that of the WT F_2 segregants ($P < 0.001$, data not shown). In addition, both single mutations significantly delayed ($P < 0.001$) the time when all seeds were dry and ready for harvest, *rms5* by 4 days and *rms2* by 11 days relative to WT plants (Table 1). The harvest time for the double mutant was similar to that for *rms2* plants (Table 1). Mutant plants had more pods and seeds than WT

plants but fewer seeds per pod (Table 1) and smaller pods (Fig. 3). These effects were strongest in *rms2* and especially *rms2 rms5* plants.

Double mutant rms *combinations not showing transgression*

The four crosses WL5237 (*rms1-1*) x K164 (*rms4-1*), K524 (*rms2-1*) x K487 (*rms3-1*), K487 (*rms3-1*) x K164 (*rms4-1*), and WL5918 (*rms1-3*) x WL6042 (*rms3-3*) gave little or no evidence of transgression. The F_2 population of the latter cross contained two plants with branching indices slightly exceeding the parental range, but F_3 progeny tests gave no evidence of a genuine transgressive phenotype. In all four F_2 populations, segregation was in good accordance with a 9 WT: 7 *rms* dihybrid ratio. A small F_2 was also grown from cross K524 (*rms2-1*) x K164 (*rms4-1*). No indication of transgression was obtained but with n = 24 we can only accept this conclusion with about 80% confidence.

Our present results are summarized in Table 3 together with other reported results for *rms* double-mutant combinations. Seven out of fifteen combinations remain to be tested.

Table 3. Summary of present results and other reported results for crosses segregating at two *Rms* loci showing the two mutant alleles involved and whether branching in the double mutant falls within the parental range (no transgression) or significantly exceeds the parental range (transgession).

	rms2	***rms3***	***rms4***	***rms5***	***rms6***
rms1	**1-1 2–2** **Transg. (7,15)**	**1-3 3–1** **No transg.**	**1-1 4–1** **No transg.**		
rms2	**–**	**2–1 3–1** **No transg.**	**2–1 4–1** **No transg.** **F_2 n = 24 only**	**2–1 5–2** **2–1 5–3** **Transg.**	
rms3	**–**	**–**	**3–1 4–1** **No transg.**		**3–1 6–2** **Transg. (14)**
rms4	**–**	**–**	**–**		
rms5	**–**	**–**	**–**	**–**	

Discussion

The four branching mutants *rms1, rms2, rms3* and *rms4* have now been subjected to fairly extensive study including use of grafting to examine long distance signalling (4-8, 15, 16). Based on these results, Beveridge (3) has suggested that *Rms2* may be concerned with the regulation of a novel root-to-shoot signal while *Rms1* may be concerned with a second graft-transmissible signal other than cytokinin or auxin moving in a root-to-shoot direction. The action of *rms3* and *rms4* is not yet clear, but they appear to act largely in the shoot (3, 5). Not enough information is currently available on *Rms5* to permit speculation as to its action. Unlike *rms1* to *rms5, rms6* mutants branch only from the basal nodes, and grafting studies show the primary action of *Rms6* may be confined to the shoot (14).

A transgressive phenotype occurred in double mutants *rms1 rms2* (7, 15), *rms2 rms 5* (Fig. 2, Table 2) and *rms3 rms6* (14). The transgression was very marked in the case of *rms2 rms5* and occurred with two different alleles, *rms5-2* and *rms5-3*. These results indicate *Rms2* probably acts in a different pathway to *Rms1* and *Rms5*, and *Rms3* acts in a different pathway to *Rms6*. However, it should be kept in mind that transgression can occur where two genes act in the same biochemical pathway if both steps are only partially blocked, either as a result of a leaky mutant allele or genetic redundancy for the step.

There was no evidence of transgression in the case of double mutants *rms1 rms3, rms1 rms4, rms2 rms3* and *rms3 rms4*. Likewise for *rms2 rms4*, but the small numbers here justify only 80% confidence in the result. These five results suggest *Rms3* and *Rms4* may act in the same pathway. They could also imply *Rms1* and *Rms2* act in this pathway. However, the transgressive phenotype of the *rms1 rms2* double mutant, and evidence from physiological studies (3), indicate that *Rms1* and *Rms2* act in different ways not only to each

other but also to *Rms3* and *Rms4*. This example highlights the fact that while double mutant phenotypes provide useful information on gene interaction, the results can be difficult to interpret with any certainty.

The entire branching control process likely involves a very extensive sequence of events. The branching index used here, ratio of total lateral length to main-stem length, gives only one view of a double mutant. Detailed morphological, physiological and biochemical studies of double mutants may reveal further information on the nature of the interaction. Likewise, molecular analysis of the *rms* genes could provide crucial information on their nature and likely mode of action.

Acknowledgement: We thank Ian Cummings and Tracey Jackson for technical assistance, Dr. Christine Beveridge and Suzanne Morris for comment on the manuscript, and the Australian Research Council for financial support.

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