Branching in pea: double mutants of *rms7* with *rms1* through *rms5*

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Mutants *rms6* and *rms7* in pea have increased branching from basal nodes (6, 10). In contrast, mutants *rmsl* through *rms5* have increased branching from both basal and aerial (upper stem) nodes (1-3). A start has been made on checking double-mutant phenotypes. In some cases, the double mutant expresses an additive phenotype with branching more strongly enhanced than in either single mutant, e.g. *rms2-1 rms4-1*, *rms2-1 rms5-2*, *rms3-1 rms6-2* and *rms6-1 rms7-1* (6, 8-10). In other cases, epistasis occurs and the double-mutant phenotype does not transgress beyond the range of the single mutants, e.g. *rms1-1 rms4-1* and *rms2-1 rms3-1* (8).

In the present study, the phenotype of the double mutants of *rms7* with *rmsl* through *rms5* was examined in tall *(Le)* plants grown in the glasshouse under an 18-h photoperiod (for details see 8). This strategy was designed to allow identification of double-mutant plants regardless of whether they were clearly obvious from an additive double-mutant phenotype or hidden by epistasis of one or other mutant allele. The strategy makes use of the following information gleaned from years of observation of branching in pea. 1) Basal branching is expressed more strongly in dwarf *(le)* than tall *(Le)* plants (4, 7, 8). 2) In contrast to dwarf plants, tall plants with WT (wild-type) branching genes invariably fail to produce secondary stems from a basal node under the 18-h conditions used. 3) Tall *rmsl* through *rms5* plants always produce aerial laterals under these 18-h conditions. Thus in an F_2 population, any tall plant with a major secondary stem and no aerial laterals could be considered as homozygous for *rms7*. In cases where double-mutant plants were not exposed in F_2 by an additive phenotype, F_3 progenies could be grown from the homozygous *rms7* plants and any F_3 plants expressing strong growth of aerial branches would be exposed as double mutants.

In accordance with this strategy, dwarf line M3T-475 *(rms7 1)* was crossed with tall lines Wtl5240 *(rms1*-5, ex Kaliski), K524 *(rms2A*, ex Torsdag), K487 *(rms3-1* ex Torsdag), K164 *(rms4-1*, ex Torsdag) and HL298 *(rms5*-3). HL298 was specifically bred for this purpose from a cross between tall cv. Torsdag and Wtl5241 *(rms5-3*, ex dwarf cv. Paloma). Further details on these mutant lines are given by Arumingtyas et al. (2).

The *rmsl*-5 *rms7-1* double mutant was found to have an additive phenotype (Fig. 1). Tall F_2 plants of cross Wtl5240 *(rms1-5)* x M3T-475 *(rms7-1)* could be partitioned into four branching classes corresponding to WT, *rms7*, *rms1*, and *rms1 rms7* double-

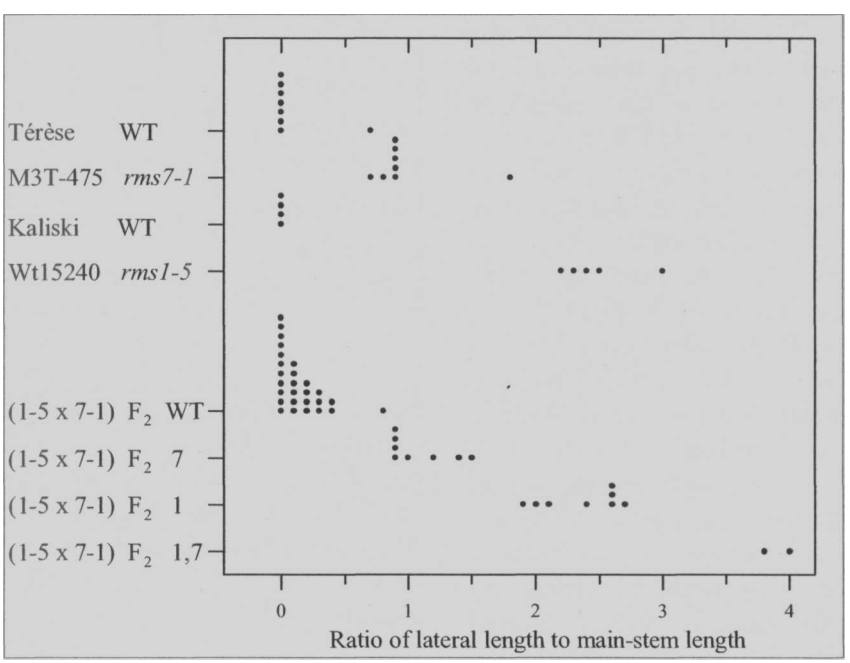

Fig. 1. Distribution of the branching index 'ratio of lateral to main-stem length' for cv. Terese, M3T-475 (rms7-1), *cv. Kaliski, Wtl5240* (rms1-5), *and tall F_2 plants from the cross Wtl5240 x* M3T-475. *The F_2 data are subdivided into four branching phenotypes representing WT,* rms7, rms1, *and double-mutant* rmsl rms7 *plants. Data are from mature plants; photoperiod 18 h.*

mutant plants. There was a quantum increase in the ratio of lateral to main-stem length from WT to *rms7* to *rms1* to *rms*1 *rms7*. The observed F_2 numbers of 27 WT, eight *rms7*, eight *rms1* and two *rms1 rms7* plants are in good accordance with a di-hybrid 9: 3: 3: 1 ratio ($P > 0.9$). The additive phenotype of the double mutant was confirmed by growing F_3 progenies from *rms7* F_2 plants (data not shown). The tall *rms1*-5 *rms7-1* segregates in the F_3 population had a branching index 42 % higher than the WT15240 *rms1-5* single-mutant controls grown with this generation.

Over half the tall WT F_2 plants of cross WT15240 x M3T-475 produced some lateral branches in contrast to the complete absence of branching in the tall WT control Kaliski (Fig.l). The WT F_2 plant with the rather high branching index of 0.8 was confirmed to be WT by growing F_3. The branching in these WT plants was comprised principally of short, late emerging, aerial laterals; no WT plant produced a secondary stem from a basal node. The late outgrowth of aerial laterals on many of the tall WT F_2 plants may partially be explained by the fact that Terese and M3T-475 tended to flower at node 18 while Kaliski and Wt 15240 generally flowered at node 16. A two-node delay in flower initiation allowed increased opportunity for aerial lateral outgrowth. (NB. The late emerging aerial laterals referred to here are a normal part of the first reproductive cycle and not to be confused with second-growth laterals that emerge if plants fail to undergo monocarpic senescence).

Growth of laterals from the cotyledonary node (node 0) was rare in the tall F_2 plants of cross Wtl5240 x M3T-475 and occurred only in two of the eight *rms7* plants and one of the two double-mutant plants.

The tall F_2 population of cross K524 *(rms2-1)* x M3T-475 *(rms7-1)* gave a clear separation into 21 WT, seven *rms7*, seven *rms2* and two probable *rms2 rms7* plants, numbers that closely fit a 9: 3: 3: 1 ratio (Fig. 2). The two F_2 plants with the high branching indices of 3.8 and 4.4 were backcrossed to K524 and M3T-475; the F_1 results gave supporting evidence these two plants had a double-mutant genotype (data not shown). Interestingly, the double-mutant F_3 plants had a branching index only 8% higher than the K524 *rms2-1* controls grown with them (Fig. 2). Thus there is evidence that the *rms2-1 rms7-1* double mutant has an additive phenotype. However, while the F_2 data indicated a clear quantum increase in branching, the F_3 data showed only a small quantitative increase in branching over the *rms2A* single mutant.

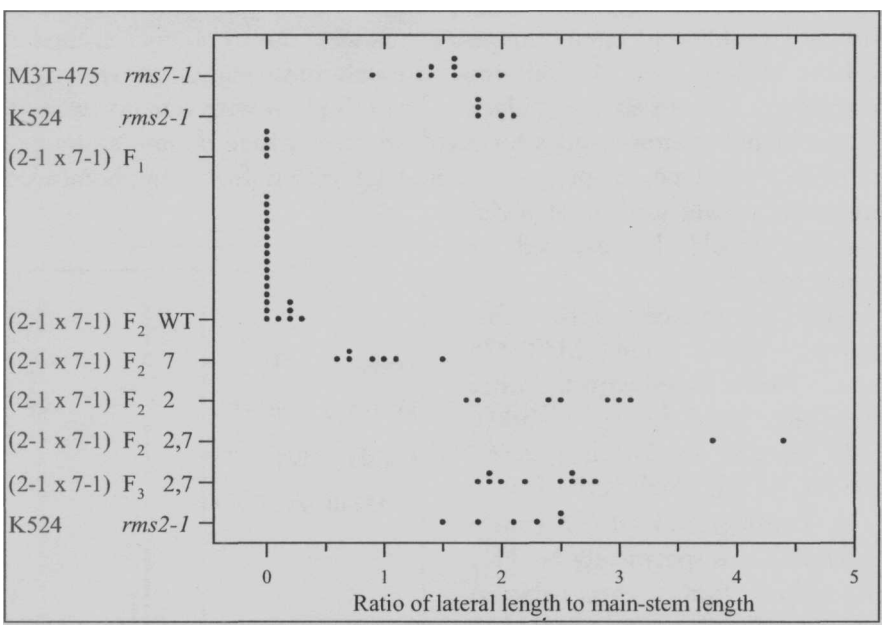

Fig. 2. Distribution of the ratio of lateral to main-stem length for M3T-475 (rms7-l), *K524* (rms2-l), *and the* F_1, F_2 *and* F_3 *plants of cross K524 x* M3T-*475. F_2 data are subdivided into four branching phenotypes representing WT,* rms7, rms2 *and* rms2 rms7 *plants. F_2 and* F_3 *data are from tall plants only. The plants represented in the upper seven rows were sown July* 31*, 2000 and in the lower two rows July 30, 2001. Data are from mature plants; photoperiod 18 h.*

The F_2 of cross K487 *(rms3-1)* x M3T-475 *{rms7-1*) gave no evidence of transgression (data not shown). An *rms3-1 rms7-1* double-mutant line was obtained in F_3 and F_4 via a clear *rms7* F_2 plant. The branching index of the double-mutant plants did not appear to be enhanced beyond the range of vigorous, single-

mutant, *rms3-1* plants. However, the double mutant did combine features from both single mutants: the aerial lateral growth of *rms3-1* plants and the tendency to produce laterals from the cotyledonary node shown by *rms7-1* plants. Out of 73 tall *rms3-1 rms7-1* plants, one third produced one or more laterals from node 0, and many of these laterals continued growth into secondary stems. In contrast, basal laterals were not produced from node 0 of the K1487 *rms3-1* mutant: when present, basal laterals grew from nodes 2 and/or 1 of K487 plants.

No transgression for the ratio of lateral to main-stem length occurred in the F_2 of cross K164 *(rms4-1)* x M3T-475 *(rms7-1)* (data not shown). Homozygous *rms4-1 rms7-1* plants were obtained in the F_3 and F_4 via clear *rms7* F_2 plants. The branching index of tall *rms4-1 rms7-1* plants did not transgress beyond the upper range of the K164 *rms4-1* single mutant. Five per cent (3/59) of double-mutant plants produced a lateral from the cotyledonary node, a feature not seen in the single mutant *rms4-1.*

F_2 data for cross HL298 *(rms5-3)* x M3T-475 *(rms7-1)* indicated an additive double-mutant phenotype (Fig. 3). The tall F, population could be partitioned into 33 WT, three *rms7,* ten *rms5,* and two probable *rms5 rms7* double-mutant plants with a substantially higher branching index than either single-mutant parent. The phenotypic contrast between the *rms5* single mutant and the double mutant is not wholly revealed by the index 'ratio of lateral to main-stem length'. Expression of *rms5-3* in a tall background seems fairly weak. Three out of six HL298 plants produced no basal lateral of any consequence; likewise three out of ten tall *rms5* F_2 plants. These *rms5* plants were not really identifiable as *ramosus* mutants until outgrowth of aerial laterals commenced three to four weeks after sowing. In contrast, the presumed double-mutant plant with index 3.5 (Fig. 3) had four basal laterals exceeding 10 mm by day 11 (two from node 0, one from node 1 and one from node 2) and all four shoots continued growth to become secondary stems. These observations provide further support for the view that *rms5-3 rms7-1* plants have an additive phenotype that combines the aerial branching of *rms5-3* plants with the enhanced basal branching of *rms7-1* plants.

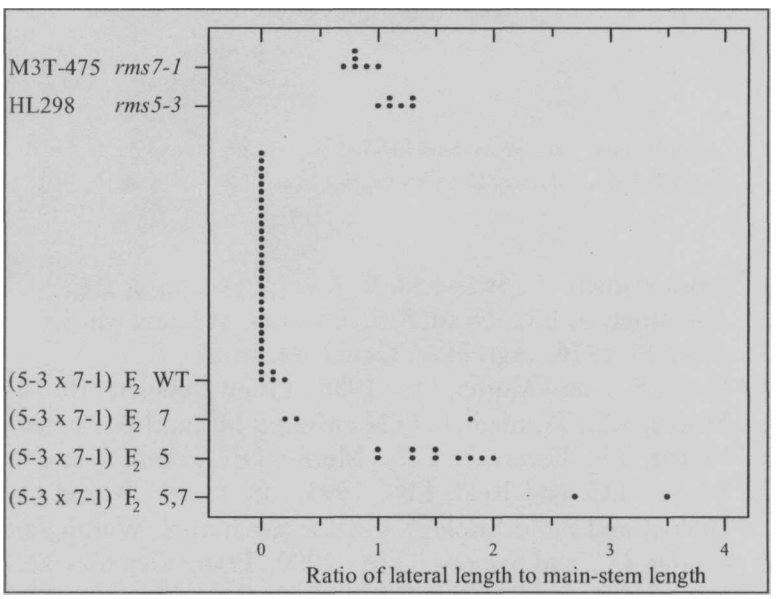

Fig. 3. Distribution of the ratio of lateral to main-stem length for M3T-*475* (rms7-l) *and HL298* (rms5-3), *and tall F_2 plants from the cross HL298 x M3T-475. The F_2 data are subdivided into four branching phenotypes representing WT,* rms7, rms5, *and double mutant* rms5 rms7 *plants. Data are from mature plants; photoperiod 18 h.*

Segregation for branching phenotype in the F_2 of cross HL298 x M3T-475 (Fig. 3) is in accordance with a 9: 3: 3: 1 ratio ($P > 0.1$). However, numbers in the *rms7* class are below expectation and it seems highly likely that some tall *rms7* plants in this F_2 failed to produce a basal lateral and were indistinguishable from WT plants. In a tall background, results from several crosses showed that *rms7-1* behaved as a weak mutant lacking the full penetrance of classic Mendelian mutants. In the F_3 of cross Wtl5240 *{rms1-5)* x M3T-475 (*rms7-1*), only 38% (8/21) of tall *rms7* plants had a phenotype distinguishable from WT. In the F_3 of crosses K164 (*rms4-I)* x M3T-475 and K487 (*rms3-1*) x M3T-475, penetrance of *rms7-1* fell to 15% (3/20) and 6% (2/36), respectively. With a penetrance that low, the *rms7-1* mutation may well have escaped detection had it been induced in a tall cultivar. We were also fortunate to have obtained *rms7* numbers right on Mendelian expectation in two F_2 populations (Figs 1 and 2). Clearly, penetrance of *rms7-1* varied from planting to planting.

In summary, a clearly additive double-mutant phenotype was observed for *rms1-5 rms7-1* (Fig. 1) and *rms5-3 rms7-1* (Fig. 3), *rms2-1 rms7-1* showed variable levels of transgression (Fig. 2), and *rms3-1 rms7-1* and *rms4-1 rms7-1* did not transgress, respectively, beyond the upper range of the *rms3-1* and *rms4-1* single-mutant parents. Interestingly, *rms1* and *rms5* both produced an additive phenotype with *rms7* (Figs 1 and 3), which fits well with evidence that *RMS1* and *RMS5* regulate the same novel branching signal (5). However, *rms1-5* and *rms5-3* had a lower branching index than *rms2-1*, *rms3-1* and *rms4-1* when all five mutants were planted and grown together (data not shown). Thus *rms1-5* and *rms5-3* have more room to show enhanced branching and an additive phenotype in combination with *rms7-1*.

The basal branching mutants *rms6-1* and *rms7-1* produced an additive double-mutant phenotype, suggesting that *RMS6* and *RMS7* may operate in different pathways (6). That view is supported by the fact that *rms3-1 rms6-2* was found to have an additive phenotype with strongly enhanced branching (10), whereas no evidence of transgression was found here for the *rms3-1 rms7-1* double mutant.

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