Identification of genes affecting root mass and root/shoot ratio in a JI1794 x 'Slow' RIL population

Weeden, N.F. and Moffet, M.

Dept. Plant Sci. and Plant Path.
Montana State Univ., Bozeman, MT

Relatively few genes have been described in pea that influence root size or architecture. Contin and Marx (3) investigated uprooting resistance and taproot thickness in several segregating populations. They found a positive correlation between the two characters as well as between these and later flowering, branched-stem types; however, neither gene designations nor locus positions were presented. A number of root architecture mutations have been described, often with altered nodulation phenotypes (1, 4); however, such mutants are not responsible for most of the variation in root morphology observed in the pea germplasm collection (6). We became interested in the genetic determinants of root mass in the *Pisum sativum* ssp *elatius* line JI1794 when this line appeared to have a greater susceptibility to common root rot (produced by *Aphanomyces eutieches*) than did most cultivated types (unpublished observations). We had also observed that JI1794 produced a smaller root system than cultivated types, and we desired to know if the size of the root system played a role in resistance to common root rot and other soil organisms. The purpose of this study was to investigate the genetic basis of the differences in root mass observed between JI1794 and the *P. sativum* ssp. *sativum* line 'Slow.'

Material and Methods

The JI1794 x 'Slow' population used in this study consisted of 53 F_2-derived recombinant inbred lines (RILs) now at the F_{14} generation. It is the population on which the linkage data forming the basis of the consensus map of pea was generated (10). A QTL analysis of seed size has been performed on this population (9), providing the opportunity to examine the possible relationship between genes influencing seed size and those producing greater root growth. 'Slow' is a typical *P. sativum* ssp. *sativum* line with dwarf (*le*) stature, large leaflets (*Fo*) and a vigorous root system. JI1794 is tall (*Le*) but has at least one other gene that produces the shorter 'pumilo' phenotype (height 60-80 cm). JI1794 also has thinner stems, narrower leaflets, and a less robust root system.

Ten seeds of each parent (JI1794 and 'Slow') and four seeds from each of 42 RILs were nicked and placed in a 1:1 perlite:vermiculite mix in a glasshouse. The remaining 11 RILs from this population produce weak, relatively unproductive phenotypes and were not used in the study. Each seed was place in a separate 13 cm diameter pot to provide plenty of soil volume and facilitate sampling of roots. As not all seed germinated immediately it was necessary to use a different measure of age than number of days after planting. We used node number as a measure of comparative age. All parental plants and three healthy plants from each RIL were harvested when eight true leaves were present on the main stem. If the plant had multiple stems we used the equation $X + \frac{1}{2} Y = 8$ (where X = number of leaves on primary stem and Y = number of leaves on secondary stems). Preliminary studies within lines that gave simple and branched phenotypes indicated that this formula permitted sampling plants at approximately the same age (unpublished results).

Each plant was carefully removed from its pot, the roots washed clean of soil mix and the roots and upper portion sampled by cutting the main axis immediately below and above the cotyledon attachment site, respectively. The two portions of the plant were blotted on paper towels, air dried for 15 min and weighed to the nearest 0.1 g on a Mettler pan balance. Average root weights and root/shoot ratios were calculated for each parent and RIL. These data were used for comparison of allelic means and for single factor analysis of variance. We used this approach instead of the traditional QTL analysis because of the relatively few RILs available. We anticipated that we would be able to resolve the effects of only a few major genes (if any) affecting this character. Markers spaced at approximately 10 cM intervals were used to provide the genotype of each RIL in each region of the linkage map.

A root mass/shoot mass ratio was obtained for each plant analyzed and these values were averaged within lines. The average root/shoot values were subjected to the same comparison of allelic means and analysis of variance described above for the root mass data.

The linkage map for the JI1794 x 'Slow' RIL population has been partially described in Weeden et al. (10). The primer sequences for the markers mentioned in this paper are as follows: B448 (5'-GTTGTGCCTG), S20 (5'-TGAACCGCCG), Myb26F (5'-GCACGAGTTCACCTTTCA) and EnodF (5'-TAGACAAATCTTCGACAGTCGTGG).

Results

Root weights for JI1794 averaged 0.48 +/- 0.20 g and those for 'Slow' averaged 1.89 +/- 0.62 g (Table 1). Average root mass among the RILs ranged from 0.09 g to 1.76 g. When the root mass data were compared with the segregation of markers across the linkage map, a significant correlation was observed with the region on linkage group III containing the *Le* locus (Table 1). Dwarf plants (*le*) had larger roots than did tall (*Le*) plants. The maximum for this effect fell within 3 cM of *Le* (Fig. 1) and dropped to insignificant values within 5 to 10 cM from this locus.

Bfruct	B448a	Myb_{900}	*Le*	S20d	EnodF-a
0.3313	0.3605	0.4135	0.4108	0.2758	0.2069
P=0.02	P=0.008	P=0.002	P=0.002	P=0.034	P=0.10

Fig. 1. A portion of linkage group III around Le presenting the difference in average mean weight of roots between lines homozygous for the 'Slow' allele at the respective marker and lines homozygous for the JI1794 allele. The P values given are for Student's t test. The markers B448a, Myb_{900}, S20d and EnodF-a are all RAPDs. The position of an unlabeled RAPD is shown just to the left of B448a. The marker Bfruct is an STS marker described in (1). The distance between Myb_{900} and Le is approximately 3 cM.

The effect of the *Le* region was sufficiently large as to obscure small effects produced by other regions. However, by treating tall plants and dwarf plants as separate subgroups and performing the same analysis on each subgroup, a region on linkage group VII near *Amy* revealed a significant ($P<0.05$) difference between allele means (Table 1). The effect of this region on root mass was barely significant but is included here because we also observed an effect of this region on root/shoot ratio.

Table 1. Difference in means for root mass (gm) in JI1794 x 'Slow' RILS

Effect of Le region on linkage group III				
	Allelic mean for root mass			
Locus	**Lines with JI allele**	**Lines with Slow allele**	**Difference**	**P**
Le	**0.70 ± 0.29**	**1.11 ± 0.40**	**0.41**	**0.002**
Effect of region on linkage group VII after separation into le and Le subgroups.				
Subgroup	**Allelic mean for root mass**			
Region	**Lines with JI allele**	**Lines with Slow allele**	**Difference**	**P**
le	**0.95+/- 0.37**	**1.33 +/- 0.29**	**0.38**	**0.047**
Le	**0.54 +/- 0.19**	**0.78 +/- 0.28**	**0.24**	**0.02**

The difference between parental lines was much less when mass measurements were converted to root/shoot mass ratios. 'Slow' gave an average ratio of 1.25 (n=10) and that for JI1794 was 1.12 (n=9). The parallel analysis of the genetic basis of variation in root/shoot ratio identified regions on linkage groups VII

and III. The region on linkage group VII appeared to be identical to that revealed in the root mass analysis, being centered in the *Amy* region. The region on LGIII was very near *M* at the opposite end of the linkage group from *Le.* The magnitude of the effect on the root/shoot ratio produced by the *Amy* region was larger than that region's relative effect on root mass. The difference between means was significant ($P<0.005$) without consideration of the genotype at *Le*. Indeed, the *Le* region did not have a significant effect on the root/shoot ratio. The effect of the *M* region on the root/shoot ratio was even greater ($P \leq 0.001$, Table 2) than that of the *Amy* region. However, the allele that increased the root/shoot ratio in the *M* region came from the JI1794 parent

Table 2. Differences in means for root/shoot ratio at *Amy* and *M* in JI1794 x 'Slow' RILS

	Mean for root/shoot ratio			
Locus	**Lines with JI allele**	**Lines with Slow allele**	**Difference**	**P**
Amy	**0.87 +/- 0.31**	**1.24 +/- 0.46**	**0.37**	**0.004**
M	**1.22 +/- 0.46**	**0.83 +/- 0.27**	**0.39**	**0.001**

The presence of a gene near *M* in JI1794 that increases the root/shoot ratio suggests that plants exhibiting a larger root/shoot ratio than that observed for 'Slow' could be generated by combining the allele from the *M* region of JI1794 with the 'Slow' allele from the *Amy* region. In the RIL population such genotypes did give the highest average root/shoot ratio (1.4), but this average fell well within the higher values obtained for the 'Slow' parent. Thus, we did not observe transgressive variation for high root/shoot ratios. In contrast, extremely low average root/shoot ratios (to 0.29) were obtained for the genotype containing the 'Slow' allele in the *M* region and the JI1794 allele in the *Amy* region. The average for the genotype (0.64) was significantly below that for JI1794.

Discussion

The significant difference in root mass between JI1794 and 'Slow' permitted an investigation of the genetic determinants of this character in an RIL population for which a saturated linkage map was available. As part of a preliminary study, we investigated whether wet or dry weight measurements would be preferable. Our results indicated that the relative weights did not change but that the variance was greater in the dry weight data (data not presented). Thus for simplicity in experimental design we used only wet weights in the analysis of the population. A second issue that could have biased the analysis was the removal of 11 lines with low vigor from the population before initiating the experiments. Examination of segregation ratios for individual markers demonstrated that these ratios were very similar in the total population and in the 42-line subset except for a region on linkage group V centered at the locus *Gp*. The 42-line subpopulation was slightly deficient in yellow-podded genotypes compared to the entire population. Our analysis of root mass and root/shoot ratio did not identify an effect near this region of the linkage map, and we do not believe elimination of the 11 lines compromised our analysis.

The analysis identified a major influence of the *Le* region on linkage group III on root mass. The tightness of the linkage to *Le* suggests that the product of the *Le* locus (gibberellin 3β-hydroxylase) (5, 8) might be responsible for the observed effect on root mass. However, at present there is very little evidence that gibberellins have an effect on root growth, and we therefore prefer to postulate that the effect is due to a gene closely linked to *Le*. The absence of an effect of this region on root/shoot ratio indicates that the gene has an overall effect on plant growth. Lines with the allele from 'Slow' apparently grow more vigorously and show increased weight gain in both roots and shoots than do lines with the allele from JI1794. The effect could be ascribed to the larger seed produced by 'Slow,' but the *Le* region was not identified as possessing a seed weight QTL in the study by Timmerman-Vaughan et al. (9), and many of the dwarf lines in the population have relatively small seeds.

The conversion of the data to root/shoot ratios virtually eliminated the influence of the *Le* region, and enhanced that of the *Amy* region. This latter region thus appears to both stimulate root growth and influence partitioning of the photosynthate between root and shoot. In contrast, the region near *M* was only observed in

the root/shoot analysis and presumably is only involved in the partitioning of carbon between the upper and lower portions of the young plant.

The two regions affecting root/shoot ratios both contain genes involved in the photoperiod response. The primary photoperiod-determining gene *Sn* is located very close to *Amy*, and the locus *Hr*, controlling the magnitude of the plant's response to photoperiod maps very close to *M*. When Contin and Marx (3) identified a correlation between tap root diameter and late-flowering, branching genotypes they undoubtedly were identifying the region near M. Hence we confirm and provide additional details regarding their observations, although as yet we have not been able to confirm that either *Sn* or *Hr* are segregating in the population because a third locus, *E*, is segregrating and under our growing conditions (16 hr day, 8 hr night) has the greatest influence on flowering time.

We were able to identify only one possible overlap between regions influencing root mass or root/shoot ratio and those affecting tolerance to common root rot. Three regions of the pea genome, one on linkage group IV (11), one on linkage group VI (2) and one on linkage group VII (7) have been associated with root rot tolerance. The region on linkage group VI was the one suspected of producing the high susceptibility seen in JI1794 (unpublished data). Our results suggest that a gene having a major influence on root mass or root/shoot ratio in the JI1794 x 'Slow' population is not present on linkage group VI. The relationship between the gene on linkage group VII affecting root/shoot ratio and that influencing tolerance to common root rot requires further investigation. Both genes map near *Amy*, but the QTL influencing tolerance was mapped in an entirely different cross.

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