

## Map position of the *FRO1* locus in *Pisum sativum*

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Iron acquisition in pea, as well as in other dicots and the non-grass monocots, involves a two-step reduction and transport process referred to as Strategy I (8). The root iron reductase in pea [designated *FRO1* (9)] is a plasmalemma-localized protein that reduces rhizospheric ferric iron (usually as Fe[III]-chelates) to ferrous iron (Fe<sup>2+</sup>), prior to the membrane uptake of Fe<sup>2+</sup> through the divalent metal transporter, RIT1 (2, 6). Thus, it is an important regulator of whole-plant iron status, especially under alkaline soil conditions where available iron is limiting (8). It also is a potential breeding target for the development of improved cultivars that would be less susceptible to conditions of iron deficiency stress.

Because of our interest in this gene, we wished to map the *FRO1* locus and therefore attempted to develop a *FRO1*-specific CAPS marker that could be used with the JI1794 x Slow mapping population. Over 1,000 segregating markers have been mapped in this recombinant inbred population (1, 11), permitting new markers/loci to be located with high precision. In order to obtain sequences for the *FRO1* alleles in both JI1794 and Slow, genomic DNA was extracted from root tissues [hydroponically grown plants (6)] of genotypes Slow and JI1794 using the DNeasy Plant kit (Qiagen, Inc., Valencia, CA, USA). A series of PCR primer sets were then designed from the available *PsFRO1* cDNA sequence (Genbank Accession AF405422), and PCR reactions were performed with DNA from both genotypes. The sequencing of a number of overlapping PCR products allowed us to determine the genomic sequences for *FRO1* in each parent; these are available as GenBank Accessions AY747697 and AY747696 for genotypes Slow and JI1794, respectively. Although several nucleotide polymorphisms were identified, we selected the region from nucleotides 702 to 1623 in Slow (equivalent to nucleotides 706 to 1623 in JI1794) as a good region for an easily distinguishable CAPS marker. The PCR product in JI1794 contains a polymorphism at nucleotide 1288 (intron) that can be cleaved with *EcoRI*. Treatment of the PCR products with this restriction enzyme yields a full-length PCR product of 922 nucleotides in Slow and two products of 583 and 335 nucleotides in JI1794.

To map the *FRO1* locus in the JI1794 x Slow RIL population, PCR reactions were performed with genomic DNA isolated from 51 F<sub>12</sub> individuals. The primers used were: forward: AGAACATGGCCCTAAAGTGACGTAA; reverse: CCCTTAGAGAAGTTGAGTTCAACGG. Products were treated with *EcoRI* and separated on 2% agarose gels to score genotypes in each line. The map position of *FRO1* also was determined in the F<sub>8</sub> RIL population (49 lines) generated from the cross MN313 x JI1794 and in two BC<sub>1</sub>F<sub>6</sub> populations: [Sparkle x WL808] x Sparkle (42 lines) and [Delta x WL808] x Delta (37 lines). In the MN313 x JI1794 population the enzyme *Hinf* produced a polymorphism, and in the [Delta x WL808] x Delta population, the enzyme *Hsp92* II was used to reveal a polymorphism in the gene sequence.

### Results

The desired fragment of about 920 bp was amplified in all parents tested (JI1794, WL808, Slow, MN313, Sparkle and Delta). In some amplifications a secondary fragment at about 900 bp was observed after electrophoresis; however multiple fragments were not observed after restriction digests, suggesting that this secondary fragment was an artifact of the electrophoresis process. The *EcoRI* site was present in the alleles possessed by JI1794, WL808, MN313 and Delta, whereas alleles in Slow and Sparkle lacked an *EcoRI* site. MN313 was found to lack a *Hinf* site possessed by JI1794, and WL808 displayed a different pattern of *Hsp92* II fragments than the other parents.

Segregation at *FRO1* did not depart significantly from the expected 1:1 (in the F<sub>2</sub>-derived RILs) or 3:1 (in the BC-derived RILs) ratios. In the JI1794 x Slow RIL population *FRO1* mapped about 13 cM distal to

**Table 1. Joint segregation analysis of *FRO1* with P393 in four pea populations**

Population <sup>b</sup>	N	<i>FRO1</i> ,P393 genotype of line <sup>a</sup>				Linkage $\chi^2$ (expected ratio)	Percent Recomb.
		P <sub>1</sub> P <sub>1</sub>	P <sub>1</sub> P <sub>2</sub>	P <sub>2</sub> P <sub>1</sub>	P <sub>2</sub> P <sub>2</sub>		
JI1794 x Slow	51	23	7	5	16	20.3*** (1:1:1:1)	12
MN313 x JI1794	49	18	4	7	20	14.9*** (1:1:1:1)	11
[D x 808] x D	37	28	2	4	3	7.6** (9:3:3:1)	15
[Sp x 808] x Sp	42	34	3	2	3	13.1*** (9:3:3:1)	12

<sup>a</sup> P<sub>1</sub> = Homozygous for allele from first parent listed, P<sub>2</sub> = homozygous for allele from second parent listed  
 \*\* = P<0.01; \*\*\* = P<0.001

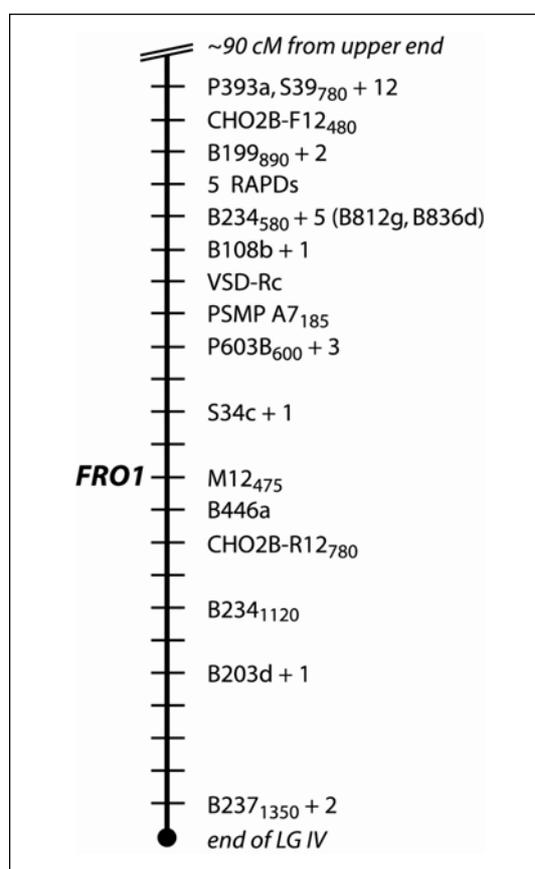
<sup>b</sup> D = Delta, 808 = WL808, Sp = Sparkle

P393a and about 6 cM distal to the microsatellite PSMP-A7<sub>185</sub> (Table 1; Fig. 1). This region represents the most distal portion of the lower arm of LGIV as depicted on the consensus map (11). The segregation pattern of *FRO1* did not display linkage to any other region of the linkage map. In the remaining three populations *FRO1* also displayed linkage with markers on LG IV (Table 1). In the MN313 x JI1794 population, *FRO1* showed weak linkage with a region on LG V. This linkage did not increase in intensity in either direction along LG V, nor was it confirmed in any other population examined.

### Discussion

The location of *FRO1* near the distal end of the non-satellite arm of LGIV (chromosome 7) makes this gene an important anchor marker on the pea linkage map. Other anchor markers for this region include the isozyme ALATp (11) and three morphological markers, *age*, *wsp* and *sil* (10, 12). Although *wsp* and *sil* are excellent markers for crosses in which they segregate, lines possessing these mutations are rarely used as parents to generate populations for mapping or QTL studies. The root mutation, *age*, is more difficult to score and is not recommended as an anchor marker. Only two alleles at *Alatp* are known, and one of these is rare (13). In contrast, we identified four distinct alleles of *FRO1* in a relatively limited, albeit wide survey of the pea germplasm. Two of these alleles are present in cultivated germplasm (MN313 and Sparkle), suggesting that many other alleles could be identified in a more intensive survey of the gene sequence in other cultivars.

The JI1794 x Slow RIL population possesses the most saturated map of the four populations we used for mapping. *FRO1* mapped unambiguously in a cluster of RAPD markers on LGIV, co-segregating with one of them. In the other crosses, *FRO1* represented the most distal marker on



**Fig. 1. Position of *FRO1* on the lower end of LG IV. *P393a* is located at the top of the figure about 90 cM from the other (upper) end of LG IV. Each cross bar represents 1 cM. The other markers are RAPDs mapped in the JI1794 x Slow population except PSMP A7, which is a microsatellite. The Arabic numerals after the '+' signs indicate the number of additional RAPDs mapped to that location in the JI1794 x Slow population. Sequences for the RAPD primers can be requested from NFW.**

linkage group IV and did not display tight linkage (<5 cM) with any marker. In all cases the most closely linked anchor marker was P393, approximately 13 cM from *FRO1*. Although in one population (MN313 x JI1794) *FRO1* also exhibited moderate linkage (15-20 cM) to a region on LG V, this linkage was judged to be coincidental because it remained relatively weak regardless of the marker on LGV we tested and failed to be confirmed in any of the other crosses.

Mapping the *FRO1* (iron reductase) locus to LGIV is an important step in our continuing investigations into the molecular regulation of plant iron homeostasis. This information will be instructive as we map other iron-related loci in pea, such as those for the ferrous iron transporter, *RIT1*, or for two iron-hyperaccumulating mutations, *brz* and *dgl*. The *brz* mutant (7), known for bronzing of the leaves, and the *dgl* mutant (3) known for degenerative leaves, have both been shown to over-accumulate iron due to an up-regulation of root iron reductase activity (5, 6). However, physiological evidence suggests that both mutations induce a shoot-derived, phloem-mobile signal that up-regulates *FRO1* and other genes in the roots, rather than being alterations in the *FRO1* gene, *per se* (4, 5). The *Brz* locus is known to be located at the opposite end of LGIV from *FRO1* (11), and it will be interesting to see whether *RIT1* and *Dgl* reside near the *FRO1* locus or assort independently.

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