Mapping the *st* locus in respect to three molecular markers on Linkage Group III

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The overwhelming majority of genetic studies in recent years have been based on DNA markers. Polymerase chain reaction (PCR)-based DNA markers have been recognized to be powerful tools for rapid construction of genetic maps. However, morphological markers still remain very convenient due to their ease of scoring and allowing larger plant numbers to be analyzed.

A pea consensus linkage map was published in 1998 (1) which included mainly morphological and isozyme data known to date. integration of new molecular, biochemical and morphological data (2) into existing maps has been possible through the use of a number of anchor genes (3). The *st* gene on Linkage Group III is a convenient genetic marker manifested as reduced stipules (4) that is clearly seen in young seedlings. The recommended anchor markers in LGIII are *Aat-c, Adh-1, M, st, b,Lap-1* (3). In the present work, we mapped the *st* locus with respect to the morphological marker *b* and three molecular markers based on the following DNA sequences available from public databases: AF255058 *Pisum sativum* 33 kDa ribonucleoprotein gene, complete cds; AF280748 *Pisum sativum* phospholipase C gene; and AJ832139 *Pisum sativum* sym7 gene for GRAS family protein.

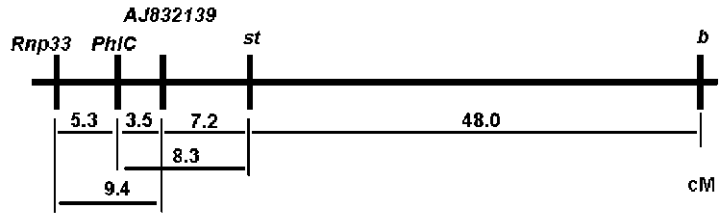
The latter gene is defined in the database as " sym7" (5); however, it seems to be different from the marker with the same designation "sym7" described by (6) and mapped on LGIII distally to *b* (1). To avoid confusion we designate it here as *AJ832139,* while the former two markers are designated as *Rnp33* and *PhlC* as in (7, 8). The molecular markers used were assessed with the use of CAPS (9) and the following primers and restriction enzymes were used:

Primers used	Primer sequence	Restriction enzyme	Length of PCR-product (bp)	Fragment lengths in WL1072 (bp)	Fragment lengths in VIR320(bp)
Rnp33 If Rnp33_2r	5'ATGTCTGTAACTTCCACCACT 5'ctgtcttcagcaacacttact	TruI	2034	622 296 a number of smaller fragments	approx. 400 approx. 200 a number of smaller fragments
PhlC If PhlC_9r	5' CACAGAGAATGAAGCACAATC 5'ccttcaagctttccgagcta	HpaII	963	694 269	351 343 269
AJ832139 14f AJ832139_13r	5'GCGACTTAGCTCGAGTGATA 5'tttgcaaagcttaccggaac	BstFNI	958	551 407	551 206 201

One hundred twenty-two plants of an F_2 population from the cross, WL1072/VIR320, where WL1072 carried *st* (reduced stipules) and *b* (pink flowers), were analyzed for the genotype with respect to *Rnp33, PhlC* and *AJ832139,* as judged on fragment lengths formed after endonuclease treatment of PCR-products and characterized for *st* and *b*. Approximately 10 seeds collected from St- plants were sown to assess the allelic state of the *st* locus. The F_2 progenitors of the F_3 families segregating for normal/reduced stipules

clearly seen in young seedlings were regarded as heterozygous. Mapmaker 3.0 software was used to construct the genetic map of the LGIII segment in Figure 1.

Figure 1 Fragment of the linkage map of LGIII.



In this study we mapped three molecular markers closely linked to st. One marker, AJ832139, had not been previously mapped. The choice of *st* was due to its ease in scoring and convenient use in mapping experiments related to the central region of LGIII. The *b* locus appears to be quite distant to *st* in our population.

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