Characterization of microsatellite loci using selected pea accessions and recombinant inbred lines (RILs)

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Most microsatellite DNA sequences are comprised of short, rapidly evolving tandem arrays located in untranslated DNA. They are unlikely to be affected directly by natural selection, making them highly informative neutral molecular markers (4-5). In a previous study (6), we developed and characterized novel pea microsatellite loci and evaluated their applicability as polymorphic molecular markers primarily by employing the Randomly Amplified Microsatellite Site (RAMS) protocol (3). In the present study, we extend these analyses by examining banding patterns produced by the microsatellite-specific primer sequences cited in the original RAMS project (3). Once again, RAMS profiles for a variety of pea accessions are used to assess band pattern variability, and a Sequence-Tagged Microsatellite Site (STMS) is characterized and mapped using Recombinant Inbred Lines (RILs).

Materials and Methods

DNAs from 17 pea accessions representing the range of the genus *Pisum* are amplified with each of 15 primer sets (3) and separated on polyacrylamide gels to evaluate detectable differences using the RAMS method (see 6). Clearly discernable polymorphic and monomorphic bands between 90-300 bp in size are scored as "present", "absent" or "uncertain" for each accession. Primer sets that provide at least one polymorphic band are surveyed using a more comprehensive set of 64 pea DNAs.

A STMS for primer set PSMPA6 is isolated by applying an increased primer annealing temperature (60° C). The STMS locus is evaluated for a similar set of 17 pea DNAs using 3% agarose gels stained with ethidium bromide and viewed with UV transillumination. The locus is mapped on the pea genome using the MapManagerQTX computer package (2) and a set of 57 RILs derived from a cross between parent plants A1078-234 and PI179449.

Results and Discussion

Eight of the 15 primer sets examined in this study provide only monomorphic bands when evaluated on the initial 17 individuals selected to represent the genus *Pisum*. The remaining seven primer sets reveal varying degrees of polymorphism for the same accessions and produce 59 total bands when scored across the more comprehensive set of 64 pea DNAs. Thirty-one of these bands are polymorphic, while 28 of the bands are monomorphic. Primer PSMPA7 yields the greatest number of bands (14 bands), of which 12 bands are polymorphic (Table 1 and Fig. 1). Primer PSMPB16 yields the fewest number of bands (6 bands), of which 5 bands are polymorphic (Table 1). None of the primers yields only polymorphic bands when evaluated for all 64 pea accessions.

The STMS bands amplified with primer set PSMPA6 appear to follow some general pea phylogenetic relationships. Little or no amplification of the STMS band is evident for *P. fulvum*, *P. sativum* ssp. *abyssinicum* or *P. sativum* ssp. southern *humile* (one of the two *P. fulvum* individuals may display a weak band near

Table 1. Number of polymorphic bands produced by selected microsatellite primers.

Primer	No. Bands – across Taxa	No. Polymorphic bands – across Taxa
PSMPA6	7	2
PSMPA7	14	12
PSMPA9	7	2
PSMPB16	6	5
PSMPC20	11	4
PSMPAA31	7	1
PSMPAD144	7	5

154bp), while all of the *P. sativum* ssp. northern *humile*, ssp. *elatius* and ssp. *sativum* display one or more strong bands between 154 and 298bp (Fig. 2).

Because the PSMPA6 STMS site appeared to segregate with plant height in ssp. *sativum* accessions during the survey process, the STMS marker is analyzed further using a set of 57 RILs. The results reveal that the polymorphic bands do segregate according to plant height with the single exception of RIL 46, a short plant phenotype that produces the "tall" STMS marker (Fig. 3). Upon combining the STMS data with previously gathered morphological, isozyme, RAPD and ISSR data, tight genetic linkage (within 0.9 cM) is established between the PSMPA6 STMS and morphological marker *Le*.

The addition of these pea microsatellite-based molecular markers to current data sets should be useful for a number of applications, including both the delineation of relationships among cultivated peas and their wild relatives and the development of highly-detailed genetic linkage maps.

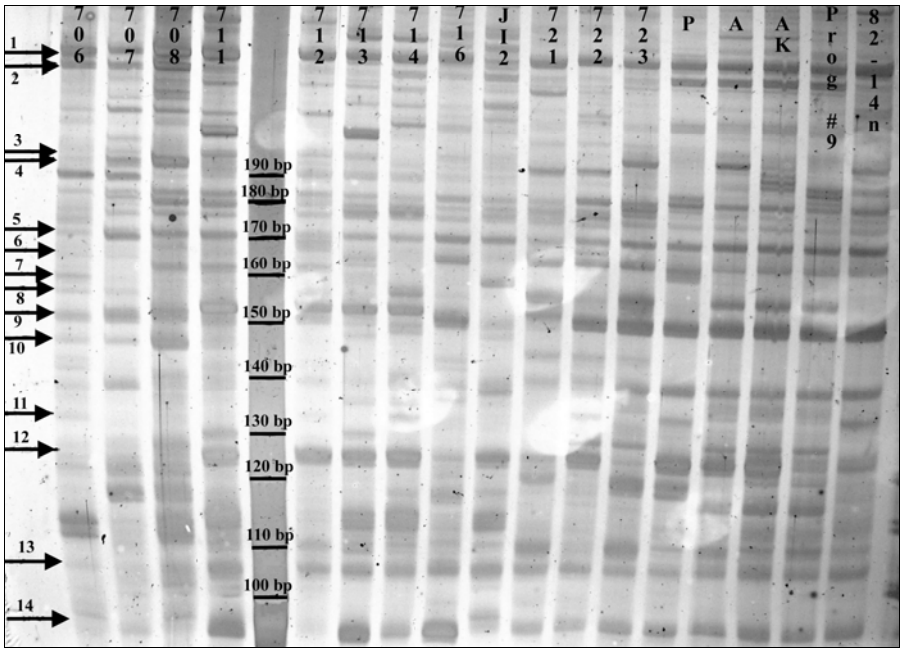

Fig 1. RAMS band patterns produced using primer set PSMPA7 with pea DNA. From left to right: fulvum *(706, 707 and 708), southern* humile *(711, 712, 713 and 714), northern* humile *(716),* abyssinicum *(JI2),* elatius *(721, 722 and 723) and* sativum *(P=PI179449, A=A1078-234, AK=cv. Alaska, Prog #9= cv. Progress #9 and 82-14n). JI denotes accessions from the John Innes collection, population isolates 706-723 are from the Ben Ze'ev and Zohary (1) collection, cv. Alaska is from J. Mollema and Son, Inc. (Grand Rapids, MI), cv. Progress #9 is from Ferry-Morse Seeds (Mountain View, CA) and accessions 82-14n, PI179449 and A1078-234 were kindly provided by G. Marx and N. Weeden. Both monomorphic bands (1 and 5) and polymorphic bands (2-4 and 6-14) are observed. The marker lane contains a 10-bp molecular size standard. The 6% polyacrylamide gel is treated with silver stain and preserved in cellophane. Digital image is captured using a Nikon CoolPix L5 digital camera mounted above a white light box. Molecular marker sizes, arrows and accessions are added using Adobe PhotoShop v. 6.0.*

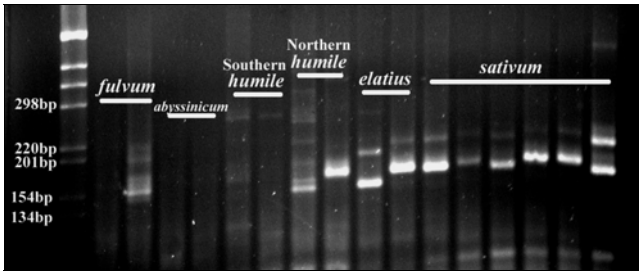

Fig 2. Primer PSMPA6 STMS band patterns detected in pea DNA. From left to right: fulvum *(703 and 707),* abyssinicum *(JI2 and JI225), southern* humile *(713 and 714), northern* humile *(716 and JI1794),* elatius *(721 and 722) and* sativum *(JI228, JI264, JI787, JI1035, JI1372 and cv. Alaska). JI denotes accessions from the John Innes collection, population isolates 703-722 are from the Ben Ze'ev and Zohary (1) collection and cv. Alaska is from J. Mollema and Son, Inc. (Grand Rapids, MI). PCR products are run on a 3% agarose gel and stained with ethidium bromide. The digital image is captured using a Gel Logic 200 Imaging System with UV transillumination. Molecular marker sizes, arrows and accessions are added using Adobe PhotoShop v. 6.0.*

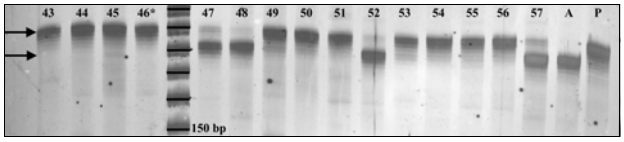

Fig 3. Primer PSMPA6 STMS band patterns for a selection of Recombinant Inbred Lines derived from an initial cross between accessions PI179449 and A1078-234. Parent PI179449 is a tall plant and displays a band at ~190bp. Parent A1078-234 is a short plant and displays a band at ~180bp. RIL individuals 43, 44, 45, 49, 50, 51, 53, 54, 55 and 56 are all tall individuals that display the 190bp-"tall" band. RIL individuals 47, 48, 52 and 57 are all short individuals that display the 180bp-"short" band. RIL individual #46 is a short plant that displays the "tall" band, thus suggesting genetic recombination between the STMS locus and the morphological marker Le. The 6% polyacrylamide gel is treated with silver stain and preserved in cellophane. Digital image is captured using a Nikon CoolPix L5 digital camera mounted above a white light box. Molecular marker sizes, arrows and accessions are added using Adobe PhotoShop v. 6.0.

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