

A mitochondrial DNA marker frequently found in wild peas

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Earlier we reported on a chloroplast DNA marker polymorphic in wild peas (1). In the present work a mitochondrial DNA marker is characterized in a set of accessions which includes 31 wild and 18 cultivated samples (see Appendix). The list of accessions tested is extended compared to that reported in (1). The primers were designed 5'-TGGTAATTGGTCTGTTCCGATTCT-3' and 5'-CCACTGCTTGAAGTGATTGTTACG-3' to match the nucleotide sequence of the EMBL X14409 accession (pea mitochondrial *coxI* gene for cytochrome oxidase subunit I). A fragment of 1200 bp was amplified using the following cycling parameters: initial denaturation at 95°C for 1 min followed by 38 cycles including denaturation at 94°C 59 sec, annealing at 56°C 45 sec, elongation at 72°C 1 min. Five microliters of the reaction were treated with one unit of *PsiI* endonuclease for 2 hours at 37°C in a volume of 12 µl and electrophoresed in 1.5% agarose gel in TAE buffer. We found a polymorphism for the presence/absence of the *PsiI* recognition site, so that PCR products obtained from some accessions were digested into two fragments of 260 and 940 bp, while other samples produced a single band of 1200 bp.

We assayed 49 specimens of wild and cultivated peas, which represent most of the presently recognised taxa. The digestion profiles of PCR products amplified from the *coxI* gene of some pea accessions are shown in Fig. 1.

We found that the restriction site for *PsiI* in the mitochondrial gene *coxI* is present in all eight accessions of *Pisum fulvum* Sibth et Smith and in all four accessions of *P. sativum* ssp. *abyssinicum*. Following Townsend (2) and Davis (3), we consider all wild representatives of *Pisum sativum* L. (except for *P. sativum* ssp. *abyssinicum* (A. Br.) Berger) within the same subspecies *P. sativum* ssp. *elatius* (Bieb.) Schmahl. *sensu lato*. In total we analysed 23 accessions of this subspecies and found that 11 of them have the restriction site while 12 do not (see Appendix). The restriction site was found in neither accession of *P. s. ssp. transcaucasicum* Govorov examined nor in any of 12 accessions of *P. s. ssp. sativum*. Noteworthy, in 44 of 49 accessions analyzed, the presence of the recognition site for *PsiI* in the mitochondrial *coxI* gene coincided with the presence of the recognition site for *AspLEI* endonuclease in the plastid gene *rbcL* (1, and unpublished), the studied fragments being amplified from the same samples of genomic DNA extracted from a single plant for each accession. The five exceptions were VIR320*, VIR2123, L90, Ps008-120689-0202 (which had the restriction site in the mitochondrial *coxI* but not the restriction site in the plastid *rbcL*), and accession VIR1975 in which the situation was opposite. We conclude that the restriction site in the *coxI* gene, as well as that in the plastic gene *rbcL*, were both present in the closest common ancestor of the genus *Pisum*. Both have been lost in at least one lineage of *P. sativum* ssp. *elatius*, and this lineage gave rise to the cultivated *P. sativum* ssp. *sativum*.

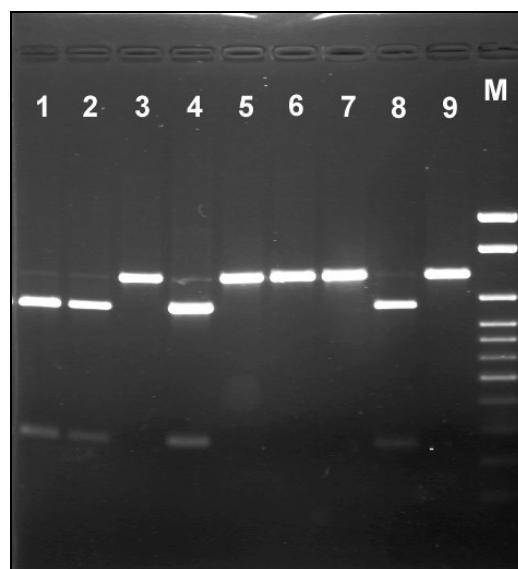


Fig. 1. Restriction fragments formed after *PsiI* digestion of PCR-amplified *coxI* gene from some pea samples. Lanes: 1—VIR2759, *P. s. ssp. abyssinicum*; 2—WL1446, *P. s. ssp. abyssinicum*; 3—VIR4871, *P. s. ssp. transcaucasicum*; 4—WT6, *P. s. ssp. abyssinicum*; 5—VIR3954, *P. s. ssp. sativum*; 6—VIR2593, *P. s. ssp. sativum*; 7—VIR3429, *P. s. ssp. sativum* ("Pisum jomardi"?); 8—VIR320*, *P. s. ssp. elatius*; 9—VIR1975, *P. s. ssp. sativum*; M—molecular weight marker 100-1000 bp + 1.5 kb + 2 kb.

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1. Bogdanova, V.S. and Kosterin, O.E. 2005. *Pisum Genetics* 37: 40-42.
2. Townsend, C. 1968. *Kew Bull. Roy. Bot. Gard.* 21: 435-358.
3. Davis, H. 1970. *Flora of Turkey and the East Aegean Islands*. Edinburgh, v. 3.

Appendix:

List of accessions analysed for the presence of *PsiI* site in *coxI* mitochondrial DNA marker:

Pisum fulvum Sibth et Smith:

L93, VIR6070, VIR6071, WL2140, JI2203, WT301, WT303, L96

P. sativum ssp. *abyssinicum*:

VIR2759, WL1446, WT6, JI1876

P. sativum ssp. *elatius* (Bieb.) Schmahl. with the restriction site:

VIR320, VIR320*, VIR2521, VIR2524, WL2123, Ps008-120689-0202, Pe013-190785-0105, JI261, L90, LI00, LI01

P. sativum ssp. *elatius* (Bieb.) Schmahl. without the restriction site:

VIR1851, VIR4014, VIR7327, VIR7328, VIR7329, JI1794, CE1, CE2, Ps002-050689-0302; P012-050785-0102, P016-290685-01, P017-270685-0105

P. s. ssp. transcaucasicum Govorov:

VIR3249, VIR4871

P. s. ssp. sativum:

VIR1975, VIR2516, VIR2172, VIR2593, VIR3424, VIR3429, VIR3439, VIR3913, VIR3954, VIR7006, VIR7163, Sprint-1