A chloroplast DNA marker frequently found in wild peas

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As part of a study of chloroplast inheritance in pea we searched for a marker that could distinguish the wild pea accession VIR320 (*Pisum sativum* L. ssp. *elatius* (Bieb.) Schmahl., Palestine) from other pea forms. We used a PCR-RFLP approach and tested two pairs of PCR primers in combination with three restriction endonucleases. The combination we report on here involves a primer pair that amplifies a portion of the coding region of the chloroplast *rbcL* gene (large subunit of ribulose-bisphosphate carboxylase) and the restriction enzyme *Asp*LEI . The primers were designed to match the X03853 EMBL database accession: 5'-TTATTATACTCCTGACTATCAAACC-3' and 5'-TACAGAATCATCTCCAAATATCTCG-3'. The following cycling parameters were used: initial denaturation at 95°C for 1 min followed by 35 cycles including denaturation at 94°C 59 sec, annealing at 58°C 59 sec, elongation at 72°C 1 min. A 15 μl sample of the reaction was treated with 20 units of *Asp*LEI endonuclease for 2 hours at 37°C in a volume of 30 μl and electrophoresed in a 1.5% agarose gel in TAE buffer. The expected size of the amplified product was 1129 bp.

Most cultivated lines generated the expected 1129 bp fragment that lacked a restriction site. However VIR320 plastid DNA gave two fragments of about 800 and 300 bp after treatment with *Asp*LEI (Fig. 1). Partial sequencing the *rbcL* gene in VIR320 showed that the restriction site was produced by a T to C nucleotide substitution in position 393 from the start codon, or position 325 from the beginning of the primer used. We assayed 27 samples of wild and cultivated peas, representing most of the presently recognized taxa. The electrophoretic pattern of DNA fragments formed upon *Asp*LEI restriction of *rbcL* amplification product is shown in Fig. 1, presence or absence of the restriction site is indicated in Table 1. In addition, Table 1 includes both the initial taxonomic designation of an accession and its taxonomic status accepted here. In particular, following Townsend (2) and Davis (1), 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.

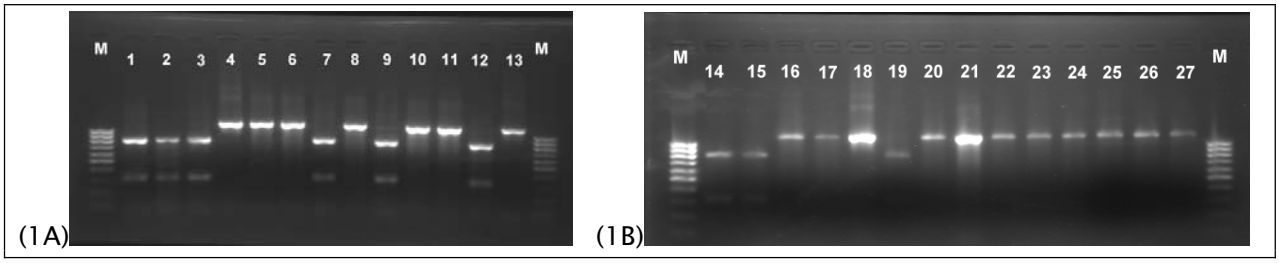

Figure 1. Restriction fragments formed upon AspLEI digestion of rbcL amplification product from different pea accessions. Lane numbers correspond to No in Table 1. M – molecular weight marker 100-1000 bp.

We found that the restriction site for *Asp*LEI in the gene *rbcL* is present in both accessions of *Pisum fulvum* Sibth et Smith, both accessions of *P. sativum* ssp. *abyssinicum*, in 4 of 11 accessions of *P. sativum* ssp. *elatius* (Bieb.) Schmahl., in neither of 2 accessions of *P. s.* ssp. *transcaucasicum* Govorov and in only one of 10 accessions of *P. s.* ssp. *sativum* (see Table 1). These data lead us to conclude that the restriction site probably was present in the ancestor of the genus *Pisum* and has been lost in some lineages of *P. sativum* ssp. *elatius*. A majority of the *P. sativum* ssp. *sativum*. germplasm appears to have inherited the derived (absence of restriction site) form of the cpDNA genome.

Table1. Pea accessions tested for the presence of *Asp*LEI recognition site in *rbcL* gene.

No	Accession number	Collection location	Wild or cultivated	Original taxonomic designation	Taxonomic designation used by authors	Presence of *Asp*LEI recognition site
1	L93	Unknown	Wild	Pisum fulvum	Pisum fulvum	YES
2	VIR6070	Palestine, foothills 30 km SW of Jerusalem	Wild	Pisum fulvum	Pisum fulvum	YES
3	L100	Unknown	Wild	Pisum humile	Pisum sativum ssp. elatius	YES
4	JI1794	Golan Heights	Wild	Pisum humile	P. sativum ssp. elatius	no
5	Ps008	Turkey, Siirt (coll. F. Muehlbauer)	Wild	Pisum humile	P. sativum ssp. elatius	no
6	VIR7329	Turkey ("received from USA")	Wild	P. sativum ssp. syriacum	Pisum sativum ssp. elatius	no
7	VIR320	Palestine (received from Sutton, France, by I. N. I. Vavilov in 1922)	Wild	P. sativum ssp. syriacum	P. sativum ssp. elatius	YES
8	VIR320*	- " -	Wild (?)	P. sativum ssp. syriacum	P. sativum ssp. elatius	no
9	VIR2521	Palestine, Kinereth, 1923	Wild	P. sativum ssp. syriacum	P. sativum ssp. elatius	YES
10	VIR4014	Azerbaijan, Lenkoran, a winter wheat field	Wild	P. sativum ssp. elatius	P. sativum ssp. elatius	no
11	VIR1851	Georgia, Tbilisi, Botanical Garden	Wild	P. sativum ssp. elatius	P. sativum ssp. elatius	no
12	VIR2524	North Galilea, in maccia Tarschich et Peccia	Wild	P. sativum ssp. elatius	P. sativum ssp. elatius	YES
13	CE1	Crimea, Simeiz (coll. Trusov and Kosterin, 1990).	Wild	P. sativum ssp. elatius	P. sativum ssp. elatius	no
14	VIR2759	Unknown	Cultivated	P. sativum ssp. abyssinicum	P. sativum ssp. abyssinicum	YES
15	WL1446	Unknown	Cultivated	P. sativum ssp. abyssinicum	P. sativum ssp. abyssinicum	YES
16	VIR3249	Georgia, Gori	Cultivated	P. sativum ssp. transcaucasicum	P. sativum ssp. sativum	no
17	VIR4871	Georgia	Cultivated	P. sativum ssp. transcaucasicum	P. sativum ssp. sativum	no
18	VIR3913	The Pamirs, Shugnan District, Shakh-Dara, Pavdysh kishlak	Cultivated	P. sativum ssp. asiaticum	P. sativum ssp. sativum	no
19	VIR1975	Afghanistan, Gerat	Cultivated	P. sativum ssp. asiaticum	P. sativum ssp. sativum	YES
20	VIR3954	Tadjikistan, Shugnan District, Emch kishlak	Cultivated	P. sativum ssp. asiaticum	P. sativum ssp. sativum	no
21	Sprint-1	A laboratory line derived from cv. Avanti and VIR7036 (Nepal)	Cultivated	Hybrid P. sativum ssp. asiaticum x P. sativum ssp. sativum	P. sativum ssp. sativum	no

Table 1 *(continued).* Pea accessions tested for the presence of *Asp*LEI recognition site in *rbcL* gene.

No	Accession number	Collection location	Wild or cultivated	Original taxonomic designation	Taxonomic designation used by authors	Presence of *Asp*LEI recognition site
22	VIR2593	Cyprus, Nikosia	Cultivated	P. sativum ssp. sativum	P. sativum ssp. sativum	no
23	VIR3429	Egypt, Assuan	?	P. sativum ssp. sativum (characters as in "P. jomardi")	P. sativum ssp. sativum	no
24	VIR7163	Lebanon	Cultivated	P. sativum ssp. sativum	P. sativum ssp. sativum	no
25	VIR7006	Syria, Damask	Cultivated	P. sativum ssp. sativum	P. sativum ssp. sativum	no
26	VIR2516	Palestine	Cultivated	P. sativum ssp. sativum	P. sativum ssp. sativum	no
27	VIR2172	Palestine, Prosch-Pina Agelet Hoschahas	Cultivated	P. sativum ssp. sativum	P. sativum ssp. sativum	no

N – lane number of Fig. 1.

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1. Davis, H. 1970. Flora of Turkey and the East Aegean Islands. Edinburgh, v. 3.
2. Townsend, C. 1968. Kew Bull. Roy. Bot. Gard. 21: 435-358.