

Variations of mitochondrial DNA found in a single protoplast- and seed-derived plants of Lotus corniculatus L.

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The mitochondrial DNAs (mtDNAs) of a single protoplast- and seed- derived plants of birdsfoot trefoil, Lotus corniculatus L. cv. Viking were analyzed by Southern blotting.

In a preliminary experiment, the mtDNAs of eight callus lines derived., from different individual plants were examined. Extracted mtDNAs were digested by a restriction endonuclease, EcoRI, and hybridized with three mixed probes, atpA, rrn26 and coxI. As a result, many different fragment patterns were observed among the callus lines (Fig. 1).

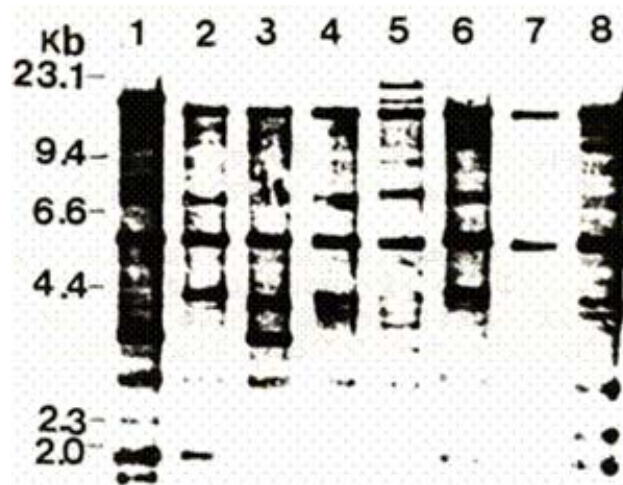


Fig. 1. mtDNAs of 8 Lotus corniculatus callus lines derived from different individual plants were digested by EcoRI and hybridized with mixed probes of atpA, rrn26, and coxI.

Specific fragments of certain calli may be explained by the polymorphism of mtDNA, because birdsfoot trefoil is a cross-pollinating species and cv. Viking is a synthetic cultivar. However, the possibility that some of the specific fragments may have originated from mtDNA mutation which occurred during callus culture cannot be ruled out.

The mtDNAs of two individual plants derived from seeds and five protoclonal plants in a single protoplast-derived population were analyzed. One gram of leaves was sampled and extracted total DNA was analyzed by the CsCl-EtBr method. We used 6 kinds of restriction endonuclease and 8 mitochondrial genes as probes (Table .1).

Table 1. Southern blotting of mtDNAs of planta in cv. Viking and protoclonal population

RE	Probe	rrn26	rrn18	coxI	coxII	CoxIII	nadI	atpA	atp9
EcoRI		ND	ND	S,P	ND	ND	ND	S	ND
BamHI		ND	ND	ND	ND	ND	ND	ND	ND
HindIII		ND	ND	ND	ND	ND	ND	S	ND
PstI		ND	ND	ND	ND	ND	ND	S	ND
SmaI		ND	ND	ND	ND	ND	ND	ND	ND
SaII		ND	ND	S,P	ND	P	ND	ND	ND

S: Southern blotting patterns of mtDNAs of two seed-derived plants, S-3 and 0-61, in cv. Viking are different. P: Protoclonal plants are different from the pattern of seed-derived plants. ND: No difference between two seed derived plants and protoclonal plants. RE: Restriction endonuclease.

Seed-derived plants, S-3 and 0-61, showed different hybridized fragment patterns in 13% of all the combinations of restriction endonucleases and mtDNA probes. This result clearly suggests that there is a polymorphism in the mtDNA of birdsfoot trefoil populations derived from seeds. On the other hand, most of the fragment patterns of protoclonal plants analyzed by Southern blotting were the same as those of plant S-3 derived from a seed. Only 2 (4%) of all the combinations of restriction endonucleases and probes showed fragment variations. However, all of the varied fragments were identical to the fragments of plant 0-61 (Fig. 2).

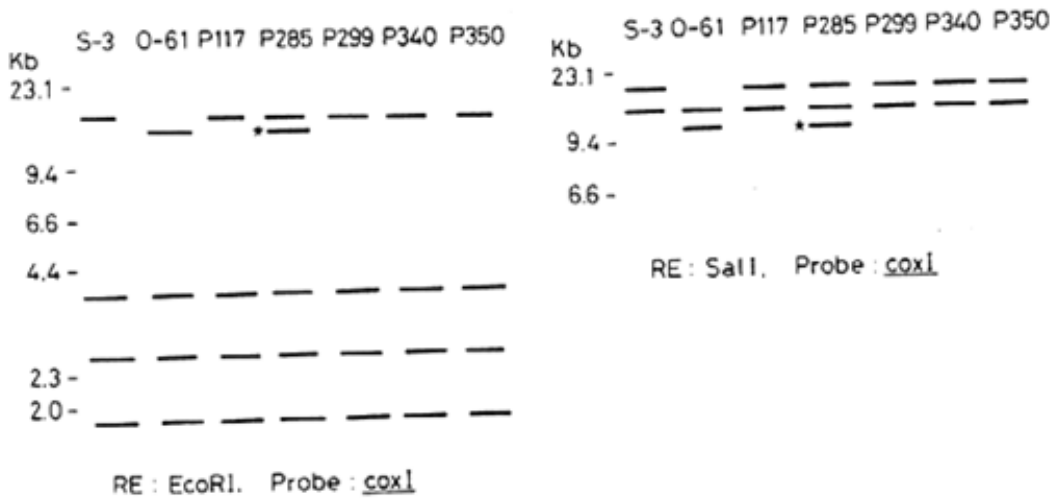


Fig. 2. Southern blotting of mtDNAs of cv. Viking and protoclones derived from 0-61: Plants from the population of cv. P350: Protoclones. \*: Variant fragment, plants in the populations of a single protoplast. S-3 and Viking; P117, P285, P299, P340, RE: Restriction endonuclease.

This fact indicates that variations may occur frequently at the same location of the mitochondrial genome or else the variations may occur in constant directions. The low occurrence of variation of mtDNA may be caused by a loss of totipotency in callus cells in which mtDNA mutation occurred.

#### References

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- 2) Milligan, B.G. 1989. Purification of chloroplast DNA using hexadecyltrimethylammonium bromide. *Plant Molec. Biol. Rep.* 7:144-149.