

Somatic Cell Hybrids between Birdsfoot Trefoil and Soybean

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The materials used were calli of birdsfoot trefoil (Lotus corniculatus L.), cv. Viking and soybean (Glycine max (L.) Merr.), cv. Harosoy. Asymmetric somatic cell fusion of the two parents and its culture were examined. Protoplasts of birdsfoot trefoil were treated with 5 or 10 mM iodoacetamide to inhibit cell division, and protoplasts of soybean were irradiated by 24 - 30 kR X-rays to eliminate chromosomes. The protoplast fusion treatment with polyethylene glycol was performed by the same method of Niizeki et al. (1985).

Somatic chromosome analysis of calli obtained by the protoplast fusion was carried out for the identification of hybrids. The chromosome number of birdsfoot trefoil is 24 and that of soybean is 40. The cells of the calli had various chromosome numbers, but most of the cells had only the chromosomes of birdsfoot trefoil. This means that the soybean chromosomes were eliminated by the effect of X-irradiation. However, some of the cells maintained a few soybean chromosomes which could be distinguished from birdsfoot trefoil chromosomes by their smaller size.

Table 1. Analysis of peroxidase isozyme and shoot formation from the calli obtained by asymmetric protoplast fusion of birdsfoot trefoil and soybean

Callus line (A-1 to C-7)	Isozyme	No. of obtained shoot	Callus line (D-1 to D-11)	Isozyme	No. of obtained shoot
A-1	B	0	D-1	B+S	6
-2	B	0	-2	B	2
-3	B	0	-3	B	12
B-1	B	0	-4	B	0
-2	B+S	0	-5	B	0
-3	B	0	-6	B	0
-4	B	43	-7	B	0
-5	B	0	-8	B	16
-6	B	11	-9	B	1
C-1	B	0	-10	B	4
-2	B	0	-11	B	1
-3	B	7			
-4	B	2			
-5	B	0			
-6	B	21			
-7	B	0			

B+S, mixed banding pattern from both parents. B, banding pattern of birdsfoot trefoil.

The peroxidase isozyme in twenty-seven calli were analyzed by the method using polyacrylamide gel. Two calli, B-2 and D-1, showed a mixed banding pattern from both parents suggesting that the calli had at least one soybean chromosome having a gene for the peroxidase isozyme. But the soybean chromosome with the peroxidase isozyme gene might be eliminated from the other calli which indicated the same banding pattern as birdsfoot trefoil (Table 1 and Figure 1).



Fig. 1. Electrophoretic banding pattern of peroxidase isozyme of birdsfoot trefoil(B), soybean(S) and their asymmetric hybrid(H) calli. Unique bands of parents are indicated by arrow heads.



Fig. 2. A, a regenerated plant from a callus of birdsfoot trefoil. B, a regenerated plant from a fused callus of birdsfoot trefoil and soybean.

Shoot formation from the hybrid calli occurred on Miller's basal medium (1963) containing 2g/l yeast extract after 8 weeks of culture (Table 1). The shoots were then transferred into the medium of Nitsch and Nitsch (1969) without the growth regulator. This resulted in the shoots proliferating roots. These plantlets were transferred to soil in pots. The morphology of the hybrid plants is shown in Figure 2.

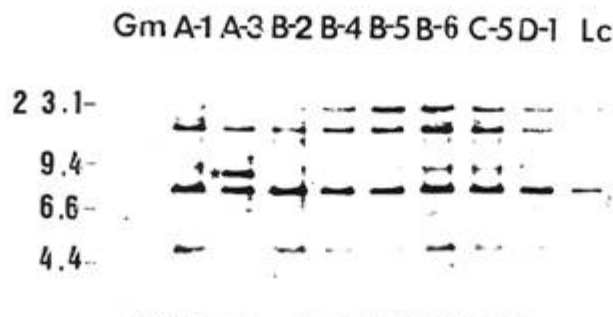


Fig. 3. Mitochondrial DNA was digested by PstI and hybridized by a probe of *atpA*. Gm, *Glycine max*; Lc, *Lotus corniculatus*; A-1, A-3, B-2, B-4, B-5, B-6, C-5, D-1, hybrid callus lines. *, indicates a unique fragment.

Table 2. Southern blotting of mtDNA in 8 hybrid calli of *Glycine max* and *Lotus corniculatus*

Restriction enzyme	Probe		
	<i>coxI</i>	<i>rrn26</i>	<i>atpA</i>
EcoRI	A-3	A-3	A-3
BamHI	A-3	A-3, B-5	A-3, B-5
HindIII	ND	ND	A-3
PstI	A-3	A-3	A-3
SmaI	A-3	A-3	A-3
SalI	A-3	A-3	A-3

A-3 and B-5 hybrid callus lines show that these callus lines have unique fragments which are different from those of parents. ND, no difference from pattern of *Lotus corniculatus*. The other 6 callus lines did not show any differences from pattern of *Lotus corniculatus*.

Mitochondrial DNA (mtDNA) of 8 hybrid callus lines were analyzed by Southern blotting. Six restriction enzymes and three probes were used in this experiment (Table 2). Six callus lines showed only the specific fragments of birdsfoot trefoil. However, two callus lines, A-3 and B-5, showed unique fragments in many combinations of the restriction enzymes and probes (Figure 3 and Table 2). These results suggest that the

mtDNA of the two parents were sorting out and the mtDNA of birdsfoot trefoil remained stable. However, the occasional occurrence of recombination between two parental DNAs or the rearrangement of one parent was also suggested by these results.

References

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