

Nuclear and Organelle DNA Behavior of *Lotus corniculatus* L. in Somatic Cell Hybrid Callus

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Protoplast fusion has been a practical method for removing the barriers of incompatibility in sexual crossing of agriculturally important plant species. On the other hands, protoplast fusion induces the heteroplasmic state of fusion partners. Therefore, it may have opportunities for recombinations and/or rearrangements of cytoplasmic genomes. In the Plant Breeding Laboratory of Hirosaki University, five asymmetrical somatic hybrid calli were produced by protoplast fusion in interfamilial combinations of rice, strain A-58, and soybean, cv. Harosoy (Niizeki and Kita 1981, Niizeki *et al.* 1985), of rice and birdsfoot trefoil, cv. Viking (Niizeki *et al.* 1992a), and of rice and alfalfa, cv. DuPuit and cv. Rangelander (Niizeki *et al.* 1992b) and two intergeneric combinations of soybean and birdsfoot trefoil (Niizeki *et al.* 1990) and of birdsfoot trefoil and alfalfa (Niizeki *et al.* 1989). They had been researched for their karyotypes, isozymes, ribulose-1,5-bisphosphate carboxylase (RuBisCo), and morphological traits.

In this study, interfamilial and intergeneric asymmetrical somatic hybrids were analyzed for their nuclear DNAs, mitochondrial DNAs (mtDNA) and chloroplast DNAs (cpDNA) by the Southern hybridization method. The aim of this study is to obtain fundamental information of the nature and behavior of their nucleus and organelle DNAs.

I. Analysis of nuclear DNAs

Nuclear DNAs of somatic hybrids were analyzed with respect to rDNA. In the Southern blots of interfamilial somatic hybrids, all hybrid cell lines had the identical banding patterns of leguminous parents except for one hybrid line of rice and soybean. The deletion of one DNA fragment of soybean was observed in the hybrid of rice and soybean. In the case of intergeneric hybrids, the banding patterns of birdsfoot trefoil were detected in all of the hybrid cell lines of soybean and birdsfoot trefoil. However, the deletion of one DNA fragment of birdsfoot trefoil and the addition of one fragment of alfalfa were observed in the somatic hybrid cell of birdsfoot trefoil and alfalfa (Fig. 1, Table 1). These results show that the intergenomic translocation may have occurred between the chromosomes of somatic hybrids.

II. Analysis of mtDNAs

In the Southern blots of mtDNAs using of three mitochondrial genes as probes, the novel fragments were detected in several cell lines of four somatic hybrids except the hybrid of birdsfoot trefoil and alfalfa. In addition, the presence or deletion of both parental fragments were observed in the mtDNAs of somatic hybrids of rice and soybean and of rice and alfalfa. These results indicate

that some kinds of alterations such as intermolecular and/or intramolecular recombinations of mtDNAs occurred in several cell lines of four somatic hybrids.

III. Analysis of cpDNA

In the Southern blots of cpDNAs, the banding patterns of cell lines of five somatic hybrids were identical with those of leguminous species cell lines, using of four chloroplast genomic DNA fragments as probes. No novel fragments were observed at all. Therefore, it was suggested that the chloroplasts of five somatic hybrids sorted out unidirectionally, and that any kinds of recombinations and/or rearrangements of cpDNAs did not occur in the five somatic hybrids.

From these results, unidirectional nuclear genome elimination have occurred in the nuclei of five interfamilial and intergeneric somatic hybrids. However, chromosomal recombinations may have occurred in the nuclei of somatic hybrids of birdsfoot trefoil and alfalfa. Some kinds of recombination and/or rearrangement may have occurred in mtDNAs of four combinations of somatic hybrids, but alterations of cpDNAs may not have occurred. Therefore, it will be possible to expect improvement of the nucleus and mitochondria in these five interfamilial and intergeneric somatic hybrids.

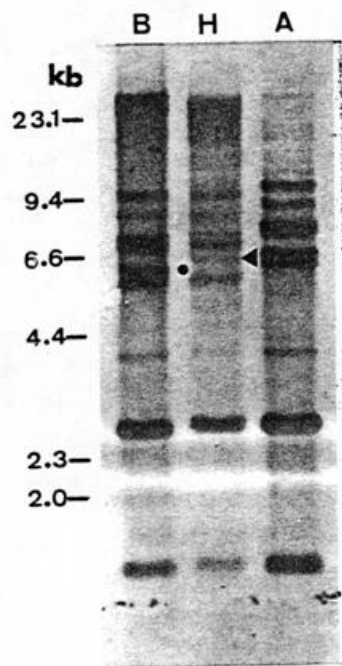


Fig.1. Southern blot of DNAs of a somatic hybrid cell line(H) between birdsfoot trefoil and alfalfa, and its parental birdsfoot trefoil(B)and alfalfa(A) cell lines. Total DNAs of hybrid and parental cell lines were digested by *Bam* HI and hybridized with rDNA,*pRR217*. Deletion of birdsfoot trefoil fragment and co-migrated fragment with that of alfalfa are indicated by dot and arrowhead, respectively.

Table 1. Restriction fragment length polymorphism of nuclear DNAs in a somatic hybrid cell line(H) between birdsfoot trefoil and alfalfa, and their parental birdsfoot trefoil(B) and alfalfa(A) proved with *pRR217*

Hybridized fragments*	Cell lines		
	B	H	A
<i>Bam</i> HI			
11.0			+
10.1	+	+	
9.4			+
9.0	+	+	
8.0			+
7.8			+
7.4	+	+	
7.2	+	+	
6.8		+	+
6.6			+
6.5	+		
6.0	+	+	
4.0	+	+	+
2.9			+
2.7	+	+	
0.4	+	+	+

*: Fragment sizes are indicated in kb.

+: Presence of fragment.

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