

Stability of Somaclonal Variation  
in *Lotus corniculatus* L.

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It is assumed that somaclonal variation occurs, due to the lack of some genetically controlled mechanisms in cultured calli or cells. It is probable that these somaclonal mutations accumulate from one cell cycle to the next. Birdsfoot trefoil (*Lotus corniculatus* L.), cv. Viking is a suitable plant for the investigation of somaclonal variation because of the high totipotency found in its cultured cells. This is true, even when they are derived from a single protoplast. Therefore, it is a useful plant for comparative studies between protoplast- and seed-derived populations.

In our study (Niizeki et al. 1990) it was shown that somaclonal variation is very useful in the improvement of quantitative traits in this species. The populations of  $P_2$  and  $P_3$  generations were obtained by the open pollination of a regenerated  $P_1$  protoclonal population and of a  $P_2$  population, respectively. Seven traits indicated in Table 1 were investigated in the  $P_1$ ,  $P_2$  and  $P_3$  generations. Few significant differences were found among the three generations in each trait, with the exception of dry matter yield and pollen fertility. There was a drastic increase in dry matter yield in  $P_2$  which then decreased in  $P_3$ . The low yield for  $P_1$  may have been caused by the low number of shoots that grew on the poorly developed crown root of the regenerated protoclones. That of  $P_3$  may have been caused by the low number of shoots and the short plant height caused by under-average temperatures in the summer season of 1993. While the large number of shoots made it substantially impossible to count them each year, plant height was investigated in  $P_2$  growing in both 1992 and 1993 (Table 2). They were significantly shorter in 1993 than that of 1992. In regard to pollen fertility, Niizeki (1993) showed that it drastically increased in  $P_2$  and  $P_3$ . This may have been caused by the elimination of gametes with abnormal chromosomal configurations in the  $P_1$  protoclones.

With regard to major genes, the nuclear DNAs were analyzed by using 2 restriction enzymes, *Hind*III and *Bam*HI, and the major genes, a small subunit of RuBisCO, phenylalanine ammonia-lyase and ribosomal DNA, pRR217. In this experiment, it was found that these genes were very stable, without any variations. However, heterochromatin parts consisting of satellite DNA revealed a considerable number of variations in a Southern blot analysis using restriction enzymes, *Hind*III and *Eco*RI, and a probe of (GGAA)<sub>3</sub>. From these results, the two alternative assumptions considered are as follows. 1. The mutation of major genes is probably very rare. 2. The plants containing mutations in the major genes are eliminated during acclimatization. Precise analyses which appear likely to solve this problem are now under way.

Table 1. Mean values on seven traits of three generations after regeneration from single protoplast-derived callus

	1	2	3	4	5	6	7
	cm	cm	mm	mm	mm	g	Arcsin $\sqrt{\%}$
P <sub>1</sub>	28.2a	2.5a	1.4a	11.0a	5.8a	8.3a	58.5a
P <sub>2</sub>	28.9a	2.9a	1.5a	12.0a	7.5a	17.3b	77.1b
P <sub>3</sub>	27.3a	2.8a	1.5a	11.4a	7.3a	9.6a	75.0b

1: Plant height, 2: Length of internode, 3: Stem diameter, 4: Leaflet length, 5: Leaflet width, 6: Dry matter yield, 7: Pollen fertility. Two values with different letters in the same column differ at 5% level after Duncan's multiple range test.

Table 2. Values on six traits of P<sub>2</sub> generation in 1992 and 1993

	1	2	3	4	5	6
	cm	cm	mm	mm	mm	g
1992	28.9±5.4	2.9±0.5	1.5±0.2	12.0±1.3	7.5±1.0	17.3±5.4
1993	25.1±4.3	2.9±0.7	1.4±0.3	12.3±1.8	7.3±1.4	7.4±1.8
t-value	3.03	0.31	0.90	0.90	0.76	9.42
	0.001<p<0.01	n.s.	n.s.	n.s.	n.s.	p<0.001

1 - 6 : The same traits as those in Table 1. n.s. : Not significant.

#### References

- M. Niizeki, R. Ishikawa and K. Saito 1990. Variation in a single protoplast- and seed-derived population of *Lotus corniculatus* L. Theor. Appl. Genet. 80 : 732-736.  
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