MORPHOLOGICAL VARIATION IN PLANTS REGENERATED FROM LONG-TERM CALLUS CULTURE OF PEA

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Pea callus tissue was derived from apices of var. 'Ranny zeleny-33' using the method of Gamborg, et al. (1). In these conditions a friable, light-green, rapidly growing morphogenetic callus was obtained. The callus was transferred to fresh medium every 1-1/2 to 2 months over a two-year period. To secure a large number of shoots, two-year-old callus was placed into 250 ml Erlenmeyer flasks either on the same medium or on a medium with added benzylaminopurine content (2 mg/1). To avoid getting adventitious shoots from the same meristematic zones, 3-5 cm long shoots were taken from flasks along with the pieces of callus from which they had been derived.

Shoot tips were grafted in the greenhouse. From about 200 shoots grafted, seeds were obtained from 70 Regenerants (R_0).

Some of the grafted regenerants had shortened internodes and altered leaf and flower morphology.

Seeds from the grafted regenerants (from 1 to 15 seeds from each regenerant) were planted in the field. Segregation of a chlorotica mutant was observed in one of 65 lines (4 green : 1 chi plants). Another line segrated for a waxless mutant (2 normal : 1 waxy plants). It is likely that other mutations had occurred but , because the population sizes were small, they remained undetected. Therefore analysis of the R_2 generation is required.

The most interesting results were obtained while studying some physiological and quantitative characters. In 70-80% of the R_1 Lines the following changes were found: more robust habit as compared with the initial variety (IL); dark green leaves unlike the light green leaves of the IL; oblong leaflets as compared with oval; earlier or later flowering time.

For example, 85.5% of the plants of var. Ranny zeleny formed theit first flower on node 10 (14.5% on nodes 9 and 11). Among the R_1 regenerants only 27% of the plants flowered at node 10, while the rest (73%) flowered at nodes 8, 9, 11, 12 and 13 (Fig. la, b).

We have not seen direct correlations among these changed charac ters. For instance, some plants with more vigorous habit were late flowering lines and some were early.

The same changes that occurred in R1 also were present in R2 and there was no segregation of the changed characters among the plants and the families belonging to the same line. For example, all the plant among three families of line No. 1 had statistically significant differences ($P > 0.999$) in the length/width ratio of the leaflets. Thus the change in leaflet shape (from oval in the IL to oblong) was an inherited difference (Table 1).

The study of flowering time of nine lines of the R2, general ion has shown that five lines flowered more frequently on node 9 (from 8 to 10, very rarely on node 11), and four lines flowered at node l3 (from 11 to 14) (Fig. 1 c, d). These data indicate that variability of flowering time among R1 lines did not result from physiological and epigenetical events, but had genetic basis.

Studies of karyotypes in callus tissues, pollen fertility, and meiosis in regenerants, which is to be reported, demonstrated that the variability of regenerants was not caused by changes in ploidy or by large chromosomal aberrations.

1. Gamborg, 0.L., F. Constabel, and J.P. Shyluk. 1974. Physiol. Plant. 30:125-128.

Table 1. Length/width ratio of leaflets from the inital line (Ranny zeleny) and from regenerants.

Genotype	Number of plants	Leaflet length/ leaflet width
Ranny zeleny	50	1.5 ± 0.01
R regenerants		
Family 1	16	1.6 ± 0.02
Family 2	10	1.6 ± 0.02
Family 3	11	1.7 ± 0.03

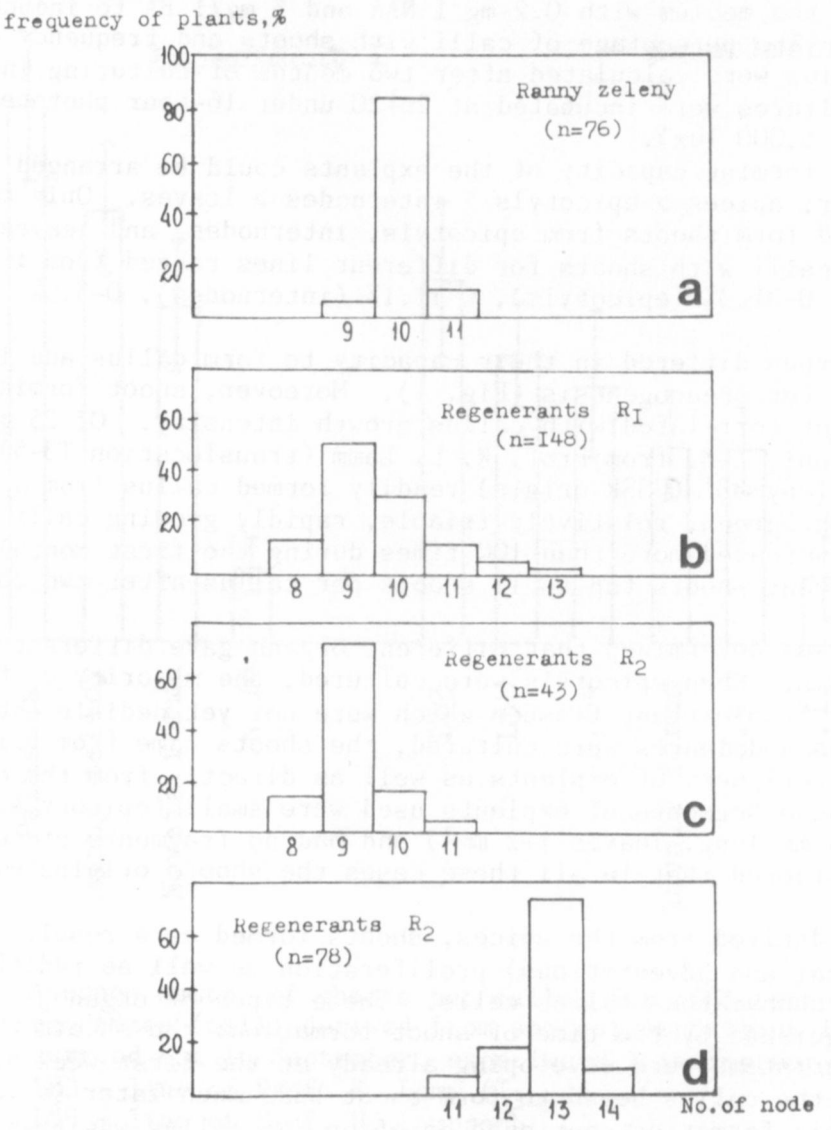

Fig. 1. Distribution of number of nodes to first flower in the initial line (Ranny zeleny) and in R1 and R2 regenerants. n = total number of plants.