STUDY OF SHOOT FORMING CAPACITY IN PEA CALLUS CULTURE

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The morphogenetic capacity of callus cultures derived from apices, epicotyls, internodes, and leaves of aseptic seedlings of different pea genotypes was studied. The induction of callus and shoot regeneration from apices of 3- to 5-day-old seedlings was obtained using the method of Gamborg et al. (1). The culture medium contained casein hydrolysate 2 g/1, sucrose 30 g/1, NH NO 0.8 g/1, alpha-naphtylacetic acid (NAA) 0.2 mg/1, and benzylaminopurine (BA) 0.1 mg/1.

Segments of epicotyls, internodes, and leaves from 4- to 10-day-old seedlings cultured under the same conditions produced very poor callus. To induce callus we therefore cultured these explants on the medium containding NAA 2 mg/1, BA 1 mg/1. After four weeks callus cultures were transferred to the medium with 0.2 mg/1 NAA and 5 mg/1 BA to induce organogenesis. The percentage of calli with shoots and frequency of shoots per callus were calculated after two months of culturing in all cases. All cultures were incubated at 26 +/- 2C under 16-hour photoperiod (approximately 5,000 lux).

The shoot forming capacity of the explants could be arranged in the following order: apices > epicotyls > internodes > leaves. Only certain genotypes could form shoots from epicotyls, internodes, and leaves. The percentage of calli with shoots for different lines ranged from : 7.1-8.5% (apices), 0-31.32 (epicotyls), 0-11.1% (internodes), 0-5.0% (leaves).

The genotypes differed in their capacity to form callus and in their capacity lot organogenesis (Fig. 1). Moreover, shoot forming capacity was not correlated with callus growth intensity. Of 25 genotypes studied only L-83 from Prof. R. L. Lamm (translocation T3-5) and var. 'Ranny zeleny 3V (USSR origin) readily formed callus from apices. These were light green, relatively friable, rapidly growing calli (their fresh weight Increased more than 100 times during the first month) that could produce many shoots (up to 11 shoots per callus after two months).

Also, it was determined that different organs gave different types of organogenesis. When epicotyls were cultured, the majority of the shoots derived from explant tissues which were not yet dedifferentiated. When internodes and leaves were cultured, the shoots came from friable callus on the peripherv of explants as well as directly from the explant tissues. Because segments of explants used were small (epicotyls, internodes 2-4 mm long, leaves 1-2 mm^2) and had no fragments of aeristems, we considered that in all. these cases the shoots originated de novo.

In calli derived from the apices, shoots formed as a result of meristem (apical and adventitious) proliferation as well as redifferentiation of parenchymatous callus cells. These types of organogenesis can be distinguished by the time of shoot formation: the shoots from preexisting meristems were developing already at the first week of culturing, while the callus began to form shoot buds much later (after 3-4 weeks) and their formation continued on after the callus was transferred to the fresh medium.

Most transplanted pea calli died, but in some cases we managed to obtain transplantable calli from apices, epicotyls, internodes, and leaves which hadn't lost their organogenic capacity. Some of them have already been cultured for more than three years.

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

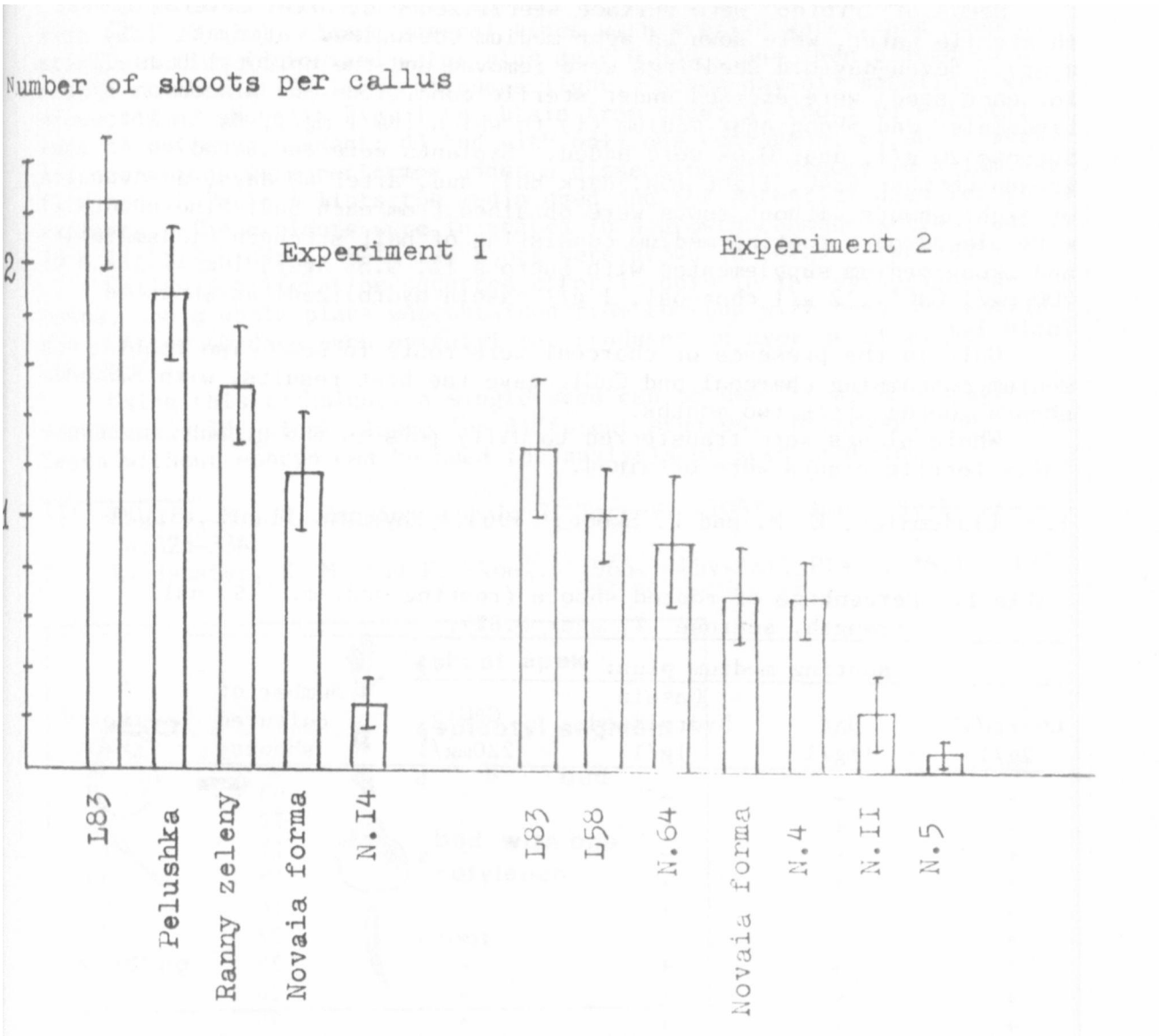

Fig. 1. Average number of shoots per callus for different pea genotypes (calli derived from apices; experiment 1 was carried out in September, experiment 2 in February).
L83 - line of Prof. R. Lamm (T3-5)
L58 - line of Prof. H. Lamprecht (T4-5)
Lines N. 5, 4, 11, 14, 64 - chlorophyll and morphological mutations from collection of Dept. of Genetics and Selection (Moscow State Univ.)
Novaia forma - line marked with genes af, i, r, tl
Ranny zeleny-33 - grain pea var.
Peluska Falenskaia - fodder pea var.