Manifestation of the *Lf* locus in tissue culture of pea

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Tissue and cell cultures provide excellent opportunities for studying the influence of nutrients on the morphological features of cultivated cells, the rate of cell division, and the regeneration capacity of plant cultures. The use, in *in vitro* experiments, of isogenic lines which differed for particular known genes allowed us to estimate the effect of these genes on the cultural characteristics mentioned above, and the dependence of this effect upon the composition of the culture media and conditions of cultivation. In this paper we report on the effect of genotype at the *Lf* locus and media composition.

Material and Methods

Earlier we obtained somaclonal variants from tissue culture of the pea cultivar Ranny Zeleny dealing with flowering behaviour (1). These somaclonal changes resulted from mutations at the *Lf* and *Sn* loci (7). Thus we had available for study the following isogenic lines which differed only in two genes:

LfLf snsn - initial cultivar Ranny Zeleny,
LfLf SnSn - line R1 (somaclonal variant),
lflf SnSn - line R9 (somaclonal variant),
lflf snsn - the line selected from the F_2 population of cross R9 x Ranny Zeleny.

We studied the effect of exogenous growth regulators, NAA (napthalene acetic acid) and BA (benzyladenine), and genetic factors (dominant and recessive alleles of the *Lf* locus) on the growth of primary tissue cultures. The significance of data was tested by a Multifactor Analysis of Variance (Stratgraphics Statistical Graphics System, version 3 -computer program by Statistical Graphics Corporation, U.S.).

Tissue cultures were induced from shoot apices of 3-day-old sterile pea seedlings using the Gamborg method (2). All aspects of the experiment were carefully equalised, starting from the use of seeds of similar age, selection of seedlings with a similar degree of development, and ending with the use of explants of as near as possible equal weight The average fresh weight of explants was about 3-4 mg. Explants were cultivated on Gamborg et al media (2) in individual glass tubes (2 cm in diameter). Each treatment was replicated in 10-20 tubes. After randomization, the explants were cultivated at 26 ± 2°C in a 16 h photoperiod. Fresh weight was measured after 4 weeks.

Results and Discussion

In the preliminary experiment we found that the major factor which determines the difference in growth between tissues of genotype *Lf* and *lf* was the content of synthetic auxin in the culture media, not the cytokinin content. These results were confirmed in the following experiments.

Table 1. Analysis of variance for callus weight (experiment 1).

Source of variation	d.f.	Mean square	F	P
Lf (Lf; lf)	1	1251.61	0.920	0.349
NAA (0.2 mg/l; 1 mg/l)	1	379.77	0.279	0.604
2-Factor interaction *Lf*-NAA	1	11144.28	8.190	0.005
Residual	116	1360.73		

Table 2. Effect of NAA content of the medium on callus weight of genotypes *Lf* and *lf* (experiment 1).

Genotype	NAA-content (mg/l)	Count	Mean ± SE (mg)
Lf	0.2	31	129.5 ± 5.6
	1	28	152.7 ± 6,7
lf	0.2	30	142.0 ± 7.9
	1	31	126.6 ± 6.4

Table 3. Analysis of variance for callus weight (experiment 2).

Source of variation	d.f.	Mean square	F	P
Lf(Lf, If)	1	19618.19	7.998	0.006
BA (0.01; 0.1; 1 mg/l)	2	380180.38	154.990	<0.001
2-Factor interaction *Lf*-BA	2	2130.54	0.869	0.422
Residual	114	2452.93		

Table 4. Effect of B A content of the medium on callus weight of genotypes *Lf* and *lf* (experiment 2).

Genotype	BA-content (mg/l)	Count	Mean ± SE (mg)
Lf	0.01	23	112.0± 6.5
	0.1	11	161.5 ± 3.2
	1	23	280.0 ± 14.3
lf	0.01	21	133.1 ± 9.6
	0.1	19	171.4 ± 11.0
	1	23	319.9 ± 11.8

In the first experiment we tested the influence of two NAA concentrations on callus weight (0.2 and 1 mg/l NAA; 0.1 mg/l BA). The experiment showed that the major contribution to the variation in callus weight was made by the interaction between the factors *Lf* and NAA ($P < 0.01$, Table 1). These data indicated that alleles *Lf* and *lf* predispose the callus to respond differently to NAA. In the medium with a high auxin content, genotype *Lf* grew better than genotype *lf* whereas in the medium with a low auxin content genotype *lf* grew better than *Lf* (Table 2).

In the second experiment the influence of different BA content on callus weight was studied (0.01, 0.1 and 1 mg/l BA; 0,2 mg/l NAA). BA content of media was the main factor which contributed to the variation of callus weight ($P < 0.001$, Table 3). Callus growth increased with increasing concentration (Table 4). This can be explained by the fact that cytokinins stimulate cell division both *in vivo* and *in vitro* (5). No interaction between genotype at the *Lf* locus and BA content of the medium was observed. Thus genotypes *Lf* and *lf* responded similarly to the different content of cytokinin in culture media. A significant influence of the *Lf* locus on callus growth was observed ($P < 0.01$, Table 3) which is consistent with the results of the first experiment, where it was shown that genotype *lf* grew better than *Lf* in a medium with a low NAA content (0.2 mg/l in this case).

To study in more detail the effect of different NAA concentrations on the growth of genotypes *Lf* and *lf*, primary callus of lines R1 (*Lf*) and R9 (*lf*) was cultivated on media with a range of NAA concentrations from 0.05 to 1.5 mg/l (the concentration of BA was 0.1 mg/l in all 8 media variants). There was a significant interaction between *Lf* genotype and NAA concentration ($F = 1.827$, $P < 0.01$). Genotype *lf* grew better than *Lf* at concentrations of NAA from 0.05 to 0.4 mg/l whereas genotype *Lf* grew better than *lf* at concentrations of 1.0 to 1.5 mg/l (Fig. 1). At concentrations 0.6 and 0.8 mg/l there was no significant difference in growth between the two genotypes (Fig. 1).

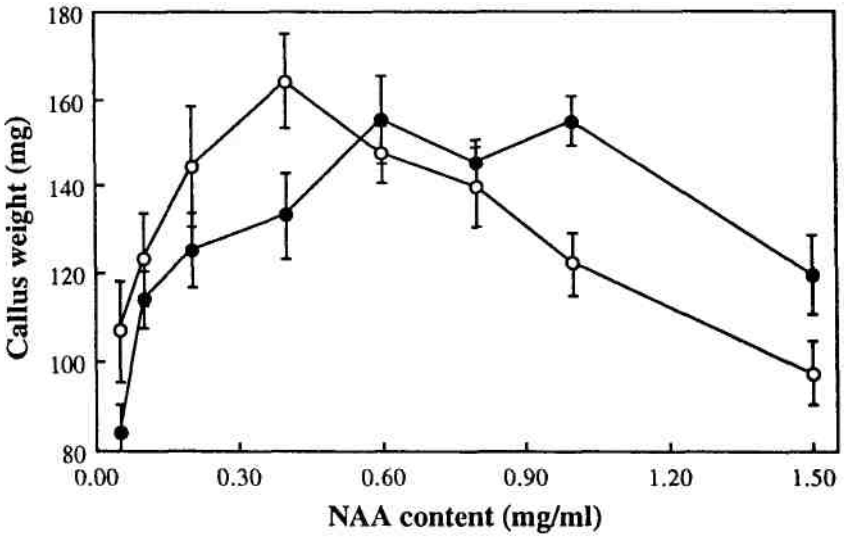

Fig. 1. Mean callus weight plotted against NAA content of the medium for *Lf* (●) and *lf* (○) explants. SE bars are shown for all means; n = 7-17, mostly 8 or 9; data from experiment 3.

Thus expression of the *Lf* locus in tissue culture is manifest by an interaction between the *Lf* genotype and NAA concentration. Stimulation of callus growth by auxin can be connected with cell elongation as well as with activation of cell division (3, 8). However, an effect of a flowering gene on callus growth was unanticipated.

Among 9 genes which control flowering in pea, locus *Lf* occupies a special place because it determines the minimum node of flower initiation (6). Murfet (6) has proposed that allele *Lf* confers a higher threshold level for the flowering signal necessary to trigger flowering than allele *lf*. Thus the *Lf* locus may act in the apical meristem of plants to control the synthesis of a receptive protein, which interacts with the flowering signal and switches on the reproductive program of development. Our studies show that the *Lf* locus operates not only at the shoot apex but it also controls auxin responsiveness of callus cells *in vitro.* Similarly as *in vivo,* our data show that a higher threshold level of the growth regulator NAA is necessary to trigger active callus growth in genotype *Lf* than *lf*. These data can be interpreted as an indication that auxin plays the role of a flowering signal and that one of the auxin-binding proteins (ABP) is a product of, or controlled by, the *Lf* locus.

Jacobsen and Hajek (4) have shown the existence in pea of several ABPs with different characteristics and locations (membrane and cytoplasmic). The spectrum of ABPs may be distinct in different genotypes and in different tissues, and it can vary during ontogenesis (4). A study of the ABPs of isogenic lines of pea with different flowering genes would permit the correctness of our supposition regarding the action of *Lf* to be tested.

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1. Ezhova, T.A., Bagrova, A.M. and Gostimski, S.A. 1990. Pisum Newsl. 22:15-17.
2. Gamborg, O.L., Constabel, F., and Shyluk, J.P. 1974. Physiol. Plant. 30:125-128.
3. Gautheret, R.J. 1955. Ann. Rev. Plant Phys. 6:433-484.
4. Jacobsen, H.-J. and Hajek, K. 1987. *In:* Molecular Biology of Plant Growth Control, Alan R. Liss, Inc., pp. 257-266.
5. Miller, C.O., Skoog, F., von Saltza, M.H. and Strong, F.M. 1955. J. Am. Chem. Soc. 77:1392.
6. Murfet, I.C. 1985. *In:* Halevy, A.H., ed., Handbook of Flowering Vol. 4, CRC Press, Boca Raton, pp. 97-126.
7. Murfet, I.C. and Ezhova, T.A. 1991. Pisum Genetics 23:19-25.
8. Went, F.W. 1928. Rec. Trav. Bot. Neer. 25:1-116.