Manifestation of the *lf* gene in callus cultures from different tissues of pea seedlings

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The *lf* gene operates in the shoot apex to determine the threshold level of flowering signal necessary to trigger flower initiation (4). Earlier we reported (1) on the influence of the *lf* gene on the growth of callus tissue induced from apices of pea seedlings. It was shown that higher levels of the synthetic auxin NAA (napthalene acetic acid) were needed in the culture medium to trigger optimum growth in *Lf* than *lf* callus. It was therefore of interest to find out whether the genotype at the *lf* locus would also influence the growth of callus cultures induced from other tissues of pea seedlings, e.g. stems or roots.

Material and Methods

Tissue cultures were induced from shoot apices, epicotyls and roots of pea seedlings of genotypes *Lf* and *lf* [lines regenerated from cv Ranny Zeleny (1)] using the Gamborg method (2). The influence of NAA content (0.2 or 1 mg/L) on callus weight was studied. The BA (benzyladenine) content was the same in all experiments (0.1 mg/L). The experiments and their statistical analysis were carried out as reported earlier (1).

For scanning electron microscopy (SEM) of callus cells, calluses were fixed in 4% glutaraldehyde in 0.16 M sodium phosphate buffer (pH 7.3-7.4) at 4°C for at least 12 h and dehydrated in a graded ethanol series. Calluses were then critical point dried using CO_2.

Results and Discussion

Different tissues of pea seedlings required different auxin content for successful callus induction. Root tissues formed callus only on media with ≥1 mg/L NAA. Induction of callus from epicotyl tissues was observed in media with high (1 mg/L) as well as low (0.2 mg/L) NAA content. However, in the latter instance callus cultures grew slowly and showed some necrosis. Tissues from the apices of pea seedlings produced light-green rapidly growing callus in media with both high and low NAA content (Figs 1, 2). Obviously, such results can be explained by the different content of endogenous auxin in different parts of pea seedlings (3), as well as by different abilities of callus cultures induced from apices, epicotyls and roots to synthesize endogenous auxin. Some indirect indication of the lower ability for auxin production in callus cultures from roots was obtained via SEM examination of callus cells. It is known that auxins induce not only the activation of division, but elongation of cells as well (3). Callus cells induced from roots were of roundish shape and on average half to two-thirds the size of cells from apices and epicotyls.

Our research showed that the genotype at the *lf* locus considerably influenced the growth of callus. Callus cultures of genotype *lf* in a medium with 0.2 mg/L NAA grew better than *Lf* callus irrespective of the origin of the culture (Fig. 1) and we did not find a significant genotype x tissue interaction in this experiment (Table 1). In contrast, callus growth of genotypes *Lf* and *lf* in a medium with a high content of NAA depended upon the type of tissue used to induce the callus (genotype x tissue interaction statistically significant, $P<0.001$; Table 2). Callus of genotype *Lf* grew slightly better than *lf* callus when the callus was induced from apices (in this experiment the difference was not statistically significant) but in callus cultures induced from epicotyls and roots genotype *lf* grew better than *Lf* ($P<0.01$ and $P<0.001$, respectively; Fig. 2).

Table 1. Analysis of variance for callus weight (experiment with 0.2 mg/L NAA)

Source of variation	d.f.	Mean square	F	P
Lf (*Lf; lf*)	1	34054.9	13.09	<0.001
Tissue (Apex; Epicotyl)	1	1576152.5	605.76	<0.001
Interaction (*Lf* x Tissue)	1	2005.4	0.77	0.390
Residual	209			

Table 2. Analysis of variance for callus weight (experiment with 2 mg/L NAA)

Source of variation	d.f.	Mean square	F	P
Lf (*Lf; lf*)	1	33580.2	20.74	<0.001
Tissue (Apex; Epicotyl; Root)	2	248525.3	153.49	<0.001
Interaction (*Lf* x Tissue)	2	13601.7	8.40	<0.001
Residual	70	1619.2		

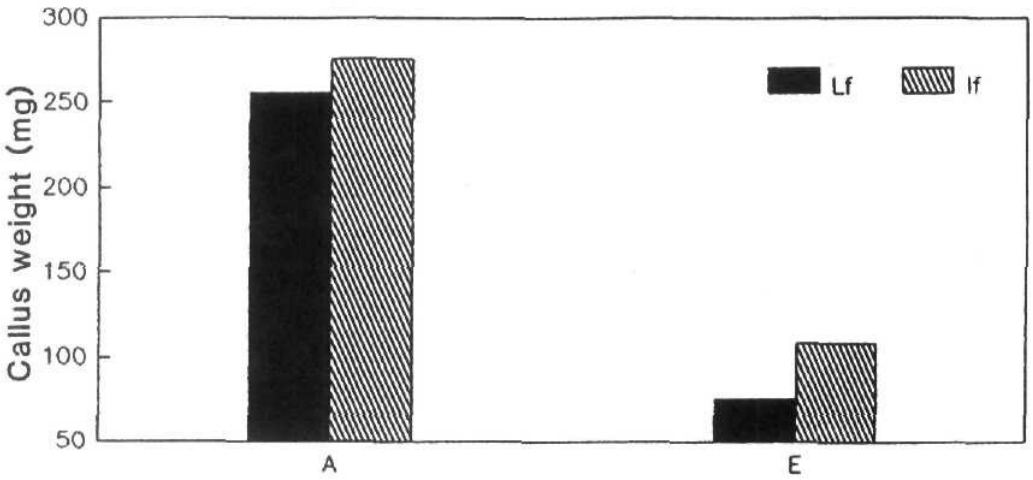

Fig. 1. The growth of *Lf* and *lf* callus induced from apices (A) and epicotyls (E) on a medium with 0.2 mg/L NAA.

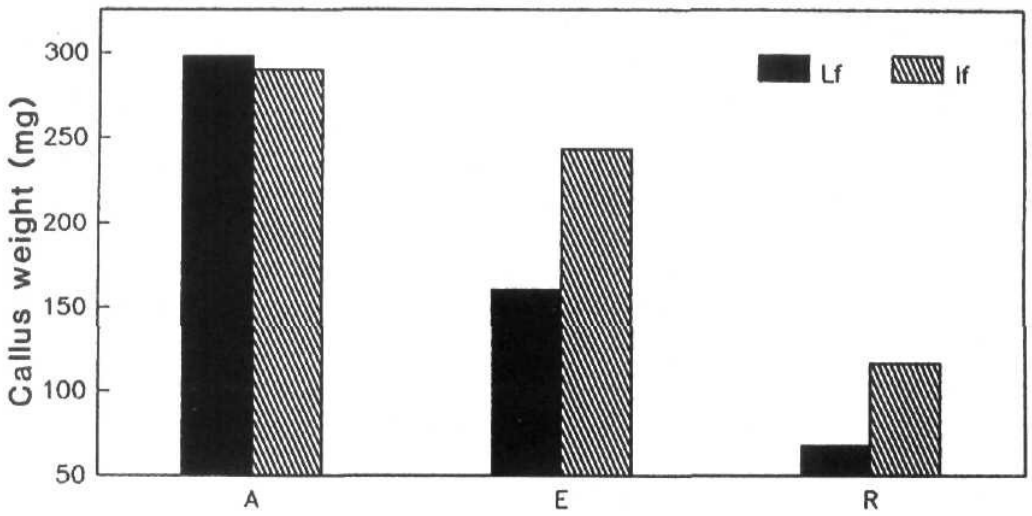

Fig. 2. The growth of *Lf* and *lf* callus induced from apices (A), epicotyls (E) and roots (R) on a medium with 1 mg/L NAA.

Earlier it was shown that callus induced from apices of genotype *Lf* grew better than callus of genotype *lf* on media with high (1-2 mg/L) NAA content whereas on media with low NAA content genotype *lf* grew better (1). It can be supposed that in callus cultures induced from epicotyls and roots the total auxin content (exogenous plus endogenous) in 1 mg/L NAA medium is sufficient for intensive callus proliferation of genotype *lf*, but is not enough for similarly intensive growth of *Lf* callus.

Thus, our experiment proved that the difference between isogenic *Lf* and *lf* lines can be observed not only in callus obtained from apices, but also in callus induced from epicotyls and roots. It can be assumed that the cell differentiation during callus initiation can induce expression of the *lf* gene whereas expression of this gene is spatially and temporally specific at the whole plant level.

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