REGENERATION OF GENE LINES 0F PISUM SATIVUM FROM CALLUS CULTURES

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This study was begun with the hypothesis that the ability to regenerate plants from somatic cell cultures of Pisum might have a genetic basis, and further that the requisite alleles which allow regeneration would be more likely to be found in the more primitive pea lines. This hypothesis has support in other crop species; certain less selected varieties of maize (1) and tomato (2) have been found to show an increased ability to regenerate from callus over the currently used crop varieties. On this basis, the standard Pisum varieties 'Frosty' and 'Alaska' were compared for the ability to regenerate from callus culture with 14 lines obtained from the collection of G. A. Marx, Geneva, N.Y.

Seeds of 16 lines were surface sterilized by soaking in 10% chlorox and 0.1% sodium dodecyl sulfate for 5 minutes, rinsing with sterile distilled water, dipping in 95% ethanol, and then rinsing again in sterile distilled water. The seeds were placed on Murashige and Skoog (3) medium with no hormones and solidified with 0.9% agar. After 2 to 3 days incubation in the dark at 26°C, the radicle emerged. As soon as this was observed, the seeds were dissected and the embryo was removed. Cylindrical sections of the epicotyl approximately 2 mm in length were placed on a callus inducing medium consisting of Murashige and Skoog salts and vitamins plus 2 mg of naphthalene acetic acid and 1 mg of benzyladenine per liter of medium. Sections of epicotyl were also placed immediately on regeneration medium consisting of the same salts and vitamins but with 0.2 mg of indoleacetic acid and 5 mg of benzyladenine per liter. Callus plates were kept in the dark at 26°C, and regeneration plates were kept at room temperature under a cool white fluorescent light of intensity approximately 2500 lux under a 16 hour/8 hour light/dark regime.

The epicotyl sections of different lines gave rise to callus at different rates, but all lines grew, approximately doubling in every month. The callus was transferred to fresh callus medium every month, and every other month a portion was subcultured and placed on regeneration medium. Thus each line was tested for regeneration from 0 months callus (epicotyl), 2 months growth as callus, 4 months growth, and 6 months (most recent test). On the regeneration medium, the greenest, most organized structures were subcultured monthly onto fresh regeneration medium until well defined shoots with leaf nodes formed. When they occurred, typically 2 to 3 months after initiation of regeneration, these well formed shoots were rooted by slicing them off just below a leaf node; the lowest leaves were removed, and then the shoot bottom was dipped in sterile naphthalene acetic acid, 1 mg per ml, to a level just above the bottom node. The shoot was planted in medium without hormones with the shoot bottom immersed in the agar to a level just above the lowest leaf node, and then kept at room temperature under light as in shoot regeneration. After approximately a month, when roots had emerged from the buried node, the plantlets were transferred to sterile soil, after the agar had been gently washed away. The plantlets were gradually brought to greenhouse conditions with special care at initially keeping the humidity very high.

Table 1 shows the results of this comparative study of regeneration among pea lines used. Listed are the various lines tested, and the last month of callus culture from which they were able to regenerate. Six were able

to regenerate from the epicotyl sections, and also from 2 months of callus culture. Four were able to regenerate from 4 months, and one is regenerating from 6 months of culture. Although these lines did not regenerate from rapidly growing callus, as there were at most 6 doublings in the line that worked after 6 months callus culture, they demonstrate the validity of the hypothesis that cell culture characters may have a genetic basis in peas. This suggests the program of crossing the best lines to obtain a cultivar that has very good regeneration properties, using this cultivar to make cell culture mutants, and then regenerating the new mutants for further study as a whole plant via crosses with standard multiply marked lines. Preliminary observations on the 4 best lines suggest that there is no common morphological characteristic, although there clearly must be some common physiological traits.

Table 1. Regeneration capacity of 16 Pisum lines.

Line	Regeneration ability
Frosty	None
Alaska	None
A478-50-2 1/	After 4 months as callus
B77-254	None
B77-256	None
B77-257	None
B77-258	None
B77-259 2/	After 6 months as callus
B77-262	None
B77-263	None
B77-266 3/	After 2 months as callus
B77-270	None
B77-273 4/	After 2 months as callus
B77-276 5/	After 4 months as callus
B77-279 6/	After 4 months as callus
B77-285	None

Partial gene characterization:

1/ le, A, D^{co}, wlo, sil, b, bt, U, R, i

2/ Le, A, D^{co}, Td Int or Ser, fr or fru or both, F, Fs or F Fs, R, I

3/ le, A, D^{co}, probably pro, fine stems and leaves, Pl, M, ar? R, I } wild or

4/ le, A, d? " " " " " " Pl, M, ar? R, I } primitive

5/ le A, d, Pa, Bt, anthocyanin flecks on leaflets, wiry vine, } forms
F or Fs, R, I

6/ le, a, habit resembling modern, commercial type, Pl, R, I, ar?

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