

few outstanding cases and segregation and selection from F2 - F5, with backcrosses and their segregations) during the last three and a half years has clearly pointed to recessive epistasis and dominance as the main causes of the heterosis observed in crosses of fasciated with non-fasciated lines.

1. Loenning, W. E. 1982. Theor. Appl. Genet, (in press).

THE INHERITANCE OF THE ABILITY TO REGENERATE PLANTS FROM CELL CULTURES
OF PISUM SATIVUM L. — A PRELIMINARY ANALYSIS

Malmberg, R. L. Cold Spring Harbor Lab, Cold Spring Harbor, NY USA

Previously we had reported that certain wild or primitive forms of peas had some ability to regenerate from callus cultures (1, 2). Specifically, two lines obtained from G. A. Marx (B77-259 and B77-276) were found to be able to give rise to whole plants when epicotyl derived callus was shifted to shoot inducing medium after as long as six months in culture; however, these lines showed an increasing difficulty in obtaining regeneration as a function of time, requiring progressively longer incubations on shoot medium to give rise to regeneration events. Also, because the pea callus grew very slowly, with doubling times on the order of three to four weeks, the six months regeneration window did not reflect very many cell divisions. Thus the ability to regenerate from cultures was very limited. In this note we report some preliminary results of crosses between one of the regenerating lines (B77-259) and a non-regenerating multiply marked line (A1078-234-0) also kindly provided by G. A. Marx. A partial genetic characterization of these lines is as follows:

B77-259:	<u>Le</u> , <u>A</u> , <u>D</u> ^{CO} , <u>Td</u> <u>Int</u> or <u>Ser</u> , <u>fr</u> or <u>fru</u> or both, <u>F</u> <u>Fs</u> or (<u>F fs f Fs</u>), <u>R</u> , <u>I</u> , wild or primitive form, some regeneration ability.
A1078-234-0:	<u>A</u> , <u>D</u> , <u>wb</u> , <u>k</u> , <u>s</u> , <u>st</u> , <u>b</u> , <u>f</u> , <u>le</u> , <u>fa</u> , <u>gd</u> , <u>tl</u> , <u>cd</u> , <u>fs</u> , <u>wlo</u> , <u>te</u> , <u>i</u> , <u>r</u> , non-regenerating.

By standard crossing methods we constructed F1, F2, and backcross generations. As previously described (1, 2) seeds to be tested for their callus/regeneration ability were surface sterilized and germinated on an agar medium. The epicotyls were dissected out two to three days later and cultured on callus medium for four to six months, after which they were transferred to shoot inducing medium. Successfully regenerated shoots were then rooted and transferred to soil and growth chamber conditions with a great deal of care at initially keeping the humidity high. Two problems with this experimental design seem unavoidable. First, the assay for regeneration potential can require as much as twelve months, e.g. six months in culture, four months to regenerate shoots, two months to obtain roots plus transplant to soil. This is before any linkage data could be obtained by monitoring the growth of the plants and scoring the markers. Second, non-regenerating calli cannot be scored at all for the whole plant markers, thus we lost a substantial portion of the linkage data that was present in the seeds. Initial data indicated that there was no maternal effect so subsequent data have been pooled.

F₁ seeds showed no regeneration at all. In the F₂, 32 out of 139 seeds gave rise to callus that has subsequently regenerated well formed shoots, a frequency of 23%. This suggests a recessive trait with perhaps a simple pattern of inheritance. The backcross data are consistent with this in that backcrosses to the regenerating parent sometimes regenerate, and those to the multiply marked parent never do. A problem has arisen in that not all of the regenerating shoots have successfully rooted and transplanted to soil, in contrast with the original line which always did so. This suggests a possible second genetic factor responsible for the hardiness to transplantation. Analysis of the generation is still in progress; to date we have observed segregation for genes *st*, *b*, *le*, *cp*, *tl*, suggesting that these are unlinked to the regeneration factors. Because of the small sample size, 32, which is regenerating, we may not be able to assign a linkage to the regeneration genetic factors. Also, we will need F₃ data to estimate the gene number, since 23% segregation in the F₂ could be compatible with models other than just the obvious single segregating locus. We believe that we have demonstrated some genetic basis for the difference between strains in ability to regenerate from callus.

1. Malmberg, R. L. 1979. PNL 11:21-22.
2. Malmberg, R. L. 1979. Planta 146:243-244.