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Tissue Culture Studies in Birdsfoot Trefoil

The practical application of cell culture selection to plant improvement requires a suitable callus and suspension culture system, a correlation between whole plant response and in vitro response and a pertinent selection scheme for in vitro selection.

Selection for 2,4-D tolerance in birdsfoot trefoil has been one objective of cell culture studies of the plant cell culture laboratory at Guelph. T-68 is a tolerant Lotus corniculatus line in the field to the systemic herbicide 2,4-D (Devine, T.E. et al. Crop Science, 15: 721-724; 1975) and Leo is a susceptible cultivar in the field. Leo and T-68 were compared for seedling hypocotyl elongation and callus growth rates on different levels of 2,4-D. Shoot and root production on a variety of different auxin-cytokinin concentrations was also observed. A differential response to 2,4-D was established at every stage of development under study. Attempts to isolate tolerant clones in the susceptible cultivar Leo proved difficult, due to slow and variable callus production.

To facilitate in vitro selection mass selection for callus production from genotypes selected from the trefoil breeding program was initiated. Hypocotyls were dissected from meristem tip cultures of nine genotypes. Two cm sections were placed in vials containing 10 ml of B<sub>5</sub> nutrient media with 1 mg/l 2,4-D. On the basis of subsequent visual callus production and condition the genotypes were divided into slow or fast callus producing lines. Any genotype which could not be clearly characterized into fast or slow callus lines (i.e., had replicates in both fast and slow groups)

was discarded. The fast and slow callus producing genotypes were combined into fast and slow callus lines respectively. These two lines were grown with an unselected control and an experiment on their relative callus growth rates was performed, after the callus lines had been subcultured for approximately eight months.

The initial inocula consisted of 2 grams of callus evenly spaced throughout a flask containing 50 ml of B<sub>5</sub> stock with 1 mg/l 2,4-D and .8% agar. The average callus produced by the respective line in 21 days, alternating light-dark at 25°C is shown below:

Leo Fast	13.5 g
Leo Stock	9.2 g
Leo Slow	8.5 g

The fast line may be characterized as a light green, relatively homogeneous, very friable callus which differentiates readily. The fast line has also proven much more useful in suspension when compared to stock lines (unselected). The fast line not only produces 80% free cells on suspension but also has a consistently faster growth rate. Even after light homogenization with forceps, 5 grams of the fast callus produced 118,000 cells/ml in the upper aqueous phase, compared to the stock callus which produced 21,000 cells/ml. The fast line has proven faster at differentiating, though the total number of plants produced per gram of callus is apparently not increased.

Experiments to isolate in vitro 2,4-D tolerant lines are continuing with the faster growing callus line.