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CALLUS GROWTH AND PLANT DIFFERENTIATION FROM SEEDLING HYPOCOTYLS OF
BIRDSFOOT TREFOIL CULTIVARS AND GERMPLASM

Five birdsfoot trefoil cultivars, Empire, Mackinaw, Viking, Maitland, Leo, and germplasm release T-68, developed for increased tolerance to the herbicide 2,4-D (Devine *et al.*, Crop Sci. 15: 721, 1975) were screened for their ability to form callus cultures that could subsequently be induced to regenerate whole plants from the callus. The callus cultures were initiated from seedling hypocotyls after surface sterilizing seed of the respective varieties. The culture media consisted of Gamborg's B-5 salts with the addition of 0.5-3.0 mg/l 2,4-D and from 0.5-3.0 mg/l kinetin for the callusing medium and 4.0 mg/l kinetin in the differentiation medium. Seedling hypocotyls were divided into four sections approximately 5 mm long, and placed on the callusing medium. After four weeks in culture the callus was divided into two pieces and transferred to differentiation medium providing sufficient callus had been produced from the hypocotyl section. Genotypes were observed for the presence of shoots after four weeks and further subcultured to differentiation medium. The callus cultures were again observed after four weeks for the presence of plant differentiation. Within each cultivar a genotype was classified as capable of differentiation from callus cultures if one or more of the calli developed from a single hypocotyl (genotype) differentiated shoots. The results are shown in the accompanying table.

Each of the cultivars and T-68 showed high frequencies of genotypes which were capable of callus production and subsequent plant regeneration from the callus. Callus growth from seedling hypocotyls was most limiting for Empire although 24 of 30 genotypes tested produced adequate callus. Cultivars Leo and Empire had the highest frequency of genotypes capable of differentiation with 63 and 75%, respectively, of the genotypes differentiating plants after a single transfer from the callusing to the differentiation medium. Germplasm release T-68 had the lowest frequency of differentiation after the first transfer to differentiation medium. A second transfer to differentiation medium increased the proportion, in some cases dramatically so, of genotypes which differentiated in each of the cultivars and T-68.

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Cultivar	Number of genotypes	Suff. callus for transfer (%)	Genotypes which differentiate (%)	
			First transfer	Second transfer
EMPIRE	30	80	63	77
MACKINAW	30	85	30	66
VIKING	28	85	39	71
MAITLAND	5	100	40	80
LEO	28	94	75	96
T-68	14	100	20	100

Each of the cultivars and the germplasm release T-68 contained high proportions of genotypes capable of regeneration from primary callus cultures. This is an important consideration when callus and cell cultures are being contemplated as possible procedures for use in the agronomic improvement of a species. The ease with which plants were differentiated from a variety of different genotypic sources indicates that birdsfoot trefoil is a very desirable species for further experimentation in the area of cellular genetics.