Screening of *Medicago* wild species for callus formation and the genetics of somatic embryogenesis

P D Walton and D C W Brown

1 Plant Science Department, University of Alberta, Edmonton, Alberta T6G 2P5, Canada
2 Genetic Engineering Section, Plant Research Centre, Agriculture Canada, Ottawa KIA 0C6, Canada

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Abstract. Genotypes from twelve accessions of five wild species from the genus *Medicago* were screened for callus formation and somatic embryogenesis using two tissue culture protocols. These wild species were compared with two highly regenerable genotypes of *Medicago sativa*. Embryogenesis for the wild types was very low. Wild species genotypes, which gave some callus production, were isolated.

In alfalfa previous studies have indicated that embryogenesis is genetically controlled. Of the two pairs of reciprocal crosses tested here, one provided evidence of cytoplasmic inheritance of the trait, the other did not. The parent which carried the cytoplasmic factor was A70.34. Nuclear factors are also involved in the inheritance of embryogenesis.

Keywords. *Medicago sativa*; *Medicago scutellata*; *Medicago disciformis*; *Medicago rugosa*; *Medicago lupulina*; *Medicago marina*; insect resistance; salt tolerance; tissue culture; cytoplasmic inheritance.

1. Introduction

Polyploidy and chromosome rearrangement in the genus *Medicago* have resulted in the genetic isolation of genome groups. Diploid, tetraploid and hexaploid species all exist. Also, the union of two chromosomes in the genome and the loss of one centromere region has produced species with basic chromosome numbers of both $x = 8$ and $x = 7$. The ploidy levels combined with differences in chromosome number have together provided a complete interbreeding barrier between members of this genus (Lesins et al 1970). Modern biotechnology offers the means of breaking such barriers and producing novel gene combinations of agronomic worth. Gene cloning and plant transformation, protoplast fusion, cell selection and the use of somaclonal variation have been proposed as techniques for new cultivar production in many species including alfalfa. All these methods utilize plant tissue culture techniques. Consequently, the key to initiating such changes is to determine whether species or genotypes are amenable to tissue culture protocols (Meijer and Brown 1985). For, while suitable regeneration protocols have been developed for some cultivars, all genotypes are not equally amenable to all culture methods. Genetic factors influence regeneration, so callus formation and embryogenesis are most important.

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Reisch and Bingham (1980) studied the genetic control of embryogenesis in diploid alfalfa stocks and suggested that differentiation from callus was controlled by two dominant genes. The segregation ratios they reported from crosses between genotypes with "good" and "poor" regeneration did not always fit their hypothesis (i.e. observed = 1:5; expected = 3:3 and observed = 14:52; expected = 28.8:37.1) and doubts remain as to the validity of their conclusions. Also, their classification groups for percentage regeneration were very large. All regeneration percentages over 15% were regarded as "good". The second classification group was made up of genotypes showing a lower percentage regeneration. Such wide categories could obscure gene action even in a diploid where only two genes are involved. The possibility that cytoplasmic genetic factors might control the inheritance of embryogenesis in alfalfa has not been considered in the literature. Hence this study was undertaken with two objectives; first, to examine callus formation and somatic embryogenesis of certain wild Medicago species using the A70.34 genotype as control, and second, to determine differences in the extent of embryogenesis in two reciprocal crosses using a poorly and a highly regenerative alfalfa genotype.

2. Materials and methods

2.1 Plant material

Two clones of Medicago sativa, A70.34 and A1, were used as controls. A70.34 is a genotype from the cultivar ‘Rangelander’ and has been shown to be highly regenerable via somatic embryogenesis (Brown and Atanassov 1985). A1 is a selection from Regen B, a M. sativa genotype provided by Dr E T Bingham, Madison, Wisconsin, USA. Species of the genus Medicago, not used commercially, were also tested. Six accessions from M. scutellata (PI 197356, PI 0541, PI 170541, PI 170552, PI 327446 and PI 307447), two M. disciformis lines (PI 487359 and 68-525), one member each of the species M. rugosa (PI 307443) and M. lupulina and two accessions from M. marina (VM and YM) were evaluated. This last species is interesting because it is highly salt tolerant (Lesins and Lesins 1976); a whole plant character for which it has not been possible to select successfully in tissue culture (Croughan et al 1978; Smith and McComb 1983). The remaining wild types were selected because their shoots and leaves had erect capitate stalked secretory glands. These structures provide plant resistance to the first instars of several insects which are serious pests of cultivated alfalfa (Kitch et al 1985).

For reciprocal crosses three genotypes of M. sativa, A70.34, A59 and F1.1, were used. A59 was a selection from Regen B which, on Murashige and Skoog medium, regenerates less well than A70.34. F1.1 is a highly regenerable genotype selected from M. sativa ssp. falcata (Meijer and Brown 1985). Reciprocal crosses were made in a glasshouse between plants of A59 and F1.1 and between A59 and A70.34 during the summer of 1986. All three genotypes are tetraploids.

2.2 Seed germination and tissue culture protocol

Scarified seeds were surface sterilized in a 0.2% solution of HgCl₂ (10 min) with one drop of Tween 80 and washed three times in sterile distilled water. The seeds