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ABSTRACT

INHERITANCE AND ACTIVITY OF THE EARLY AUXIN-RESPONSIVE GH3 PROMOTER AND SOMATIC EMBRYOGENESIS IN ALFALFA (*Medicago sativa* L.).

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Somatic embryogenesis in alfalfa (*Medicago sativa* L.) is an *in vitro* process of cell differentiation that is induced by 2,4-D and kinetin. The process is genotype dependent, under the control of two dominant, independent and complementary loci and presents variation within and among genotypes. The hypothetical signal transduction pathway induced by 2,4-D presumably targets auxin responsive genes involved in cell division and differentiation. To investigate gene regulation during auxin induction of somatic embryo formation, an embryogenic alfalfa plant was transformed by *Agrobacterium tumefaciens* with a binary vector carrying the GH3 auxin responsive promoter and β -Glucuronidase (GUS). Analysis of GUS activity in primary regenerants indicated that plants with more than one T-DNA insertion event did not show GUS activity, suggesting that gene silencing could be involved. Segregation analysis of T1 and T2 progeny carrying one T-DNA insertion indicated that the transgene was inherited as a single Mendelian dominant trait. However, there was variation in GUS activity among these progeny which indicated segregation of other genes involved in regulation of the GH3 promoter. The 2,4-D induced GUS activity was related to the endogenous-

auxin-induction of the GH3 promoter in T1 and T2 progeny. However, *in vitro* screening of the T1 progeny indicated that variation in somatic embryogenesis was not related to variation in GH3-GUS activity, suggesting separate signal transduction pathways and independent segregation of genes involved in these pathways. Fluorometric and histochemical analysis indicated that GH3-GUS expression was developmentally regulated during the process of somatic embryo formation. This research will contribute to the development of breeding methodologies for transgenic alfalfa and may help to identify and isolate genes involved in controlling *in vitro* somatic embryo formation.