Genetic control of in vitro regeneration in alfalfa (Medicago sativa L.)

Y. Wan,¹, E.L. Sorensen² and G.H. Liang¹
¹ Dept. of Agronomy, Kansas State University, Manhattan, KS 66506, USA; ² U.S. Dept. of Agriculture, Agricultural Research Service, Dept. of Agronomy, Throckmorton Hall, Kansas University, Manhattan, KS 66506, USA

Received 17 February 1987; accepted in revised form 14 May 1987

Key words: Medicago sativa, alfalfa, lucerne, tissue culture, callus, segregation ratio, complementary genes, genetic control

Summary

The genetic control of plant regeneration from callus culture was studied in tetraploid alfalfa (Medicago sativa L.). Seven cultivars (total 72 plants) were screened for regenerability. Ladak had the best regeneration response, in which 42% of the plants regenerated. Four regenerable plants and three nonregenerable plants were used to form 10 F₁ hybrids and three S₁ populations. Segregation ratios in the populations suggested that regenerability of alfalfa via petiole culture was under the control of two complementary genes, Rn³ and Rn⁴; the presence of both dominant genes was necessary for a plant to regenerate in a two-step culture system. The data also indicated that gene dosage influenced regeneration efficiency. Significant reciprocal effects demonstrated that the interaction between callus induction medium and callus regenerability was affected by cytoplasmic factor(s).

Introduction

Successful in vitro regeneration of many plant species has resulted from proper growth medium and environmental conditions (Murashige, 1974), as well as genetic control (Brown & Atanassov, 1985; Nesticky et al., 1983; Oelck & Schieder, 1983; Reisch & Bingham, 1980). The effects of genetic background on plant tissue culture have been increasingly noted. Tabata & Motoyoshi (1965) first reported major gene and maternal effects in callus formation from maize (Zea mays L.) endosperm. Sun & Ullstrup (1971) also found that proliferation of endosperm callus in maize was controlled by two genetic factors, with the expression of a maternal effect. Izhar & Power (1977) suggested that only a few genes were involved in genotype-specific hormone requirements for protoplast growth in petunia (Petunia sp.), and different stages of protoplast development might be controlled by different genes. Ma et al. (1987) suggested that two complementary genes were involved in regeneration from cultured immature embryos of sorghum (Sorghum vulgare). Hlasnikova (1977) studied the genetic aspects of in vitro androgenesis in tobacco (Nicotiana sp.) species and found that genetic interactions at the level of species, lines, and hybrids played an important part in promoting androgenetic efficiency. Buiatti et al. (1974) determined that callus growth and bud formation in wild cabbage (Brassica-
Ca oleracea L.) was controlled primarily by additive gene effects. Dependence of regeneration on genotype in tissue culture has been observed in legumes (Phillips, 1983; Campbell & Tomes, 1984; Kumar et al., 1983; Atanassov & Brown, 1984; Bingham et al., 1975). In alfalfa, genotypic variation in embryogenesis appears to be a widespread phenomenon. Brown & Atanassov (1985) found that embryogenesis response in cell suspensions and callus cultures derived from cotyledon explants was strongly genotype dependent. The induction of somatic embryogenesis varied among cultivars (Bingham et al., 1975; Brown & Atanassov, 1985) and genotypes of a cultivar (Kao & Michayluk, 1981; Mitten et al., 1984; Phillips, 1983). Even though the frequency of regenerating genotypes within a cultivar was high, much variation existed in the efficiency of regeneration (Mitten et al., 1984). This was attributed to the intervarietal and intravarietal heterogeneity in alfalfa which is an open-pollinated species.

Plant regeneration from callus was highly heritable in alfalfa. Regeneration increased from about 10% in standard alfalfa cultivars to 67% in two cycles of recurrent selection (Bingham et al., 1975). Reisch & Bingham (1980) found that in diploid alfalfa, bud differentiation from callus was controlled by two dominant genes, and both must be present in order to obtain more than 75% regeneration. They designated the genes as Rn1 and Rn2.

In genetic studies of tissue culture, a cytoplasmic effect was observed by some researchers. Nesticky et al. (1983) noted significant reciprocal effects in the basic analysis of combining ability and high values of reciprocal effects in corn tissue culture. They suggested that callus growth in vitro was controlled by two genetic systems, one located in the nucleus and another in the cytoplasm. Proliferation of endosperm callus of corn also was considered to be controlled by two genetic factors, with the expression of maternal effect (Sun & Ullstrup, 1971; Tabata & Motoyoshi, 1965).

These genetic studies of different species in tissue culture indicated that several major traits are probably under qualitative genetic control, and cytoplasm may make a contribution. Our study was designed to assess the qualitative genetic control of in vitro regeneration of tetraploid (2n = 4x = 32) alfalfa. The effect of cytoplasm on the interaction between callus induction medium and callus regenerability also was studied by comparing the responses of the F1 progenies of two sets of reciprocal crosses, in which two parents of each set of crosses had different responses to two induction media.

Materials and methods

A total of 72 plants from seven tetraploid cultivars: ‘Ladak’, ‘Lahontan’, ‘Grimm’, ‘DuPuits’, ‘Buffalo’, ‘Anik’, and ‘African’, was screened for regenerability. Each plant was numbered, e.g., Ladak-1, Lahontan-17, etc. Three regenerable plants and one nonregenerable plant from Ladak and one regenerable and two nonregenerable plants from Lahontan were selected as parents to produce 10 F1 populations and three S1 populations (Table 1). Populations I and II are F1 populations derived from crosses between two nonregenerable parents. Populations III to VII are F1 populations from crosses between one regenerable parent and one nonregenerable parent. Populations VIII to X are F1 populations between two regenerable parents. Populations XI and XII are S1 progenies of regenerable plants. Population XIII is S1 progeny of a nonregenerable plant. All the crosses were made in a greenhouse by hand pollination without emasculation. Parents and their progenies were grown in the same greenhouse.

A two-step sequence was used in this study. Medium 7951 (Liang et al., 1982) containing 2 ml/l 2,4-D and 0.5 mg/l kinetin solidified with Difco agar was used for callus initiation. A hormone free medium, SHAP, which is a modified Schenk & Hildebrandt (1972) medium, with an addition of 50 µm proline and 30 µm alanine was used for regeneration. Both media were adjusted to pH 5.9–6.0. Petioles of the second or third leaves from the stem apex were used as explants for callus initiation. Petioles were cut into pieces (about 0.5 cm long), which were sterilized in 75% ethanol for 15 seconds and then in 50% commercial bleach for 5 minutes followed by three washes in sterile double distilled water. Sterilized petiole segments, usually