MICROPROPAGATION OF *ASTRAGALUS MAXIMUS* WILLD.

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ABSTRACT

Micropropagation of *Astragalus maximus* Willd. through axillary bud culture was achieved 30 day-old plants. The shoots of plants were used as explants for micropropagation. The explants were cultured on MS media supplemented with BA (0.2, 0.5, 1.0 mg/l), ZR (0.2, 0.5, 1.0 mg/l) and their combinations with 0.4 mg/l NAA. After 20 days, micropropagation was strongly induced in MS medium supplemented with 0.5 mg/l ZR, ~30 shoots obtained from each explant. In all other treatments, proliferation number on per explant varied to 2-3. Propagated shoots were cultured on MS medium without plant growth regulators, MS medium supplemented with 0.2-1.5 mg/l NAA and ½ MS with 1.5 % sucrose. Root induction was only achieved on ½ MS containing 1.5 % sucrose after 20 days. Plane MS and MS medium with 0.2, 0.4, 0.8, 1.2 and 1.5 mg/l NAA did not induce root formation.

Introduction

*Astragalus* (fam. Leguminosae) species are important medicinal plants, because of their bioactive secondary metabolites which are used as immunstimulant, tonic, diuretic and an anticancer agent (1). In addition their product, gum tragacanth, a versatile food stuffs additive and pharmaceutical emulsifier, is a valuable rural crop of ecological importance derived from *Astragalus* spp. growing wild in Turkey and Iran (2). However, the breeding of *Astragalus* spp. is limited by their low productivity of seeds. Despite their medicinal importance only a few in vitro culture studies have been reported in *Astragalus* spp.: plant regeneration from callus and protoplasts in *A. adscens* (3,4), from hairy roots in *A. sinicus* (5), from somatic embryos in *A. adscens* (6), *A. melilotoides* (7) and in *A. chrysochlorus* (8).

Micropropagation has proved to be an alternative way for the multiplication of selected genotypes of several medicinal and aromatic plants (9). *Astragalus maximus* Willd. is one of the *Astragalus* species which is mainly distributed throughout eastern Anatolia (10). There has not been find any report on tissue culture and secondary metabolism of *Astragalus maximus* Willd., so far. Our overall goal is to develop a propagation procedure to study of secondary metabolites of *A. maximus* like we have done for *A. chrysochlorus*. In this paper, we report original results of investigations to establish micropropagation of *Astragalus maximus* Willd.

Materials and Methods

*A. maximus* seeds were classified by Dr. Hasan Akan (Harran University, Faculty of Arts & Science, Department of Biology, Turkey). The seeds were surface-sterilized...
in 70% ethanol for 1 min then 5% commercial bleach (domestos) for 15 min, followed by three rinses with sterile distilled water. The sterilized seeds were germinated aseptically in Petri dishes containing 25 ml aliquots of agar-solidified in MS (11), supplemented with 3% (w/v) sucrose and 0.8% agar (w/v). The pH was adjusted to 5.8 before autoclaving at 121 °C for 20 min.

30 day-old plants were used for micropropagation. Roots of plants were excised and shoots were used as explants. Shoots were placed on culture tubes (Sigma, C-5916) containing 10 ml aliquots of MS media containing BA (0.2, 0.5, 1.0 mg/l), ZR (0.2, 0.5, 1.0 mg/l) and their combinations with 0.4 mg/l NAA. After 20 days, propagated shoots were placed on culture tubes containing 10 ml aliquots of agar-solidified three different MS media for root introduction; (I) MS medium without plant growth regulators (plane MS), (II) MS medium with 0.2, 0.4, 0.8, 1.2 and 1.5 mg/l concentrations of NAA, (III) ½ MS with 1.5% sucrose. One explant were inoculated in each culture tube. Five replicates were used per treatment and each experiment was repeated three times. Seed germination and micropropagation were carried out in a growth chamber illuminated with fluorescent light (c. 1400 lux) for 16h per day at 25 °C.

Results and Discussion

Micropropagation was strongly induced by 0.5 mg/l ZR within 20 days, ~30 shoots obtained from each explant (Figure a.). In all other treatments, proliferation number on per explant varied to 2-3 (Tab. 1). It has been shown that ZR has similar effect in Astragalus chrysochlorus (12). However, in Astragalus adsurgens (3), Pittosporum napalensis (13) and Cunila galioides (14) maximum shoot proliferation rates have been reported depends to use BA combination with NAA or BA alone.

Propagated shoots were used rooting experiments. After 20 days, root induction was only achieved on ½ MS containing 1.5% sucrose (Figure b.). Plane MS and MS medium with 0.2-1.5 mg/l NAA did not induce root formation. In contrast to these results, rooting was achieved in Astragalus adsurgens by NAA treatment (3). In some; Phyllanthus species, NAA were found to be more effective in stimulating root induction but not in the others (15). This similar results suggested that genotype differences could be responsible the effectiveness of NAA on root induction. These results could provide a practical means of clonal propagation of this medically and economically important plant species and for further biotechnological applications.

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Figure. a) Shoot propagation from seedling of *A. maximus* on MS+ 0.5 mg/l ZR (*bar*: 1 cm), b) root induction of propagated shoots on ½ MS containing 1.5 % sucrose (*bar*: 1 cm)

REFERENCES