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Optimization of Callus Induction and Regeneration in Two Fenugreek Landraces as a Medicinal Plant during *in vitro* Condition

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Introduction: Fenugreek *(Trigonella foenum- graecum*) is a medicinal plant extensively distributed in most regions of the world. Fenugreek is an annual plant from the family of papilionaceae, leguminosae. Fenugreek leaves and seeds have been used extensively to prepare extracts and powders for medicinal uses. Its root, leaf and seed contain a number of important medicinal compounds such as polysaccharide, galactomannan, different saponins such as diosgenin, yamogenin, mucilage, volatile oil and alkaloids such as choline and trigonelline. Plant tissue culture is fundamental to most aspects of biotechnology of plants. Establishment of an efficient callus induction and direct regeneration protocol is an essential prerequisite in harnessing the advantage of cell and tissue culture for genetic improvement. For the successful application of the tissue culture technique in plant breeding, callus induction and plant regeneration potential of each plant must be determined. The present study was performed in order to determine the optimum concentration of plant growth regulators (IBA + TDZ) for producing of *in vitro* plantlet using cotyledon and hypocotyl as an explant for two different Iranian genotypes (Ardestani and Neyshabouri).

Materials and Methods: In this investigation, Ardestani and Neyshabouri genotypes were used for callus induction and direct shoot regeneration. The medium used in this investigation was MS (Murashige and Skoog) basal medium. Then seeds were germinated on MS medium. For callus induction and direct shoot regeneration, cotyledon and hypocotyl explants were excised from 8-day-old sterile seedlings and cultured on MS medium containing various concentrations of IBA and TDZ. In this experiment, two combinations (TDZ + IBA) were used. In the first composition, IBA had four levels (0, 0.1, 0.3, 0.5 mg l^{-1}) and TDZ had five levels (0, 0.2, 0.4, 0.6, 0.8 mg l^{-1}) and in the second composition, IBA had four levels (0, 0.05, 0.1, 0.15 mg l^{-1}) and TDZ had seven levels (0, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45 mg l^{-1}).The experimental designs were factorial based on completely randomized design with four replications. Cultures were incubated at 25° C ± 2 with a 16/8 hour (day/night) photoperiod and an irradiance of 1500 LUX using Sylvania cool white fluorescent tubes. The percentage of callus induction, direct shoot regeneration and average weight of callus were calculated for cotyledon and hypocotyl explants. All Data were analyzed using SPSS16, and mean comparisons were performed with duncan's multiple range test ($P < 0.05$).

Results and Discussion: According to our results, explants cultured on MS basal medium without plant growth regulators (control) produced no callus. However, after two weeks, callus formed in both of Ardestani and Neyshabouri genotypes from cotyledon and hypocotyl explants in the presence of IBA + TDZ plant growth regulators in most of the combinations. In hypocotyl explants of Neyshabouri genotype, the highest callus induction was obtained from the medium containing 0.15 mg l^{-1} IBA + 0.45 mg l^{-1} TDZ (96.87%). Various important factors such as genotype, source of explants and plant growth regulators significantly influence direct regeneration. Direct regeneration was obtained from hypocotyl explants for Neyshabouri genotype in combination IBA + TDZ. The highest percentage of direct shoot regeneration was observed in MS medium containing 0.05 mg l^{-1} IBA + 0.35 mg l^{-1} TDZ in hypocotyl explants of Neyshabouri genotype (37.5%). Direct shoot regeneration requires plant cells to undergo dedifferentiation which is known to be affected by not only exogenous plant growth regulators but also endogenous content of the hormones. Different tissues may have different levels of endogenous hormones and, therefore, the type of explant source would have a critical impact on the regeneration success. In our study, when cotyledon and hypocotyl explants were compared, it was clear that hypocotyl explants were much more productive for direct shoot regeneration than cotyledon explants.

Conclusions: Callus induction and direct shoot regeneration are as *in vitro* tissue culture methods**.** Plant growth regulators and types of explant and genotype are the most important factors for callus induction and direct shoot regeneration phases. Therefore, optimization of these factors is essential to establish a high frequency of callus induction, direct shoot regeneration and gene transferring to this plant. According to the

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results of this investigation, it is recommended to apply plant growth regulators that were used in this study for other landraces of fenugreek cultured in Iran and select the best genotypes in response to tissue culture conditions for using in future studies.

Keywords: Cotyledon, Explants, Hypocotyl, Leguminosae, Medium