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In vitro culture and plant regeneration of *Sesbania grandiflora*

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Abstract

Studies were carried out with the aim of evaluating *in vitro* the effects of growth regulators auxin and cytokinin on *Sesbania grandiflora* (*S. grandiflora*) regeneration potentials through callus induction. Explants were cultured on to solid medium (Murashige and Skoog (MS)) enriched with different concentrations of auxins such as indole-3-acetic acid (IAA) & 1-naphthaleneacetic acid (NAA) and cytokinins such as kinetin (Kin) & 6-benzylamino purine (BAP). The medium (MS + 0.5 mg/L BAP + 0.2 mg/L IAA) showed up to 76% regeneration activity. The shoot formation form callus was observed 60% when MS + 1.5 mg/L BAP + 0.5 mg/L NAA were applied, while for combination of MS + 0.5 mg/L Kin + 0.1 mg/L IAA, the shooting percentage was recorded up to 80% for the cotyledons explants and MS + 1.5 mg/L Kin + 0.2 mg/L NAA revealed the shoot regeneration of 68% grown on solid medium. The highest root induction of cotyledons callus was noticed in medium (MS + 0.1 mg/L IBA) and (MS+ 0.1 mg/L IAA) which was 70% and 100%, respectively. From results of present study, it is concluded that the auxins (IAA, NAA) and cytokinins (Kin, BAP) could possibly use for the root and shoot regeneration of *S. grandiflora* plant and this study also provides a basis for future studies on genetic improvement and should be applied to large-scale multiplication systems for *S. grandiflora* plant.

Key words: *Sesbania*, callus induction, root regeneration, micropropagation, plant growth regulators

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1. Introduction

The genus *Sesbania* (Family *Fabaceae*) includes herbs, shrubs and soft woody plants, but short-lived trees also existed in tropical regions of the world. *S. grandiflora* is a short-lived, quick-growing, soft woody tree [1]. It is a native to Asia and is also grown in many parts of Iraq. *Sesbania* species are generally annual or biennial, some are perennial shrubs of short duration and a few species are truly perennial. The high nitrogen fixation capacity of these species results in rapid growth, even in soils deficient in nitrogen and permits the utilization of *Sesbania* as green manure, intercropping and ground cover in agro-forestry and wood production systems [2].

Plant tissue culture has become an important tool for the isolation of somaclonal variants with high nitrogen-fixing capacity and high biomass producing leguminous trees. Unfortunately, the transplantation of plantlets developed *in vitro* to soil claims very little success in many cases [3]. Extensive efforts to develop efficient regeneration systems for many legumes have resulted in a large array of *in vitro* protocols. However, leguminous tree species are less well studied and only a few are amenable to *in vitro* culture. Researchers highlighted that inoculation of micropropagated shrub legumes shortened their acclimatization process [4], woody legumes [5] including *Sesbania sesban* [1] and *Dianthus caryophyllus* [6]. The propagation through direct shoot has been implemented successfully [7]. However, regeneration from callus still needs to be developed and regeneration of plant using callus has certain advantages over indirect methods. A callus phase is commonly included in tissue culture protocols with the objectives of generating variability to introduce new desirable traits and generating transgenic plants to introduce new traits [8]. Moreover, callus production is also a necessary step for obtaining protoplasts which is a useful tool in genetic improvement for introducing useful genes or producing new species [9-10].

The aim of the present study was to develop a method for *in vitro* regeneration of *Sesbania* plant from cotyledon under the effect of different combinations of plant growth regulators (PGR) to find out the best genotype source linked with the optimum medium conditions for the high potential of shoot and root indications.

2. Material and Methods

Sesbania grandiflora seeds were procured from fields located in Sayafia village, 20 Km south of Baghdad. Surface sterilization of seed was carried out with 1.75%

NaOCl for 5 min followed by three washing with sterile distilled water (dH_2O). Seeds were placed in vessels (8*2.5 cm) containing 10 mL MS [11] solidified with 7g/L of agar.

Cotyledons explants were placed on MS with 30 g/L sucrose and agar-agar in vessels (8*2.5 cm). Various combination of 2,4-D and BAP were used for callus induction. The pH of media was adjusted to 5.8 before autoclaving at 121°C under 1.04 Kg/cm² pressure, for 15 min. All cultures were maintained at 24±2ºC in growth chamber in dark.

The response of cotyledons to auxin and cytokinin combinations was evaluated after 25 days of culturing. The initiated callus was removed from the explants using forceps and scalpel and then pieces (35 mg) were subcultured onto fresh medium supplemented with the same combinations of growth regulators. Healthy calli were transferred to regeneration medium, consisted of MS medium supplemented with 30g/L sucrose, NAA (0.0, 0.1 or 0.5 mg/L) or IAA (0.0, 0.2 or 0.5 mg/L) and BAP (0.0, 0.5 or 1.5 mg/L) or Kin (0.0, 0.5 or 1.5 mg/L) or regenerated shoot were transferred to the root induction medium consisted of MS medium supplemented with 30g/L sucrose, IBA (0.0, 0.05, 0.1 or 0.2 mg/L) or IAA (0.0, 0.05, 0.1 or 0.2 mg/L).

The responses thus obtained were statistically analyzed as a 2- factor experiment (cultivar and medium protocol) in a completely randomized design with ten replicates. Comparisons among means were made using the Least Significant Differences test ($LSD_{0.05}$) using SAS (version 9.2) program.

3. Results and Discussion

3.1 Germination of seeds

Results showed that the percentage germination of seed on MS medium after 10 days was 50%. However, when seeds were inoculated on sterilized moistened filter paper, the germination percentage reached to 100% after 5 days of sowing (Fig. 1 and 2). The germination on to sterilized moistened filter paper was recorded to be significantly higher versus only MS medium and this less and slow germination in MS medium might be due to agar impurities and/or to the osmotic potential [13-14]. Thus, seeds for all the experiments for root and shoot induction studies were germinated on sterile moistened filter paper later.

3.2 Shoot induction

The effect of various plant growth regulators on shoot induction was investigated and results are shown in Table 1. Results indicate that MS medium supplemented with 0.5 mg/L BAP and 0.2 mg/L IAA gave highest shoot formation which was 76% higher versus control. The combination of 1.5 mg/L BAP and 0.5 mg/L NAA showed the shoot induction of 33% which indicate that low doses of BAP and IAA are best as compared to higher doses for shoot induction from callus, however, higher doses response of shoot induction was also significantly higher in comparison to control. In second combination, the IAA was replaced with NAA and it was noted that the shoot induction was high at higher doses of regulator (BAP and NAA) as compared to BAP and IAA combination. The shoot induction was 60% when BAP and NAA were applied at the rate of 1.5 mg/L and 0.5 mg/L, respectively, while it was 23% for 0.5 mg/L and 0.1 mg/L BAP and NAA application, respectively versus control (Table 2). Furthermore, it was observed that the plant growth regulators produced green healthy and long shoots when low concentrations were used, whereas at higher concentrations glossy shoots were observed. These results are in line with earlier reports that direct and callus mediated organogenesis from both hypocotyl and cotyledons of *Sesbania grandiflora* [14-15] and *Sesbania* spp [16] were better at higher concentrations of BAP and NAA or IBA. Khatter and Mohan [14] also reported 3-5 cm long shoots after 4-6 weeks of growth when growth medium was supplemented with 0.49 ML^{-1} IBA. These results are also similar to that of Allavena and Sarp [17] who induced multiple and adventitious shoots of beans in a basal MS medium supplemented with 1.5 mg/L BAP in combination with 0.5 mg/L IAA or NAA. Moreover, Nandwani and Ramawal [18] reported that high frequency of plantlet regeneration from cotyledons of soybean was obtained on MS medium supplemented with IAA and BAP regulator. Similarly, Ashis *et al.* [19] reported that tobacco plants could be easily regenerated from leaf pieces on MS medium supplemented with 1 mg/L BAP and 0.1mg/L NAA.

The shoot induction results in response of cytokinin application are shown in Table 3. From results, it was revealed that 80% of shoot regeneration can be achieved for 0.5 mg/L Kin and 0.1 mg/L IAA concentrations. However, shoot regeneration was reduced to 32% when concentrations of Kin and IAA were used at the rate of 1.5 mg/L and 0.2 mg/L, respectively which indicates that low concentration of cytokinin is more effective as compared to higher concentrations. The effect of NAA was also checked on shoot regeneration in combination of Kin and response is given in Table 4. The shoot regeneration percentage was 18% when Kin and NAA were used at the rate of 0.5 mg/L and 0.1 mg/L, respectively. However, by increasing the concentrations of Kin and NAA, the shoot regeneration increased significantly and recorded to be 68% versus control. Overall, it was observed that the auxin response for shoot regeneration was slightly higher as compared to cytokinin.

3.3 Root induction

The effect of auxin (IBA and IAA) was checked for root regeneration and results are shown in Table 5. The root regeneration was recorded to be 62% when IBA was used at the rate of 0.05 mg/L. By increasing the concentration to 0.1 mg/L, the root regeneration increased to 70% and again reduced to 59% when IBA was used at the rate of 0.2 mg/L (Table 5). The IAA application showed the root regeneration of 75%, 100% and 84% higher versus control for 0.05 mg/L, 0.1 mg/L and 0.2 mg/L concentration respectively. Overall, it was observed that the IAA was more effective as compared to IBA for the regeneration of root from callus. Similar results were observed by [20] who reported that auxins are efficient for root regeneration and 100% higher root regeneration was achieved with 0.1 mg/L IAA application.

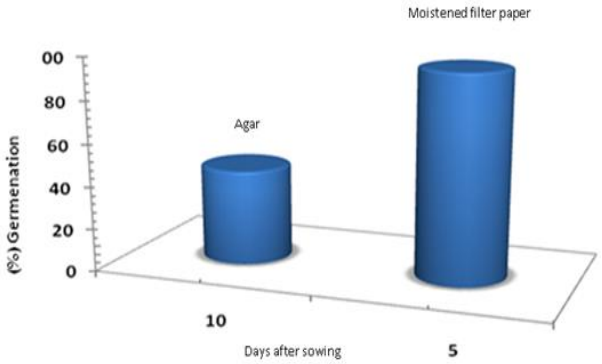

Fig. 1. Germination of *S. grandiflora* seeds on to half strength MS medium solidified with 7 g/L agar after 10 days and on to moistened sterile filter paper after 5 days of inoculation.

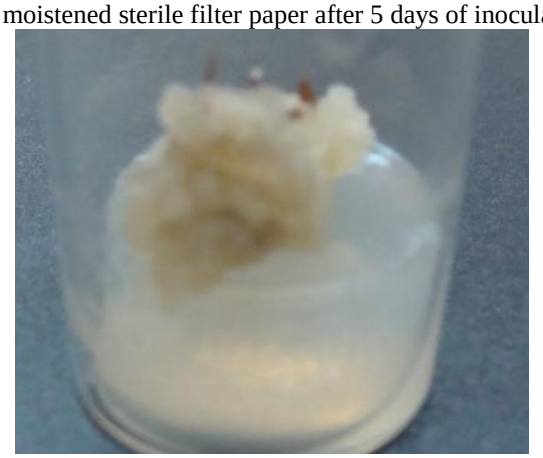

Fig. 2. 25th day of callus grown on MS medium supplemented with 2.0 mg/L 2, 4-D and 0.5 mg/L BA

Table 1. Effect of BAP and IAA on shoot regeneration from cotyledonary explants of *S. grandiflora*

BAP	IAA	Shoot formation
mg/L	mg/L	%
0	0	0
0.5	0.2	76
1.5	0.5	33
$LSD_{0.05}$		8.6

Table 2. Effect of BAP and NAA on shoot regeneration from cotyledonary explants of *S. grandiflora*

BAP	NAA	Shoot formation
mg/L	mg/L	%
0	0	0
0.5	0.1	23
1.5	0.5	60
$LSD_{0.05}$		5.4

Table 3. Effect of KIN and IAA on shoot regeneration.

Kin	IAA	Shoot formation
mg/L	mg/L	%
0	0	0
0.5	0.1	80
1.5	0.2	32
$LSD_{0.05}$		5.4

Table 4. Effect of KIN and NAA on shoot regeneration.

Kin	NAA	Shoot formation
mg/L	mg/L	%
0	0	0
0.5	0.1	18
1.5	0.2	68
$LSD_{0.05}$		6.3

Table 5. Effect of IBA and IAA on root regeneration of *S. grandiflora*

IBA	Root induction	IAA	Root induction
mg/L	%	mg/L	%
0	0	0	0
0.05	62	0.05	75
0.1	70	0.1	100
0.2	59	0.2	84
$LSD_{0.05}$	4.631	$LSD_{0.05}$	2.681

Conclusions

The effects of plant growth regulators (auxin and cytokinin) were studied *in vitro* on *S. grandiflora* to appraise the regeneration potentials through callus induction. The results indicate that the combination and the concentration of plant growth regulator are highly important in the establishment or regeneration system. All concentration and combination of plant growth regulator furnished significant responses versus control; however, 0.5 mg/L Kin and 0.1 mg/L IAA were more effective for shoot regeneration, whereas 0.1 mg/L IAA was found to be best dose for root regeneration and these treatments could possibly be used for multiplication of *S. grandiflora* plants.

References

[1] R. C. Gutteridge and H.M. Shelton. (1998). Forage Tree Legumes in Tropical Agriculture. The Tropical Grassland Society of Australia Inc.

[2] I. Ndoye, K. Tomekpe, B. Dreyfus and Y.R. Dommergues. (1990). *Sesbania* and *Rhizobium* symbiosis: nodulation and nitrogen fixation. In: ACKLIN, B.; EVANS, D.O. (Ed.) Perennial Sesbania Species in Agroforestry Systems. Waimanalo: Nitrogen Fixing Tree Association. Pp. 31-38.

[3] P.L.M. Pierik. (1988). In vitro culture of higher plants as a tool in the propagation of horticultural crops. Acta Horticulturea. 226: 25-40.

[4] P.E. Lovato, H. Schuepp, A. Trouvelot and S. Gianinazzi. (1995). Application of AMF in Orchard

and Ornamental Plants. In: Varma A. und Hock, B. (eds): Mycorrhiza. Springer, Berlin Pp. 443-468.

[5] C.P. Salamanca, M.A. Herrera and J.M. Barea. 1992. Mycorrhizal inoculation of micropropagated woody legumes used in revegetation programmes for desertified mediterranean ecosystems. Agronomie. 12: 869-872.

[6] M. Naglaa and A. Abdullah, N.M. Al-Doss, Mohamed. (2013). An assessment of in vitro culture and plant regeneration from leaf base explants in carnation (*Dianthus caryophyllus*). Journal of Food & Environment. 11: 1113-1117.

[7] P. Xu, Q. Su, X. Wang and J. Li. (2005). Studies induced adventitious shoots via tissue culture in garlic inflorescence and on the dynamics of endogenous polyamine contents during shooting. Acta Horticulture. 688: 269–272.

[8] M-M. Yan, C. Xu, C-H. Kim, Y-C. Um, A.A. Bah, D-P. Guo. (2009). Effects of explant type, culture media and growth regulators on callus induction and plant regeneration of Chinese jiaotou (*Allium* chinense). Scientia Horticulturae. 123: 124–128

[9] K. Yamashita, Y. Hisatsune, T. Sakamoto, K. Ishizuka and Y. Tashiro. (2002). Chromosome and cytoplasm analyses of somatic hybrids between onion (*Allium cepa* L.) and garlic (*A. sativum* L.). Euphytica. 125: 163–167.

[10] J. Liu, X. Xu and X. Deng. (2005). Intergeneric somatic hybridization and its application to crop genetic improvement. Plant Cell Tissue Organ Culture. 82: 19–44

[11] T. Murashige and F. Skoog. (1962). A revised medium for rapid growth and bioassays with Tobacco tissue culture. Plant Physiology. 15: 473-497.

[12] F. Bewley and D. Black. (1994). Enhanced somatic embryogenesis in *Sorghum bicolor* L. from shoot tip culture *in vitro*. Cell Development Biology. 24: 65-70.

[13] P.K. Agarwal, M. Akhtar and M. Anwar. (1997). Basmati rice progress, plant regeneration from maintained calli. Oryza. 43: 10-15.

[14] S. Khattar and H.Y. Mohan-Ram. (1983). Organogenesis and plantlet formation *in vitro* in *Sesbania grandiflora* (L.) Pers. Indian Journal of Experimental Biology. 21: 251-253.

[15] S. Shanker and H.Y. Mohan-Ram. (1990). Plantlet regeneration from tissue cultures of *Sesbania grandiflora*. Current Science. 59: 39-43.

[16] Y.X Zhao, D.Y. Yao and P.J.C Harris. (1993). Plant regeneration from callus and explants of *Sesbania* spp. Plant Cell Tissue Org Culture. 34: 253-260.

[17] H. Allavena and T. Sarp. (1981). *Agrobacterium tumefaciens* transformation of Japonica and Indica varieties of rice. Planta, 199: 612-617.

[18] F. Nandwani and K. Ramawal. (1992). Regeneration efficiency and embryogenic callus production of three cultivars of rice. Pakistan Journal of Agriculture Research. 34: 66-74.

[19] Ashis, T. Henry, H. and Blakeney, E. (2007). Varietal differences in plant regeneration from root callus tissue of rice. Journal of Plant Breed. 34: 187-196.

[20] P.K. Pindi, T. Sultana, P.K. Vootla. (2014). Plant growth regulation of Bt-cotton through Bacillus species. Biotechnology. 4: 305–315.