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Optimization of Callus Induction and Seedling Regeneration in Asparagus (*Asparagus officinalis*)

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ABSTRACT

In order to study the in vitro callus induction and plant regeneration in the asparagus plant (Asparagus officinalis) and indirect organogenesis in explants, an experiment in a completely randomized design was performed in tissue culture laboratory of faculty of agriculture, Islamic Azad University of Dezfool. In this study, the nodal explants treated with different concentrations of NAA and BAP, and it was found that the presence of two plant growth regulators NAA And BAP Is necessary for seedlings regeneration and callus induction in asparagus,. Treatment that leads to achieving the maximum amount of callus buds was MS medium supplied with 0.5 mg/lit of NAA and 1 mg/lit BAP which leads to the production of 22 buds per explants. Two different levels of 2, 4-D were used for induce roots in regenerated seedlings. The best treatment was 1.25 mg/ lit 2, 4-D with an average of 52% rooting in regenerated plants.

***Key words**: Asparagus, Tissue culture, Callus induction, seedling regeneration, plant growth regulator*

INTRODUCTION

Asparagus is a herbaceous plant belongs to *Liliaceae* family with semi-erect and branched articulated branches and thick and white roots, between 30 and 100 cm in length and 0.7 to 1 cm in thickness and dioecious perennial herb and is native to the Far East and the Mediterranean region. Almost all organs of the plant, including stems, roots, seeds and rhizomes have medicinal properties for the treatment of rare diseases such as bloody diarrhea. The powdered rhizome of this plant is used in combination with milk for baby weakness [1,2,3].

In traditional propagation method, plants are divided into 2-4 parts and each part plants separately. This type of reproduction is very slow and time consuming, and each plant can only produces 2-4 new plants. But a quick and safe solution for production and plant propagation is micropropagation through tissue culture [4].

Preliminary reports on asparagus tissue culture dates back to 1945. Among the various ways of *in vitro* regeneration in Asparagus plants, none of them has been commercially and widely and common in use because of very poor vigor seedling production [5,6,7].

The major factors responsible for the low survival rate of seedlings in Asparagus tissue culture, are tensions over *in vitro* plantlets during their life, the kind of plant growth regulators applied and glucose content in the medium [8,9].

The Asparagus plant is commonly a wild form and this is the main reason for the low rate of seed germination and rhizome reproduction in nature. The wild form of the plant species listed as endangered in the world [10,11,12,13].

MATERIALS AND METHODS

Tissue culture conditions

The culture medium condition (Temperature and light) during the callus incubation in medium was adjusted. Temperature during the growing period adjusted to 1 ± 25°c and light density adjusted to 1200 lux over the period of 16 hours light and 8 hours of darkness.

Plant material

In order to produce sterile plants, sterilized seeds were germinated in MS medium supply with 6 gr/lit agar and 30 gr/lit sucrose , at 1 ± 25°c and 16 hours of light and 8 hours of darkness photoperiod with light intensity of 3000 lux .

The seeds were prepared from Safi Abad research institute of Dezfool . The variety used in this study was Mary Washington variety of Asparagus.

Sterilization of seeds performed under the hood laminar flow. Seeds were shaken for 5 min in the sodium hypochlorite 2.5% (active ingredient) solution and then three times washes with sterile water and finally sterilized with 70° ethanol for 30 seconds.. The average viability of seeds used for this study was 93% .

Callus induction and seedling regeneration

Primary culture medium used in this study for callus induction in explants was MS medium with four different levels of plant growth regulators NAA and BAP. crown meristem of germinated seeds were used as explants in MS medium for callus induction. As the sterilized seeds were used for germination in MS medium, it does not necessary to sterilize the explants again. Thus, four different levels of NAA and BAP and a treatment without the use of growth regulators (control) used for callus induction and plant regeneration. Table 1 indicates the treatments used in this study.

Table 1 : The different treatments used for different levels of NAA and BAP .

NAA mg/lit	) mg/lit(BAP			
	0.0	0.2	0.5	1
0.0	A	B	C	D
0.15	E	F	G	H
0.25	I	J	K	L
0.5	M	N	O	P

Explants were placed on glass petri dishes with a 9 cm diameter and 25 ml culture medium. The cultures were incubated at 1 ± 25°c and 1200 lux light intensity. After four weeks (28 days) from the date of transfer of explants to the culture medium a subculture was performed in the same culture medium with the same ratio of plant growth regulators but in 50 ml glass jams instead of Petri dishes.

After 4 weeks from the date of subculture, germinated calli were transferred to regeneration medium. Regeneration medium was MS medium containing 25g/lit sucrose with two different level of growth plant regulators : 0.5 mg/lit and 1.25 mg/lit , 2,4-D. Regenerated calli cultured in glass jams containing 25 ml of regeneration medium. There are only two regenerated calli in each glass jam. Regenerated calli were selected from the treatments which regeneration occurred and each regenerated calli were culture in regeneration medium in four replications. In order to root induction , after transferring the calli into regeneration medium , glass jams were incubated in 1 ± 25°c with 16 h light with 3000 lux intensity and 8 h darkness photoperiod. Registration of callus characters and regenerated plantlets was performed 30 days after transferring the calli in regeneration medium.

In order to reach the whole regenerated plantlets, rooted calli were transfer into 50 ml glass jams.

Figure 1 : a- Primary stages of callus induction in explants , b- Somatic embryogenesis in calli

Statistical analysis

This study was performed in Completely Randomized Design with 16 treatments in 4 replications. Mean comparisons conducted with Duncan's multiple range test and all the statistical analysis were analyzed with MSTATC software. All the primary observation data were registered in Microsoft EXCEL software.

RESULTS AND DISCUSSIONS

In all 16 treatments, after 10-14 days of explant culture, callus induction signs were appear. Calli color varied from pale yellow to light green. After 20 to 24 days of explant culture, green buds or somatic

embryos on callus masses were detected. After two months of culture (One months after subculture), in 16 treatments, 5 treatments was the best callus eligible in order to transfer to regeneration medium. This 5 treatments consisted of: N, M, B, P, G. (Table 1).This 5 treatments meet the highest average number of regenerated green buds on the callus. Results demonstrate the importance of auxin as same as the cytokinin on callus induction and callus sprouting in asparagus plant which is coincidence with *Bojnauth* And colleagues results . In all 5 treatments mentioned above, the only treatment which has never been used the auxine was B. The mean comparing table (Table 2) confirmed that the statistical difference between P treatments and other treatments was significant at the 5% level, but the differences were not significant between the other four treatments.

Table 2: Duncan's Multiple Range table. Shoot number per explants

Treatment	Mean of shoots per explant
P	22 A
N	B 7.2
M	B 6.3
B	B 5.8
G	B 5.3

The study of regenerated calli in regeneration medium indicated that the 2th treatment (1.25 mg/lit 2,4-D) was the best one in order to root regeneration. In this treatment , root regeneration percentage was 52% and this is meet the *Bojnauth* et al and *Reuther* et al results . However, according to table 3 , the percentage of root production in treatment 1 is Only 27% , So , there was a significant differences between this treatments in 5% .

Table 3 : Duncan's Multiple Range table. Average of root regeneration on callus

Treatment	Mean of root production on callus
1	B 27 %
2	A 52%

The outcome of the result analysis, indicate that in order to suitable callus induction and plant regeneration and also produce strong roots in the asparagus plant, the combination of auxin and cytokinin are necessary. So, production of regenerated plants with just one of the plant regulators (Auxine or Cytokinin) is not successful and this is meet by the results of *Bojnauth et al* and *Reuther et al* studies. Therefore, it is recommended that, to increase the percentage of plant regeneration and rooting of asparagus, different concentrations of plant growth regulators and different explants of asparagus should be examine.

Figure 2 : Root regeneration on callus in Asparagus sp.

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