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Investigation of pistachio micro-propagation (proliferation) *(pistacia vera L.)*

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Abstract

Tissue culture and micro-propagation is a branch of applied sciences in plant biotechnology which is of critical significance these days. This has played a great role in increasing different species of tree, shrub, herbaceous, etc. It merits some advantages such as ease of propagation, maintenance of genetic stability, lack of time limitation and place. In this research, pistachio rootstock (Pistacia vera L.) micro propagation has been selected due to its prevalence in Iran, the heavy cost of propagation through mating, the spread of common diseases of propagation by the current propagation methods and specially the market's demand for asexual propagation. In this study, some media cultures including knop, ½ knop, Ms, ½ Ms, Dkw, ½ Dkw were investigated in factorial statistical design with two hormones called BAP in four levels (0.5, 1, 1.5, 2) mg/l and NAA in Tri levels (0,0.1,0.2) mg/l and by Tri repetition. Results showed that most suitable treatment for explant (shoot tip) sterilization of samples is using %96 ethanol and %15 vaitex and washing with water, which then was cultured for proliferation. Also, the results showed DKW media is more suitable relative to others media and high dose of BAP (2 mg/l) and low dose of NAA (0 mg/l) in proliferation is suitable.

Keywords: pistachio, Rootstock, Tissue culture, micro propagation, proliferation.

Introduction

The plant growth regulators of cytokinin group such as BAP, BA branching production, proliferation and the plant growth regulators of auxin group such as NAA,IBA in rooting stage production of micro propagation of wood trees with compounds of alchaloidi in production to other of growth regulators of mentioned group lead to better results(Darvishian,2008; Stiff and Robert,2005; Arvin, 2002). The environmental culture which is usable in micro propagation of the trees which have been taken including: knop, Nigra, Ms, Dkw and the most common organ for explant of micro propagation is bud. In order to explant sterilization of micro propagation sample we can use %96 etanol and %15vitex and bleach (Villalabos and Leung,2004).The present research is done to find out the best environmental culture and also kind and concentration of the plant growth regulator in proliferation production of domestic pistachio micro-propagation .

Materials and methods

In order to separate and prepare the explants, the best terminal buds of tissue culture plants were selected and then shoot tip of terminal bud was separated. For shoot tip sterilization of samples %96 etanol and %15 vitex were used and then they were washed with water. Next, after making the suitable environmental culture for micropropagation including MS, ½ m/s, Dkw, ½ Dkw, Knop, ½ Knop and division 20cc of environmental culture to testing pipes, the samples under the pressure of

one atmosphere under 120 centigrade, autoclave and then the environments culture are kept in refrigerators in order to be used for culture of explants in the due time. These including six types of environmental culture such as MS, ½ MS, knop, ½ knop, Dkw, ½ Dkw were investigated in factorial statistics with two plant growth regulators such as BAP in four levels (0/5,1,1/5,2) milligram per liter and NAA in three levels (0,0/1,0/2) milligram per litre, with three repetitions were studied.

Table 1.The regulators of plant growth in shooting stage milligram per litre

BAP / NAA	a1 0/5	a2 1	a3 1/5	a4 2
0 b1	a1 b1	a2 b1	a3 b1	a4 b1
0/1 b2	a1 b2	a2 b2	a3 b2	a4 b2
0/2 b3	a1 b3	a2 b3	a3 b3	a4 b3

The dishes which are cultured in the growth chamber with lighting of 300 lux with flu resent lamps were lit for 16 hours on and 8 hours off under 25 ºC and the darkness temperature of 18 ºC were inept. Based on the conducted research and the necessity for sub culture in order for the provocation and triggering the growth of shoots, one stage of subculture was carried with one week interval. The environments subculture was unchanged with the same hormone compounds and the amount of growth in different stages of culture, the number and length of shoots of explants, using the loop of growth measures as well as the pollution and the process of growth of explants were recorded. To evaluate the effect of treatments in the rate of growth and the conditions of samples using the amount of obtained growth from the first record and the last in the sub culture process, the impact of each of the treatments in the from of factorial statistics using the software package of SAS was investigated and analyzed.

Results and discussion

Based on the results of variance of the models used, the effect of each of the special treatments was estimated and the environment of culture Dkw to other environments was preferred (tables 2,3).

Table 2. Analysis of variance on the plant growth regulators and interactions the environmental culture of Dkw

F	Mean square	Total square	df	Source
1/77	0/135	0/403	3	NAA) a(
0/71	0/054	0/108	2	BAP) b(
2/08	0/158	0/95	6	Interaction a*b

Table 3. Analysis of variance on the plant growth regulators and interactions in the environmental culture of 1/2 Dkw

F	Mean square	Total square	Df	Source
0/8	0/02	0/08	3	NAA) a(
0/66	0/02	0/04	2	BAP) b(
1/19	0/044	0/2	6	Interaction a*b

The effect of BAP hormone and interaction from the cytokinin group in the first stage of shooting is more than NAA hormone from the auxin group. According to figures and results, the effect of BAP hormone and its interaction with NAA in 5% level was meaningful. But the effect of NAA was not statistically significant especially the interaction of BAP and NAA was of greater growth in the evaluation of the model which showed that adding NAA in environmental culture was the factor in the inhibitor of BAP absorption which finally leads to the inhibitor of growth and stops it. Accordingly, the results show that high usage of hormones of auxin groups must be done with care in the shooting stage.

Generally, the statistics show that the use of BAP 2milligrams per litre with the low level of NAA,(0) milligram per litre has a more proper growth in the environmental culture, Dkw (table 2). The method of the changes in the average of growth and deviation of the observation in each of the treatments in the Dkw is shown in figure 1(figure 1) . As it is shown in table 3, in Dkw the interactions of BAP and NAA are higher compared with other treatments but eventually the obtained results in comparison to 1/2Dkw was weaker. On the other hand, the increase of the Interactions of NAA and BAP in comparison with other treatments can strengthen the hypothesis while in the shooting stage, not using NAA was more effective. Figure 2 shows the changes of the growth mean and the level of deviation in the observation in each of the treatments was shown in the 1/2Dkw (figure 2).

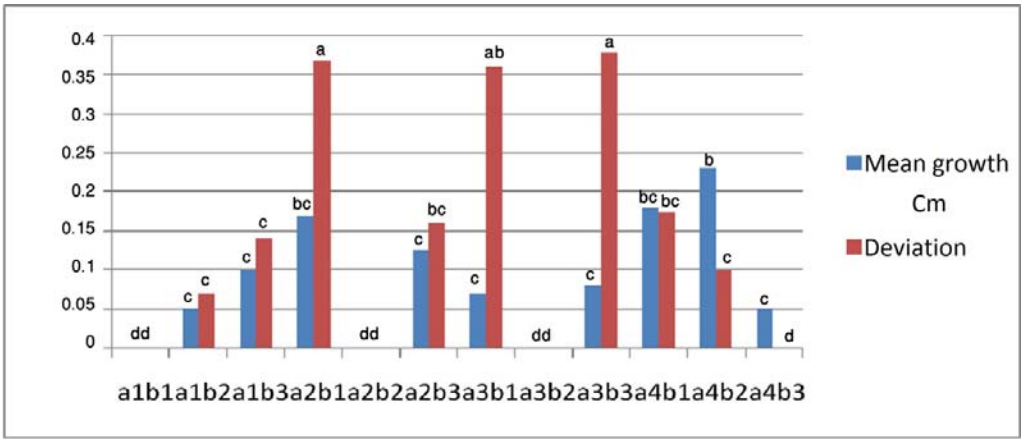

Figure 1.The growth mean and deviation in different treatments in Dkw

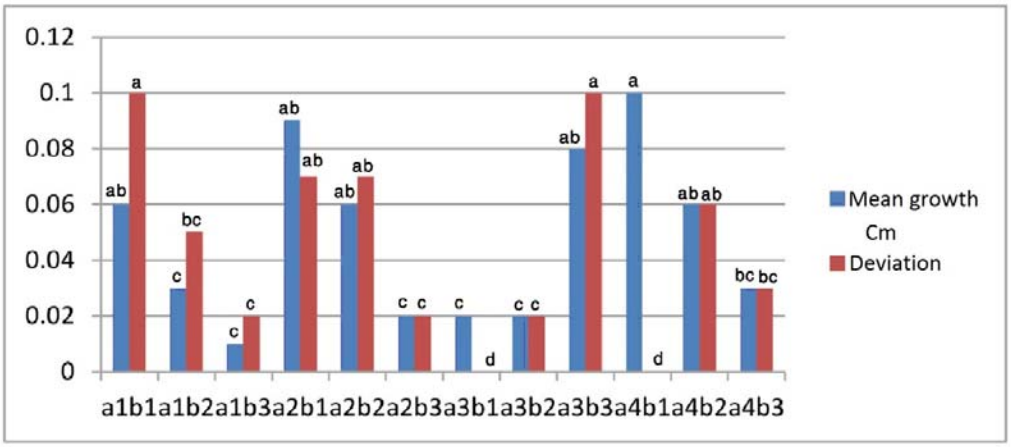

Figure 2.The growth mean and deviation in different treatments in 1/2Dkw

Conclusions

The results of this study show that Dkw is the best suitable environment for culture in the shooting stage. Moreover, among the different hormones regarding figure 1 and 2, the most growth belongs to a4b2, a4b1 including the amount of BAP and without NAA(a4b1) and the least amount of NAA(a4b2). The difference between these two results is related to the deviations and deviation of a4b1 was less. Finally, the best treatment for shooting stage in the micro propagation was Dkw with BAP(2mg/1) and without NAA(0 mg/1).

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