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EFFECT OF SOAKING PERIODS , GIBBERELLIC ACID , AND BENZYLADENINE ON PISTACHIO SEEDS GERMINATION AND SUBSEQUENT SEEDLING GROWTH (*Pistacia vera* L.)

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ABSTRACT

A nursery experiment was conducted in the College of Agriculture and Forestry, University of Mosul, Ninevah, Iraq to evaluate the effects of soaking periods (12 and 24 hours), Gibberellic Acid (0, 100 and 200 mg.l^{-1} GA_3) and Benzyladenine (0,50 and 100 mg.l^{-1} BA) on pistachio seeds germination and subsequent seedlings growth of *Pistacia vera* Ashoury cultivar.Soaking seeds for 12 hours in (200 mg.l^{-1} GA_3 + 100 mg.l^{-1} BA) showed the highest germination percentage (88.5%). Better seedling growth parameters (seedling height and diameter) were obtained with seed soaking in 200 mg.l^{-1} GA_3 for 12 hours, while the internode length was significantly improved by soaking seeds in 200 mg.l^{-1} GA_3 for 24 hours . Dry weights of seedling shoot and root were significantly increased by soaking seeds for 24 hours in (200 mg.l^{-1} GA_3+100 mg.l^{-1} BA) and 24 hours in (200 mg.l^{-1} GA_3), respectively.

INTRODUCTION

Pistacia is a genus of the Anacardiaceae family which are widely known trees and shrubs (Onay , 2000) . Pistachio seeds were used in commercial production for rootstocks (Hartmann *et al.* , 2002). Usually, pistachio don't propagate well from cuttings, so they are grafting or budding on various rootstocks due to difficulties in rooting of soft and hard cuttings (Sakoury , 1976 ; Onay , 2000). Seedlings rootstocks of *Pistacia vera* made more lateral roots and thicker stems than the other species and they can reach budding size in a shorter time (Ayfer *et al.* , 1990) .Ashoury is one of three main commercial Syrian pistachio cultivars which represents 85% of cultivars in Aleppo pistachio orchards (Padulosi and Hadj-Hassan , 1998 ; Tubeileh *et al.* , 2004).Gibberellins (GA) is an important endogenous growth regulator which has profound and diverse effects on plant growth and development. One of the roles of gibberellins is induction of seed germination and the promotion of radical elongation and mobilization of endosperm reserves during early embryo growth , as well as flower and fruit development (Hopkins , 1999 ; Peng and Harberd , 2002 ; Hartmann *et. al.* , 2002). Cytokinins is among the most important hormones in regulating cell division and has the capacity to initiate division in quiescent or nondividing cells, in addition to stimulating cell division , cytokinins also influence shoot and root differentiation in tissue culture , the growth of lateral buds, leaf expansion , chloroplast development and leaf senescence .

Benzyladenine (BA or BAP) is one of the synthetic cytokinins (Hopkins and Hüner , 2004). Many attempts have been made to use GA_3 for improving seed

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germination and subsequent seedlings growth of plants (Yousif, *et al*., 1984 on *Pistacia vera* L. seeds ; Al-Fawaier , 1994 on *Pistacia atlantica* Desf. seeds ; Khalil , 1997 on *Amygdalus arabica* Oliv. Seeds ; Beyhan , *et al.* , 1999 on hazelnut seeds ; Abdullah and Younis , 2002 on *P. atlantica* and *P. terebinthus* ; and Al-Imam and Al-Brifkany , 2006 (a&b) on three cultivars of hazelnut seeds), but there is a little information about the effects of seeds soaking especially in cytokinins and its combinations with GA_3 on seed germination and seedling growth of pistachio.The aim of the present study is to determine the effects of pistachio seeds soaking periods and different concentrations of GA_3 and cytokinins (BA) and their interaction for the improvement of the pistachio seeds germination and the subsequent seedlings growth .

MATERIALS AND METHODS

The mature seeds of *Pistacia vera* L. cultivar Ashoury were obtained from commercial Aleppo pistachio orchards on September 25, 2004 which is used in this experiment.The seeds were tested for viability before starting the experiment using the triphenyltetrazolium chloride procedure, the viability was more than 98% and the germination test was conducted in peat moss in greenhouse and germination, indexed by emergence of the radical through the testa, after three weeks at 20 ± 1^0C and 2.2 klx of fluorescent light according to Mathur *et al*. (1971) and, Bonamy and Dennis (1977). A factorial experiment was conducted to study three factors , the first was the two soaking periods, 12 and 24 hours, the second was three different concentrations of Gibberellic acid (GA_3) solutions (0,100 and 200 $mg.l^{-1}$), while the third factor was three concentrations of benzyladenine (BA) solutions (0,50 and 100 $mg.l^{-1}$) were used to study seeds germination percentage and subsequent seedlings growth . On March 1st , 2005 the pistachio seeds were sown at depth 2 to 3 cms. in black Polyethylen bag (20cm. in diameter and 60cm. length) using sand-loam soil as showed in table (1) as Page *et al*., (1982) .

Table (1): Some properties of experiment soil .

Soil pH	EC : $ds.m^{-1}$	Soluble cation M. m^{-3}		
		Ca^{++}	Mg^{++}	K^{+}
7.21	0.94	3.7	0.9	0.29
Soluble anions M. m^{-3}		available Fe	available N	Avalible P
Cl	HCO_3	Fe^{++}mg.Fe/Kg.soil	mg./Kg.soil	$M.m^{-3}$ /Kg.soil
3.0	3.04	1.17	0.45	2.39
Sand	silt	clay	organic matter	
471.5	390.1	138.4	9.4%	

Percentage of germination was recorded daily on the basis of cotyledon emergence during two months. Irrigation was applied as needed .When the experiment was ended on September 1, 2005 , the stem height , stem diameter of

five cm. above the soil surface , internode length and total dry weight of vegetative and root system were measured for all plants. Data were statistically analyzed using completely randomized design with three factors with four replicates (15 seeds per replicate). Analysis of variance and Duncan's multiple range test were used (Roger Mead and Hasted, 2003).

RESULTS AND DISCUSSION

Seeds germination(%) : Soaking seeds for 12 hours gave the highest seed germination percentage which significantly differ than seeds soaked for 24 hours (Table 2). Seeds germination percentage significantly increased with the increasing of the concentration of GA_3 and BA (Table 2). The highest seeds germination percentages were found when the seeds were soaked for 12 hours in 200 $mg.l^{-1}$ GA_3, and for 12 hours in 100 $mg.l^{-1}$ BA (Table 3) , and for 12 hour in 200 $mg.l^{-1}$ GA_3 + 100 $mg.l^{-1}$ BA (Table 4) which was 88.50%. This increase in seeds germination percentage might be related to the initial enzyme induction and to the activation of reserve food – mobilizing systems by Gibberellins which have also been used to enhance

Table (2): Effect of soaking periods , Gibberellic acid and Benzyladenins concentrations on seed germination and seedling growth parameters.

Growth Parameters	Soaking Periods (hr)		Gibberellic acid ($mg.l^{-1}$)			Benzyladenins ($mg.l^{-1}$)		
	12	24	0	100	200	0	50	100
Seed Germination(%)	77.04 a	74.22 b	71.32 c	76.70 b	78.88 a	69.57 c	74.85 b	82.48 a
Seedling height (cm)	16.18 a	16.14 a	13.42 c	17.13 b	17.93 a	17.92 a	17.92 a	15.60 b
Seedling diameter (mm)	3.25 a	3.07 b	2.74 c	3.13 b	3.61 a	3.24 a	3.18 b	3.07 c
Internode length (cm)	1.18 b	1.27 a	1.07 c	1.25 b	1.37 a	1.26 a	1.25 a	1.17 b
Vegetative dry weight (g)	9.49 b	18.65 a	11.32 c	14.76 b	16.12 a	12.01 c	14.18 b	16.02 a
Root dry weight (g)	7.55 b	13.18 a	8.99 c	10.31 b	11.79 a	9.26 c	10.50 b	11.35 a

The same letter with row indicates that there is no significant difference ($p<0.05$)

germination and stimulate carly seedling emergence and growth (Hopkins and Hüner , 2004). In spite of cytokinins dose not appear essential for seed germination but during germination , cytokinins appear to offset the effect of inhibitors, notably ABA . It has been described , as playing a permissive role in germination in allowing gibberellins to function (Hartmann *et al.*, 2002). Hopkins and Hüner (2004) stated that Gibberellins prominently involved in seed

germination and mobilization of endosperm reserves during early embryo growth as well as flower and fruit development.

Seedling Growth Parameter : Pistachio seeds soaking for 12 hours significantly increased seedlings diameter as compared with seeds soaking for 24 hours (Table 2), while internodes length increased significantly at 24 hours soaking periods as compared with seeds soaked for 12 hours (Table 2). The three seedlings growth parameters, seedling height, seedling diameter and internodes length were significantly increased with increasing the concentrations of GA_3 and decreased with increasing the

Table (3): Interaction effects of soaking periods, gibberellic acid and benzyladenins on seed germination and seedling growth parameters.

Treatments	Seed germination (%)	Seedling height (cm)	Seedling diameter (mm)	Internode length (cm)	Vegetative dry weight (g)	Roots dry weight (g)
Interaction effect of soaking periods and Gibberellic acid.						
12 hrs + 0 GA_3	72.23d	13.10 f	2.71 d	1.03 d	8.63 f	6.75 f
12 hrs+100 GA_3	77.83 b	16.73 d	3.13 c	1.15 b	9.60 e	7.83 e
12 hrs + 200GA_3	81.07 a	18.70 a	3.92 a	1.36 a	10.51 d	8.08 d
24 hrs + 0 GA_3	70.40 e	13.73 e	2.77 d	1.08 c	14.28 c	11.24 c
24 hrs + 100GA_3	75.57 c	17.53 b	3.13 c	1.36 a	19.92 b	12.80 b
24 hrs + 200GA_3	76.70 bc	17.17 c	3.31 b	1.37 a	21.73 a	15.51 a
Interaction effect of soaking periods and Benzyladenins.						
12 hrs + 0 BA	70.17 e	18.00 a	3.29 a	1.13 d	8.24 f	5.12 f
12 hrs + 50 BA	76.10 c	15.57 b	3.19 b	1.30 b	8.57 e	8.51 e
12 hrs + 100 BA	84.87 a	14.97 c	3.28 a	1.03 d	11.66 d	9.03 d
24 hrs + 0 BA	68.97 f	17.83 a	3.19 b	1.38 a	15.77 c	13.40 b
24. hrs + 50 BA	73.60 d	15.63 b	3.17 b	1.20 c	19.79 b	12.48 c
24 hrs + 100 BA	80.10 b	14.97 c	2.85 c	1.23 c	20.37 a	13.66 a
Interaction effect of Gibberellic acid and Benzyladenins concentrations						
0GA_3 + 0 BA	64.40 g	16.05 e	2.69 g	1.10 e	10.25 g	7.35 h
0GA_3 + 50 BA	70.65 F	13.20 F	2.95 F	1.13 e	11.48 f	9.61 f
0GA_3 + 100 BA	78.90 C	12.00 g	2.59 h	0.93 g	12.23 e	10.03 e
100 GA_3 + 0 BA	70.80 F	18.50 b	3.23 d	1.26 c	11.59 f	8.57 g
100 GA_3 + 50 BA	76.20 d	17.25 c	3.13 e	1.32 b	11.48 f	11.19 c
100 GA_3 +100 BA	83.10 b	16.00 d	3.04 ef	1.19 d	17.14 b	11.18 c
200 GA_3 + 0 BA	73.50 e	20.55 a	3.81 a	1.41 a	14.19 d	11.87 b
200 GA_3 + 50 BA	77.70 c	16.35 d	3.46 c	1.31 bc	15.52 c	10.69 d
200 GA_3 + 100 BA	85.45 a	16.90 c	3.57 b	1.39 a	18.67 a	12.83 a

A same letter in the column indicates that there is no significant difference ($p<0.05$)

concentrations of BA (Table 2). Results in (Table 2and 3) showed that the highest seedling height, seedling diameter and internode length were found when the seeds soaked for 12 hours in 200 $mg.l^{-1}$ GA_3 (Table 3) and seeds soaked for 12 hours in (200 $mg.l^{-1}$ GA_3 + O $mg.l^{-1}$BA) except internode length which was superior in (200 $mg.lit^{1}$ GA_3 +50 $mg.lit^{-1}$BA) (Table 4) .

This increase in seedling growth parameters with GA might be related to the fact that GA promote stem and shoot elongation through the increase of both cell division and from internodes elongation in higher plant (Harmann *et al*., 2002 ; Hopkins and Hüner (2004) ; Harris *et al.,* 2004). The role for application of cytokinins is to detached leaves will delay the onset senescence , stimulating protein synthesis and maintain protein levels, prevent chlorophyll breakdown , employing recombinant DNA techniques and cytokinins direct nutrient mobilization and retention by stimulating metabolism in the area of cytokinins application (Hopkins and Hüner , 2004).

Seedlings dry weight : Significant increase in shoot and root dry weights of pistachio seedling occurred under 24 hours seed soaking period and with increasing GA_3 and BA concentrations (Table 2). The highest shoot and root dry weights were found when seeds soaked for 24 hours in 200 $mg.lit^{-1}$ GA_3 or 100 $mg.lit^{-1}$ BA (Table 3),

Table (4): Interaction effect of Soaking periods, Gibberellic acid and Benzyladenins concentration on seed germination and seedling growth parameters**.**

Treatments	Seed germination (%)	Seedling height (cm)	Seedling Diameter (mm)	Internode length (cm)	Vegetative Dry weight (g)	Roots dry weight (g)
12 hrs+0 GA_3+0 BA	65.40 i	13.00 i	2.41 j	0.93 h i	7.09 k	3.79 k
12 hrs+0 GA_3+50 BA	70.60 i	13.20 i	2.94 g h	1.18 d	8.53 j	8.20 h
12 hrs+0 GA_3+100 BA	80.70 c d	13.10 i	2.78 i	0.97 g h	9.47 h	8.26 h
12 hrs+100 GA_3+0 BA	70.30 i	18.70 b	3.16 e f	1.13 d e	8.55 j	5.21 j
12 hrs+100 GA_3+50 BA	77.80 e	16.30 g	3.04 f g	1.27 c	8.98 i	9.32 g
12 hrs+100 GA_3+100 BA	85.40 b	15.20 h	3.20 d e	1.04 g	11.27 g	8.95 g
12 hrs+200 GA_3+0 BA	74.80 g	22.30 a	4.30 a	1.33 b c	9.80 hi	6.35 i
12 hrs+200 GA_3+50 BA	79.90 d	17.20 de	3.59 c	1.46 a	8.21 j	8.01 h
12 hrs+200 GA_3+100 BA	88.50 a	16.60 f g	3.88 b	1.30 b c	14.25 e	9.88 f
24 hrs+0 GA_3+0 BA	63.40 i	17.10 def	2.96 g h	1.27 c	13.40 f	10.91 e
24 hrs+0 GA_3+50 BA	70.70 h	13.20 i	2.96 g h	1.08 e f	14.43 e	11.02 e
24 hrs+0 GA_3+100 BA	77.10 e f	10.90 j	2.40 j	0.89 j	15.01 d	11.80 d
24 hrs+100 GA_3+0 BA	71.30 h	17.60 d	3.30 d e	1.38 b	14.63 d e	11.92 d
24 hrs+100 GA_3+50 BA	74.60 g	18.20 c	3.22 d e	1.36 b	22.12 b	13.06 c
24 hrs+100 GA_3+100 BA	80.80 c d	16.80 f g	2.88 h i	1.33 b c	23.01 a	13.41 c
24 hrs+200 GA_3+0 BA	72.20 h	18.80 b	3.32 d	1.49 a	19.29 c	17.38 a
24 hrs+200 GA_3+50 BA	75.50 f g	15.50 h	3.34 d	1.15 d e	22.82 a	13.37 c
24 hrs+200 GA_3+100 BA	82.40 c	17.20 d e	3.26 d e	1.48 a	23.09 a	15.77 b

A same letter in the column indicates that there is no significant difference ($p<0.05$)

and 24 hours in 200$mg.l^{-1}GA_3$ + 100 $mg.l^{-1}BA$ for vegetative weight only (Table 4). This increase in dry weight of vegetative and root might increase the seedlings growth. These results are in agreement with those found by (Al-Fawair, 1994 , Al-Imam and Al-Brifkany , 2006 , c) .

تأثير فترات النقع وحامض الجبرليك والبنزايل أدينين في إنبات بذور الفستق الحلبي والنمو اللاحق للشتلات (*Pistacia vera* L.)

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الخلاصة

نفذت تجربة عاملية في مشتل كلية الزراعة والغابات ، جامعة الموصل / العراق. لتقييم تاثير فترات النقع لمدة ١٢ و ٢٤ ساعة في محاليل من تراكيز مختلفة لحامض الجبرليك (GA_3) صفر و ١٠٠ و٢٠٠ ملغم.لتر$^{-1}$ والبنزايل ادينين (BA) صفر و٥٠ و١٠٠ ملغم . لتر$^{-1}$ في نسبة الإنبات والنمو اللاحق للشتلات البذرية للفستق الحلبي صنف عاشوري . بينت النتائج ان النقع لمدة ١٢ ساعة في محلول ٢٠٠ ملغم . لتر$^{-1}$ GA_3 +١٠٠ ملغم لتر$^{-1}$ BA حققت اعلى نسبة مئوية لانبات البذور والتي بلغت ٨٨% . تم الحصول على افضل صفات النمو المدروسة (ارتفاع الشتلات وقطر الساق الرئيسي) من خلال نقع البذور في ٢٠٠ ملغم . لتر$^{-1}$ حامض الجبرليك ولمدة ١٢ ساعة ، بينما بينت نتائج التجربة أن افضل طول للسلاميات من خلال النقع في ٢٠٠ ملغم . لتر$^{-1}$ حامض الجبرليك ولمدة ٢٤ ساعة . ولوحظ زيادة معنوية للوزن الجاف للمجموع الخضري والجذري من خلال نقع البذور في ٢٠٠ ملغم . لتر$^{-1}$ GA_3 + ١٠٠ ملغم . لتر$^{-1}$ BA والنقع لمدة ٢٤ ساعة في ٢٠٠ ملغم لتر$^{-1}$ GA_3 على التوالي .

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