

Indole-3-Butyric Acid and Naphthalene Acetic Acid Impacts on in Vitro Rooting of Mariana and Nemaguard Rootstocks

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Abstract: Conventional cuttings and microcuttings have a major problem in vegetative propagation of many plant species (especially the woody plants) represented by adventitious root formation. This study investigates the effects of Indole-3-Butyric Acid (IBA) alone and in combination with Naphthalene Acetic Acid (NAA) with half strength MS medium on in vitro rooting of Mariana and Nemaguard rootstocks. Optimum percentage of rooting (100%) was observed on media supplemented with 1.5 mg/l IBA for both rootstocks. For Mariana rootstock, the best result for root number (2.20) was obtained on 1.5mg/l IBA in combination with NAA 0.1 mg/l, while root elongation average (28.72mm) was stimulated at 1.5mg/l IBA and 0.2 mg/l NAA. For Nemaguard rootstock, the inclusion of 1.5mg/l IBA together with 0.1 mg/l of NAA in the culture medium significantly enhanced root number (2.50). The root length was the maximum (29.37mm) with the application of 1.5 mg/l IBA solely.

I. Introduction

Plums and prunes are belonging to *Prunus* genus, *Prunoideae* subfamily and *Rosaceae* family, plum rootstocks have the most diversity and are more compatible to the diverse climatic and soil condition, as well as resistance to some diseases and nematode root nodes (1). There are a few studies about successful *in vitro* culture of woody plants compared to other herbaceous plants In spite of the importance of stocks of fruit stone trees cultivation (2). Selection of appropriate medium, plant growth regulators and other components that stimulate the required response is very important (10). Thus it's not easy to establish a uniform procedure that can be applied to propagation within and between species, thus the influence of the medium should be studied for each genotype separately (6). Some authors recommended adding IBA into the MS (17) medium as a means to enhance root initiation and elongation (14), while some others posit that supplementation by different auxins like IBA and NAA can increase the number of rooted plants as well as the number and length of roots (13). Fotopoulos and Sotiropoulos (2005) reported achieving 100% rooting in PR 204/84 rootstock using half strength MS medium with 2.5 μ M IBA. The aim of this investigation was to study the effect of IBA and NAA on rooting of mariana and nemaguard rootstocks on *in vitro* condition.

II. Methods and Materials

This research was implemented in the laboratory of plant tissue culture, department of Horticulture-Faculty of Agriculture, University of Baghdad, from October 2015 until February 2016. Firstly, young stem cuttings from the field of both mentioned rootstocks were excised into 5 cm long sections and washed for 30 minutes with tap water, then were disinfected in laminar flow chamber by using commercial Clorox at 3% for 10 minutes and finally these explants were rinsed three times with distilled water (11).

For culture initiation, stem cuttings (approx. 4 cm long) of Mariana and Nemaguard rootstocks were cultured on MS basal medium, supplemented with 2 mg/l BA, then the multiplication was achieved through transferring the initiated shoots to full strength MS medium supplemented with 2.5 mg/l BA and 0.3 mg/l IBA. For rooting, plants were placed in MS hormone-free medium for 4 weeks in order to improve shoot elongation. 3 cm –long shoots from previous culture were transferred to 1/2 MS medium supplemented with IBA (0.0, 0.5, 1.0 and 1.5mg/l) in combination with the NAA (0.0, 0.1, 0.2 and 0.3 mg/l). All cultures were incubated in 26 \pm 1 $^{\circ}$ C with 16 hours photoperiod and 1000 lux of light intensity. Observations were performed after six weeks include the rooting percentage, number of roots and total root length.

Statistically analysis:

This experiment was carried out based on Completely Randomized Design (CRD), each treatment was done in 10 replications. SPSS 16 software was used for statistically analysis of the data, and differences among means of treatments were compared by using (LSD) Least Significance Design (21).

III. Results and Discussion

Rooting Percentage (RP): root regeneration started after 6 weeks after shoots were transferred to root induction media for both rootstocks. The dose of auxin is known to be critical in root induction (3, 12) optimum percentage of rooting (95%) was observed on media supplemented with 1.5 mg/l IBA. Highly significant ($P \leq 0.05$) interaction between the genotype and auxin was noticed, for Mariana, the highest (RP) (100%) was on media supplemented with 1.5 mg/l IBA. For Nemaguard, the highest (PR) (68%) was with 0.3 mg/l NAA. The interaction between genotype, auxin and hormonal concentration was highly significant for (RP). For Mariana, the highest percentage (100%) was observed on media supplemented with 1.5 mg/l IBA and 0.0, 0.1, 0.2 or 0.3 mg/l NAA. For Nemaguard, the optimum percentage of rooting (90%) was on media with 1.5 mg/l IBA with 0.0 or 0.1mg/l NAA. (table1)

Table 1: Influence of genotype, auxin and concentration on rooting percentage of Mariana and Nemaguard rootstocks:

Genotype X IBA conc.	NAA conc. mg/l				IBA conc. mg/l	Genotype	
	0.3	0.2	0.1	0.0			
0.22	0.40	0.30	0.17	0.00	0	Mariana	
0.55	0.60	0.40	0.60	0.60	0.5		
0.45	0.60	0.40	0.40	0.40	1.0		
1.00	1.00	1.00	1.00	1.00	1.5		
0.35	0.50	0.40	0.41	0.00	0	Nemaguard	
0.58	0.80	0.60	0.40	0.50	0.5		
0.58	0.60	0.70	0.50	0.50	1.0		
0.90	0.80	0.80	1.00	1.00	1.5		
Genotype							
0.56	0.65	0.53	0.54	0.51	Mariana	Genotype X NAA conc.	
0.60	0.68	0.62	0.58	0.51	Nemaguard		
Aver. of IBA conc.							
0.29	0.45	0.35	0.29	0.00	0.00	IBA conc. X NAA conc.	
0.56	0.70	0.50	0.50	0.55	0.50		
0.51	0.60	0.55	0.50	0.45	1.00		
0.95	0.90	0.90	1.00	1.00	1.50		
	0.66	0.58	0.56	0.51	Aver. of NAA conc.		
Genotype X NAA = 0.19 Genotype X IBA = 0.29 Genotype X NAA x IBA = 0.39					Genotype = 0.90 IBA = 0.13 NAA = 0.13 Genotype X IBA = 0.19		L.S.D 0.05

Exogenous auxins such as IBA and NAA can induce root formation in tissue culture, these auxins and their interactions with internal auxins which cannot be adequately synthesized by isolated tissues and small organs *in vitro* (22), also their need is limited at the early stages of the new roots emergence (5).

IBA superiority over other auxin sources in *in vitro* root formation due to its stability and slight sensitivity to degradation of enzymes (18). Our results regarding with adventitious root formation by exogenous auxin are in agreement with the findings of other investigators in other plant species (4, 8, 15, 20).

Roots number:

Based on the results gained (table2), genotype is one of the factors affecting the number of roots per shoot, for Mariana, the highest number was 1.26. while the maximum average (0.89) was recorded for Nemaguard. Highly significant ($P \leq 0.05$) interaction between IBA and NAA concentrations was observed, the average of roots number was ranged from 2.34 in 1.5 mg/l IBA together with 0.1 mg/l NAA to 0.62 root per shoot in 0.2 mg/l NAA.

Results in table 2 showed that the interaction between genotype, auxin and concentration varieties was significant for root number, the Mariana and Nemaguard shoots cultured on 1/2 MS media supplemented with 1.5 IBA + 0.1 mg/l NAA gave the highest number (2.5) (2.2) root/shoot respectively.

Table 2: Influence of genotype, auxin and concentration on root number of Mariana and Nemaguard rootstocks:

Genotype X IBA conc.	NAA conc. mg/l				IBA conc. mg/l	Genotype
	0.3	0.2	0.1	0.0		
0.88	1.30	0.90	1.17	0.00	0	Mariana
1.00	1.90	0.90	0.60	0.60	0.5	
1.38	1.70	1.90	1.20	0.70	1.0	
1.78	1.70	1.30	2.20	1.90	1.5	
0.21	0.40	0.30	0.17	0.00	0	Nemaguard
0.78	0.60	0.60	1.00	0.90	0.5	
0.65	0.60	0.60	0.70	0.70	1.0	
1.93	2.40	1.50	2.30	1.30	1.5	
Genotype						
0.89	1.00	0.75	1.08	0.73	Mariana	Genotype X NAA conc.
1.26	0.82	1.30	1.25	1.65	Nemaguard	
Aver. of IBA conc.						
0.56	0.87	0.62	0.68	0.00	0	IBA conc. X NAA conc.
0.89	1.25	0.75	0.79	0.75	0.5	
1.01	1.15	1.25	0.94	0.70	1.0	
1.85	2.05	1.40	9.34	1.60	1.5	
	1.33	1.00	1.19	0.78	Aver. of NAA conc.	
Genotype X NAA = 0.41 Genotype X IBA = 0.59 Genotype X NAA x IBA = 0.84				Genotype = 0.20 IBA = 0.29 NAA = 0.29 Genotype X IBA = 0.41		L.S.D 0.05

Combination of various auxins scored better responses in many crops which show sever rooting, many investigators have reported that the IBA in combination with NAA have a better impact on promoting adventitious root formation in comparison to IBA solely (9, 12, 16, 19,23).

Root length:

From table 3, root length was significantly affected by interaction of genotype and growth regulators, Mariana rootstock shoots gave highest average (16.51 mm) when cultured on 1/2 MS medium containing 1.5mg/l IBA, while they produced root with (11.82 mm) length on 1/2 MS media supplemented with 0.3 mg/l NAA.

It is important to note the interaction of auxin and hormonal concentrations, highest average (19.31 mm) was obtained from the treatment (1.5 mg/l IBA+ 0.2 mg/l NAA).

Highly root length was observed between the interaction genotype, auxin type and hormonal concentrations producing maximum root length (28.72 mm) when Mariana shoots cultured on 1/2 MS medium supplemented with 1.5 mg/l IBA. For Nemaguard, maximum shoot length was seen (29.37 mm) in treatment containing 1.5 mg/l IBA which proved to be the best treatment.

Table 3: Influence of genotype, auxin and concentration on root length of Mariana and Nemaguard rootstocks:

Genotype X IBA conc.	NAA conc. mg/l				IBA conc. mg/l	Genotype
	0.3	0.2	0.1	0.0		
1.13	2.08	1.15	0.85	0.00	0	Mariana
7.24	8.50	5.69	7.92	6.96	0.5	
6.22	8.49	5.69	5.14	5.63	1.0	
16.17	28.19	28.72	1.44	6.41	1.5	
1.34	1.75	1.33	1.94	0.00	0	Nemaguard
4.25	6.01	4.67	2.11	4.42	0.5	
7.52	4.19	7.83	8.24	9.19	1.0	
16.51	7.77	9.86	19.15	29.37	1.5	
Genotype						
7.70	11.82	10.31	4.01	4.65	Mariana	Genotype X NAA conc.
7.40	5.11	5.92	7.87	10.97	Nemaguard	
Aver. of IBA conc.						
1.08	1.84	1.17	1.34	0.00	0	IBA conc. X NAA conc.
5.77	7.28	5.20	5.04	5.66	0.5	
6.90	6.73	6.78	6.72	7.43	1.0	
16.36	18.00	19.31	10.32	17.91	1.5	
	8.46	8.12	5.94	7.81	Aver. of NAA conc.	
Genotype X NAA = 2.51 Genotype X IBA = 3.62 Genotype X NAA x IBA = 2.15				Genotype = 1.25 IBA = 1.78 NAA = 1.78 Genotype X IBA = 2.51		L.S.D 0.05

In conclusion, the results of Nemaguard rootstock experiment indicate that 1/2 MS medium supplemented with 1.5 mg/l IBA solely proved to be the best treatment in cases of RP and length of root. 1/2 MS medium supplemented with 1.5mg/l IBA plus 0.1 mg/l NAA resulted in desirable results in case of root number and for both studied rootstocks.

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