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Influence of Growth Regulators on Organogenesis of Unshiu mandarin

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ABSTRACT: Virus-free plants is very important in citrus breeding program. This study was conducted to evaluate the Influence of growth regulators on Unshiu mandarin (Citrus unshiuowari) seed rganogenesis at in vitro conditions in 2014, in a completely randomized design with three replications. In this study treatments, including 9 levels of hormones (control, BA-1, BA-2, GA-1, GA-2, IBA-0.25, IBA-0.5, NAA-0.25, NAA-0.5), respectively. In order to measure traits during development stages of randomly sampling and measurement were conducted and analyzed. Most of the highest (3.25 and 3.05 cm)to 2 mg/l GA and 1 mg/l GA respectively, the maximum length of the root (7.12 and 7.02 cm) to 0.5 mg/l NAA and IBA, the largest number of root (9.75) to 0.5 mg per liter of IBA, the largest number of shoot (1.5) corresponds to 2 mg/l and BA, the highest percentage of callus induction (70%) of the NAA was 0.5 mg .

Keyword: Unshiu mandarin, Callus induction, GA, Germination.

INTRODUCTION

Citrus is one of the most important commercial crops of the world valued for its juice and other by products (Ramdan *et al*., 2014). Many studies were undertaken with the aim of controlling the techniques being able to lead in the genetic improvement of the selected citrus fruit species (Chakravarty *et al*, 1999). Establishment of an efficient callus induction protocol is an essential prerequisite in harnessing the advantage of cell and tissue culture for genetic improvement. For the successful application of the tissue culture technique in crop breeding, callus growth and plant regeneration potential of each crop must be determined (Khaleda *et al*, 2006; Altaf *et al*, 2009).Citrus Embryos explants were most responsive to callus induction and proliferation (Alka, 2010). Thus, for biotechnological research on citrus, a reliable callus induction protocol using embryos is essential. Many of the changes in the embryo and endosperm during seed maturation and imbibition described above are controlled by plant hormones such as ABA and GA. Changes in hormone concentrations in seed play in critical role in the suppression and promotion of germination programs. Vivipary precocious germination from seed that are still developing on the maternal plant, is a good example of the consequence of aberrant hormone concentrations in seeds. In this section the mechanisms of hormone level regulation and signal translation are discussed (Chang *et al*, 2009).

For hormones, the effect of auxin on shoot regeneration was rarely concerned, though the main hormone effect on bud formation was due to the addition of BAP (Garcia *et al*., 1999). Almeida *et al*. (2003) recorded maximum number of shoots when epicotyl segments were cultured on regeneration EME medium supplemented with 25 g/l additional sucrose and 1 and 2 mg/l BAP for sweet orange and rangpur lime, respectively. Among cut modes, transversal cut, the most popular cut mode (Moore *et al*., 1992; Pena *et al.*, 2004) is simple to manipulate but produces the fewest adventitious buds. Longitudinal cut, a newly developed but infrequently used cut mode produced the most adventitious buds (Kayim *et al*., 2004). The goal of this study was to find efficient explant source, cut mode and optimize hormone combination for organogenesis of Unshiu mandarin.

MATERIAL AND METHOD

A. Plant materials
In this study Unshiu mandarin seed were obtained from Center of Mazandaran, Iran.

B. Explants sterilization
Under the laminar flow cabinet. Seeds were immersed in ethanol 70% for 10 minutes, then in sodium hypochlorite solution 5% for1 minute and finally washed three times by sterilized distilled water.

C. Hormonal compounds

The experiment was conducted as completely randomized design with three replications.

Treatment	Hormonal compounds
control	0
T1	1 mg/l GA
T2	2 mg/l GA
T3	1 mg/l BA
T4	2 mg/l BA
T5	0.25 mg/l NAA
T6	0.5 mg/l NAA
T7	0.25 mg/l IBA
T8	0.5 mg/l IBA

Explants were cultured on Murashige and Skoog (MS)basal medium (Murashige & Skoog,1962) with 5% sucrose and 0.8% agar .The pH of MS medium was adjusted to 5.7 before autoclaving at 121°C for 20 min. Callus is initiated in the dark at 26 ± 1°C.

D. Statistical analysis

Analysis of variance (ANOVA) was carried out using SAS (SAS Institute, Cary, N.C.) and MSTATC (MSTATC, East Lansing, Mich.). Treatments were compared using the protected Duncan's multiple-range tests.

RESULT AND DISCUSSION

Germination of Citrus unshiuis very dependent on growth hormones. Analysis of variance showed that Hormones treatment was significant. The results of the analysis of variance showed in the Table 1.

Table 1: Results of Analysis of variance (ANOVA).

Callus induction	Number of shoot	Number of root	Length of the root	Length of the seedling	df	Source of variation
1156.94 **	0.611*	39.81*	22.68**	3.613*	8	Treatment
16.66	0.250	8.009	2.84	0.596	26	Error
					34	Total
10.49	12.06	14.07	15.32	20.60	-	CV

*,** Significant at 5% and 1% levels, respectively.

Length of seedling: Based on the analysis of variance significant differences between hormonal treatments for germination at 5% was observed. (Table 1). The mean size of 3.25 cm and 3.05 cm and the maximum length of seedling with 2 mg/lGA(GA-2) and 1 mg/l GA (GA-1), respectively. This indicates that gibberellic acid can have a significant impact on the increase during germination. (Fig. 1).

Karimi *et al* (2012) in the study of optimized direct regeneration of tissue culture and local oranges announced that BA with a concentration of 0.2 mg/l and 0.2 mg/l GA3 was no appreciable change in the size of shoot, but in the medium containing 0.5 mg/lBA to 0.5 mg /l GA3 with an average of 0.3 cm on each shoot showed elongation.

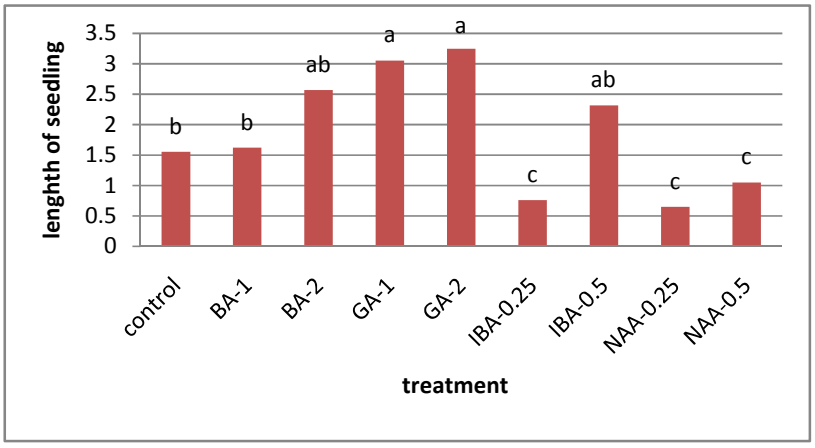

Fig. 1. The effect of hormone treatments on the length of seedling.

Length of the root: According to the analysis of variance examines the main root length, hormonal treatments had no significant effect at 1% (Table 1).Average effects of hormonal treatments, according to results of the comparison, the maximum length of the main roots of 0.5 mg/l NAA (7.12 cm) and 0.5 mg/l IBA (7.02 cm), respectively. While the minimum length of root (0.5 cm) to control (without hormones), respectively. This indicates that the use of NAA and IBA had a significant impact on root growth (Fig. 2).

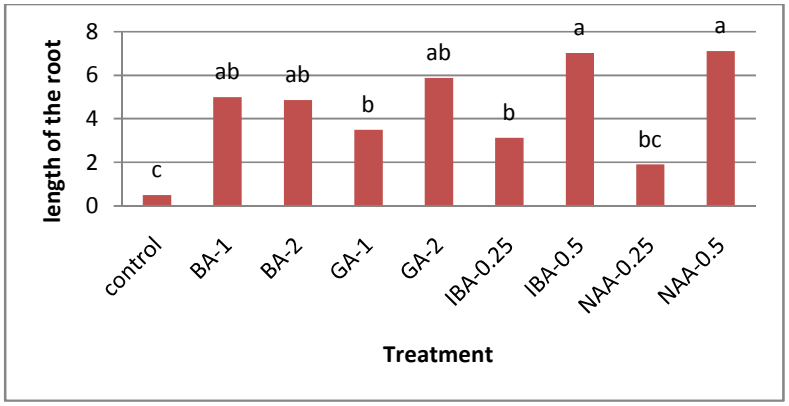

Fig. 2. The effect of hormone treatments on the length of root.

Number of root: According to the analysis of variance in this study, no significant differences in the level of 5% between hormonal treatments on the number of root (Table 1). The mean of the maximum number of root (9.75) to 0.5 mg/l IBA and the lowest number was 1.75 to the plant control.

Results showed that the hormone treatments had a significant impact in number of roots and the greatest impact on 0.5 mg/l IBA and 2 mg/l GA, respectively (Fig. 3).Similar results Karimi *et al* (2012) in the optimization of direct regeneration of tissue culture and local oranges announced.

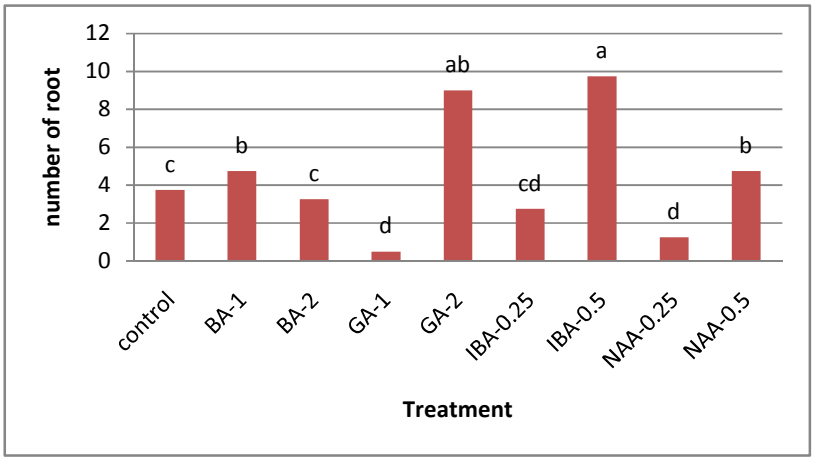

Fig. 3. The effect of hormone treatments on number of root.

Number of shoot: In accordance with the analysis of variance on the number of shoot, hormonal treatments had a significant effect on the level of 5% on the number of shoot (Table 1). The average of the number of shoot (1.5) corresponds to 2 mg/l BA. 6-Benzylaminopurine, of cytokinins and cytokinin is caused shoots (Fig. 4).This result reflects the impact of BA is as a cytokinin.

Comparison charts showing average is the best response was a concentration of 2 mg BA (Hiregoudar *et al*., 2005).lemon conducted a study on hormone BA alone was enough to produce adventitious shoots. In numerous reports as the most efficient hormone cytokinin BA effectively regenerate adventitious shoots are presented in citrus fruits (Costa *et al*., 2002).

Callus induction: The percentage of callus on hormone treatments were significantly affected (Table 1). Based on the mean percentage of callus, callus highest percentage (70%) of the NAA was 0.5 mg/l and the lowest percentage of callus of control (without hormones) with 12.5 percent. The results represent a dramatic impact on the level of one percent of the NAA hormone has been callus (Fig. 5). Pomelo regeneration showed the best callus on medium containing 5 mg/l NAA and 0.5 mg/l BA (100%) callus per explants (Rastgoo *et al*., 2009).

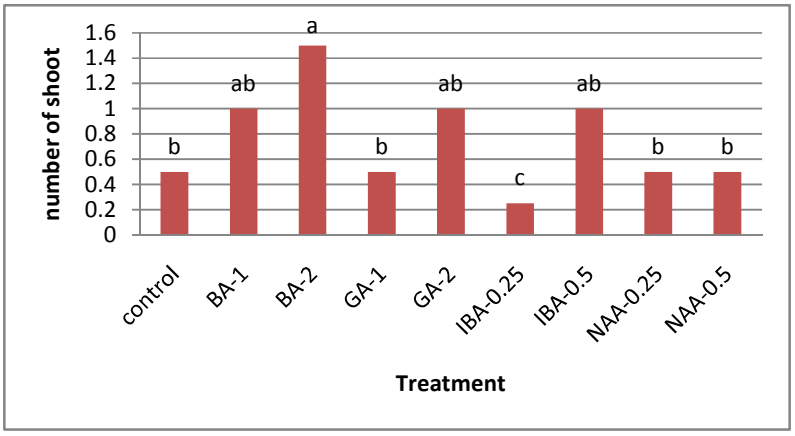

Fig. 4. The effect of hormone treatments on number of shoot.

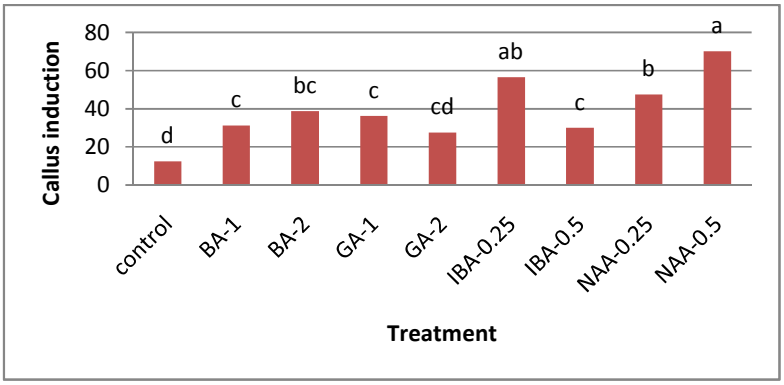

Fig. 5. The effect of hormone treatments on callus induction.

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