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Investigation of Changes in Phytohormone Levels Depending on Effects of Exogenous Indole-Butyric-Acid and Callus Formation in the Stem Cuttings of Some Apple Kinds (*Malus sylvestris* Miller)

[1]Aysel Sivaci and [2]İbrahim Yalçin
[1]Department of Biology, Faculty of Sinop Art and Science, Ondokuz Mayıs University, Sinop, Turkey
[2]Department of Biology, Faculty of Art and Science, Cumhuriyet University, Sivas, Turkey

Abstract: In this study, the effect of exogenous indole-butyric-acid and callus formation on the total indole-3-acetic acid, gibberellic acid, zeatin and abscisic acid levels in cuttings stems of apple (*Malus sylvestris* Miller) kinds (Golden delicious, Starkrimson delicious and Misket delicious) were determined the variations before and after the treatment. Total indole-3-acetic acid, gibberellic acid, zeatin and abscisic acid levels of the callus stems in the all of apple kinds were higher than those of non-callus forming. There was also increasing determined in indole-3-acetic acid, gibberellic acid, zeatin levels of calli treated with indole-butyric-acid (2000 and 3000 mg L^{-1}) and decreasing in abscisic acid.

Key words: Apple (*Malus sylvestris* Miller), callus, endogenous plant hormones, exogenous indole-butyric-acid

INTRODUCTION

The ability of propagation in stem cuttings promote by treatment with synthetic auxins and many methods for the propagation of apple cultivars and rootstocks have been successfully developed (Wilkinson and Withnall, 1970; Noiton *et al*., 1992). In cuttings which are treated with synthetic auxins such as Indole Butyric Acid (IBA), concentrations of indole-3-acetic acid at the base of cuttings can increase transiently and then decrease prior to rooting, sometimes within a time period as short as 24 h (Blakesley *et al*., 1991; De Klerk *et al*., 1999). As reported elsewhere, IBA itself or combination with other growth regulators at high concentrations increase or accelerate the rooting of plants that exhibit particular rooting difficulties (Sun and Bassuk, 1991). Many studies have reported that IBA increase root number in plants. (Wynne and Mcdonald, 2002; Henrique *et al*., 2006).

Endogenous hormone levels are important factor affecting root and callus formation in stem cuttings. Many reports have showed a positive correlation between endogenous indole-3-acetic acid (IAA) levels in cuttings and the number of adventitious roots produced per cutting (Weigel *et al*., 1984; Welander and Snygg, 1987; Alvarez *et al*., 1989). A negative correlation between root growth and endogenous abscisic acid (ABA) was reported (Pilet and Saugy, 1987). Hansen (1988) reported that gibberellins (GA) inhibit rooting of cuttings of many plant species. Feito *et al*. (1996) reported that the most active rooting systems have high levels of cytokinins (mainly (diH) zeatin and its riboside), with low IAA and ABA contents. The aim of this study is to investigate the effects of callus formation and IBA treatment on endogenous IAA, GA_3, zeatin and ABA levels in three apple kinds (Golden delicious, Starkrimson delicious and Misket delicious).

MATERIALS AND METHODS

Plant material: Rooting studies of three apple (*Malus sylvestris* Miller) kinds (Golden delicious, Starkrimson delicious and Misket delicious) were carried out with the stem cuttings collected in February 2000-2002. Stem cuttings of three apple kinds measuring 15 to 20 cm in length and 7 to 8 mm in diameter were used. Different concentrations of the IBA (2000 and 3000 mg L^{-1}) were used for the treatments. The bases of the stem cuttings were soaked for 5 s in IBA solution at different concentration and cuttings soaked in distilled water were used as control. Three groups of replicates each consisting of 15 cuttings were then planted immediately in pots filled with sand-perlite. The rooting studies were carried out climatic room in daily period at 17-24°C. Observations on morphological characters such as callus number were recorded after planting (Table 1). For each analysis, 15 cuttings were taken and split into 3 sets of 5 replicates and basal parts in stem cuttings were sampled.

Corresponding Author: Aysel Sivaci, Department of Biology, Faculty of Sinop Art and Science, Ondokuz Mayıs University, Sinop, Turkey Fax: +90 3682715524

Table 1: The callus formation (%) in Golden delicious, Misket delicious and Starkrimson delicious

Apple kinds	Control	IBA treatment 2000 mg L^{-1}	IBA treatment 3000 mg L^{-1}
Golden delicious	35±6	46±4	31±6
Misket delicious	42±5	62±6	76±4
Starkrimson delicious	37±6	43±5	34±5

Data are the mean±SD

Hormone analysis: One gram fresh stems samples of each apple kinds were placed in 100 mL methanol: chloroform: 2 N amonyum hydroxide (12:5:3 v/v/v) and homogenized by using a Kinematic Polytron homogenizer. After addition of 1 µg/100 mL butylated hydroxytoluene (BHT), the samples were frozen at -80°C for one week for further analysis. After incubation, extracts were transferred into 250 mL conical flasks and added 22.4 mL bi-distilled water. To obtain a homogenize mixture, the conical flasks were shaken 3 or 4 times. Thus, with the exception of plant growth substances, the other organics in methanol were allowed to pass into the chloroform phase. The extraction, purification and quantitative determination of total IAA, GA_3, ABA and zeatin were done according to literature methods (Unyayar *et al*., 1996; Yürekli *et al*., 2001). A scanning densitometer (DESAGA CD 60) was used to determine the amounts IAA, GA_3, ABA and zeatin (Saucedo *et al*., 1989; Yürekli *et al*., 2001). Results are the mean of three replicates.

Statistical analysis: Data which were obtained from three replications of all treatments were subjected to analysis of variance using SPSS 8.0 for Windows for all statistical analysis. Differences between means at 5% ($p<0.05$) level were considered as significant.

RESULTS AND DISCUSSION

The callus formation percentage increased with IBA treatment of three apple kinds but it is decreased at certain concentrations of IBA (3000 mg L^{-1}) in Golden delicious and Starkrimson delicious. Callus formation percentage of Misket delicious was higher than other apple kinds (Golden delicious and Starkrimson delicious) (Table 1). According to Qaddoury and Amssa (2004), the root formation in date palm offshoots was significantly improved by IBA treatment and the contents of phenolic compounds increased steeply after IBA treatment and then decreased. Sivaci and Sokmen (2006) reported that the callus formation increased by IBA treatment in *Morus* stem cuttings and it may be effective for production phenolic, anthocyanin, antioxidant activity.

The IAA contents were significantly increased with callus formation and IBA treatment in the all of apple kinds (Fig. 1; $p<0.05$). In the callus control group of

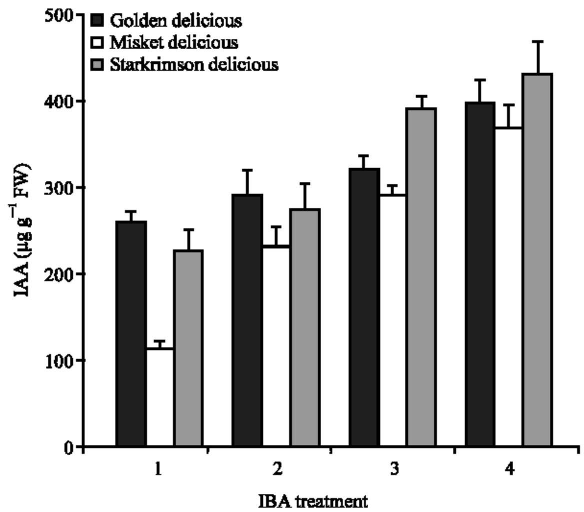

Fig. 1: Total IAA contents of Golden delicious, Starkrimson delicious and Misket delicious related to IBA treatment (1) non-callus, stem cuttings without callus formation (2) callus-control, callus formed stem cuttings, not IBA treated (3) callus formed stem cuttings, 2000 mg L^{-1} IBA treated and (4) callus formed stem cuttings, 3000 mg L^{-1} IBA treated) (FW, Fresh weight)

Golden delicious, IAA content was higher than other apple kinds (Misket deliciousand Starkrimson delicious) in non-callus (Fig. 1; $p<0.05$). The lowest IAA content was belonging to Misket delicious. The total IAA contents of the callus control group were found to be lower than that of with treatment IBA in the all of apple kinds (2000 and 3000 mg L^{-1}) (Fig. 1; $p<0.05$). The IAA contents were observed the highest at 3000 mg L^{-1} IBA in all. Liu *et al*. (1998) indicated that accumulation of endogenous IAA in soybean hypocotyl explants during adventitious root formation in NAA and IBA treatments was significant. Some reports showed that exogenous IBA could induce the changes in enzyme activities (peroxidase and IAA oxidase) and their effectors contents (phenolics) allowing the establishment of the favourable endogenous hormone balance (Ribnicky *et al*., 1996; Gaspar *et al*., 1997).

The GA_3 contents were significantly increased with callus formation in the all of apple kinds (Fig. 2). The GA_3 contents in Starkrimson delicious were higher than other apple kinds (Golden delicious and Misket delicious) in non-callus stem cuttings (Fig. 2). In Misket delicious, the content of GA_3 was lower than other kinds (Golden delicious and Starkrimson delicious) in non-callus. In the all of apple kinds, the GA_3 were significantly increased after treatment with 2000 and 3000 mg L^{-1} IBA to callus control (Fig. 2; $p<0.05$). Petridou and Porlingis

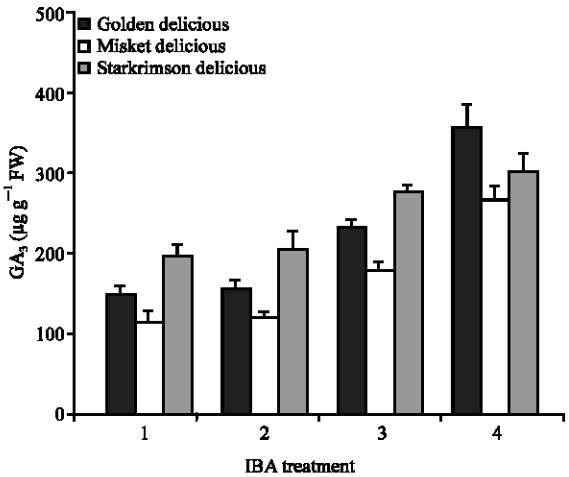

Fig. 2: Total GA_3 contents of Golden delicious, Starkrimson delicious and Misket delicious related to IBA treatment, (1) non-callus, stem cuttings without callus formation, (2) callus-control, callus formed stem cuttings, not IBA treated, (3) callus formed stem cuttings, 2000 mg L^{-1} IBA treated and (4) callus formed stem cuttings, 3000 mg L^{-1} IBA treated) (FW, Fresh weight)

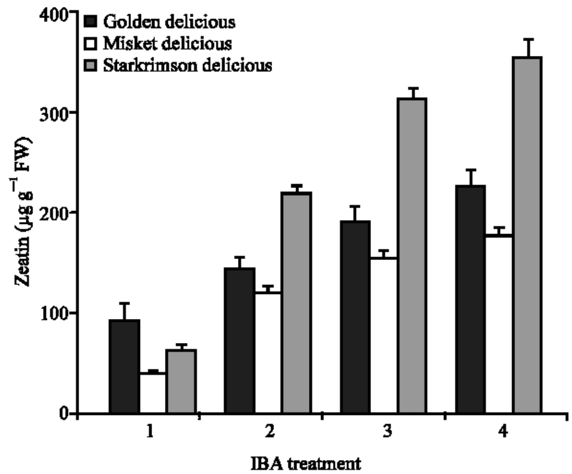

Fig. 3: Total zeatin contents of Golden delicious, Starkrimson delicious and Misket delicious related to IBA treatment, (1) non-callus, stem cuttings without callus formation, (2) callus-control, callus formed stem cuttings, not IBA treated (3) callus formed stem cuttings, 2000 mg L^{-1} IBA treated and (4) callus formed stem cuttings, 3000 mg L^{-1} IBA treated) (FW, Fresh weight)

(1997) reported that application of GA_3 promoted root development in cuttings of mung bean only in a small degree and this effect was increased by IBA, paclobutrazol and sucrose.

The zeatin contents of three kinds have been increased with callus formation and following IBA treatment (2000 and 3000 mg L^{-1}) (Fig. 3; $p<0.05$). After the treatment, the highest zeatin content was found at Starkrimson delicious with 3000 mg L^{-1} IBA and the lowest content was observed in Misket delicious in non-callus stem cuttings (Fig. 3). Debi *et al.* (2005) indicated to the effect of cytokinins on initiation, emergence and elongation of lateral root in rice. Both the kinetine and zeatin at 1 μM and higher concentration inhibited lateral root formation by inhibiting the initiation of lateral root primordia. Bollmark *et al.* (1988) showed that cytokinins inhibited the cell division during early phase of organization of adventitious root primordia in pea stem cuttings.

The endogenous ABA levels in stem tissues of all apple kinds were significantly increased with callus formation (Fig. 4; $p<0.05$). However, in the all of apple kinds, the levels of ABA were decreased by IBA treatment (2000 and 3000 mg L^{-1}). The ABA levels of in Starkrimson delicious were found lower than other apple kinds (Golden delicious and Misket delicious) at 3000 mg L^{-1} IBA (Fig. 4; $p<0.05$). Pilet and Saugy (1987) indicated that there was a negative between root growth

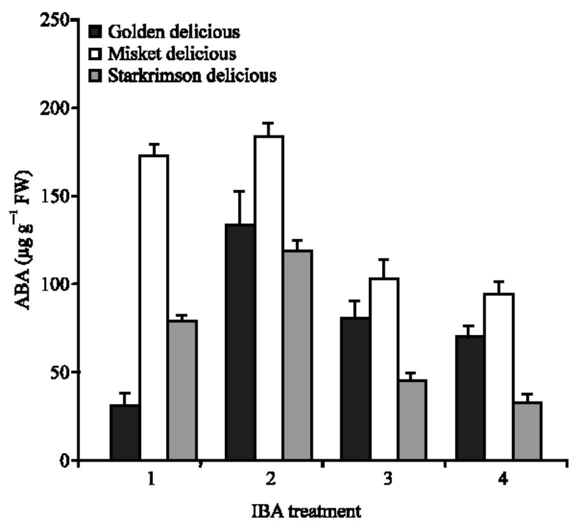

Fig. 4: Total ABA contents of Golden delicious, Starkrimson delicious and Misket delicious related to IBA treatment, (1) non-callus, stem cuttings without callus formation, (2) callus-control, callus formed stem cuttings, not IBA treated, (3) callus formed stem cuttings, 2000 mg L^{-1} IBA treated and (4) callus formed stem cuttings, 3000 mg L^{-1} IBA treated) (FW, Fresh weight)

and endogenous ABA. The inhibition effect of ABA was decreased or over come by some plant growth regulators

(Kotsias and Roussos, 2001). Feito *et al*. (1996) reported that the root formation in *Juglans regia* was promoted by high cytokinins, low IAA and ABA. Noda *et al*. (2000) indicated a negative correlation between citrus scion growth and endogenous IAA and ABA in the fibrous roots.

CONCLUSIONS

Results of the present study have demonstrated the total IAA, GA_3, zeatin and ABA levels significantly were changed by callus formation and IBA treatment in three apples of stem cuttings. Herein, we also have reported that exogenous plant growth regulator (IBA) may be effective on the callus formation by increasing of the total IAA, GA_3, zeatin and decreasing of ABA levels in three apple of stem cuttings.

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