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Studies on Rooting of Azayesh Apple Cutting Using Indolebutyric Acid and Media

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ABSTRACT

This study was conducted with the main purpose of determining the most suitable Indolebutyric acid (IBA) concentrations and rooting media for Azayesh hardwood cuttings. Azayesh apple is a dwarf rootstock. In this study, the influence of IBA concentration and rooting media on the rooting of Azayesh hardwood cuttings was examined. Four concentrations of IBA (0, 1500, 2500 and 3500 mg L^{-1}) and three rooting media (cocopeat, perlite, cocopeat+perlite 1:1 by volume) in a factorial design were applied to cuttings. The best rooting was achieved by 2500 mg L^{-1} concentration and cocopeat+perlite medium and the lowest by control IBA concentration and cocopeat medium. Based on the present study results, IBA concentration with 2500 mg L^{-1} and cocopeat+perlite medium were recommended for the rooting of Azayesh apple rootstocks.

Key words: Rooting media, hardwood cuttings, IBA concentration, cocopeat, perlite

INTRODUCTION

To date, tree size reduction using genetically dwarf trees has been a key component of high density orchard system (Vahdati *et al*., 2009). Dwarf trees can be more easily and economically pruned, sprayed, thinned, harvested and they usually come into fruiting at a much earlier age than standard tree (Tareen *et al*., 2002). Azayesh (native apple of Iran) is a very important dwarf apple rootstock due to their adaptability to the region's climate, reduction in the expense of their supply and prevention of pests and diseases from entering the country. Therefore, methods to improve its propagation are required. Hardwood cuttings are one of the least expensive and easiest methods of vegetative propagation. They are easy to prepare, are not readily perishable, may be shipped safely over long distances if necessary and require little or no special equipment during rooting (Denny and Arnold, 2001).

Rooting of cuttings is not always successful and the reasons for rooting failure are not clearly understood. Factors such as cultivar and age of the source tree; the collection date, length, diameter and degree of hardening of the cuttings; injury and heat treatments of the cuttings and the treatment concentrations of auxin-like compounds (Tsipouridis *et al*., 2003) rooting hormones,

planting time, maturity of the stock plants and propagation environment might be among the important factors affecting the rooting of stem cuttings (Bhusal *et al*., 2001). Also, the genetic variation was reflected in the differences observed among the varieties in their response to IBA concentration (Owais, 2010). And the choice of an appropriate substrate is crucial for the rooting of cuttings (Tofanelli *et al*., 2003).

The effect of IBA on root formation was reported in several apple rootstocks. Tajbakhsh *et al*. (2009) reported rooting percentage in apple cuttings (31.5%) was in 3000 mg L^{-1} concentration. Fukuda *et al*. (1988) also reported that the rooting percentage in M26 and MM106 rootstock cuttings was 7.2 and 25%, respectively, with 2000 mg L^{-1}.

Both rooting success and root quality are greatly affected by the substrate. Coir is a coconut processing by-product and renewable substitute for peat (Prasad, 1997). Coconut coir has been shown to increase rooting of several woody ornamentals (Stoven and Kooima, 1999). Ma and Nichols (2004) reported that the problems with coir extend beyond its high salinity. Their data indicate that high concentrations of phenolic compounds in fresh coir are at least partly responsible for the growth reduction observed in other studies. Cocopeat has been recognized to have high water holding capacity which causes poor air-water relationship, leading to low aeration within the medium, thus affecting the oxygen diffusion to the roots (Abad *et al*., 2002). Perlite is recognized to have a unique capillary action which makes it a superior growing medium for hydroponic cultures (Robbins and Evans, 2004). It is very useful for increasing aeration and drainage within the container because of its uniformity and lightness (Paradiso and de Pascale, 2008). Incorporation of coarser materials into cocopeat could improve the aeration status of the media (Richards *et al*., 1986; Sambo *et al*., 2008). Burnt rice hull, kenaf core fiber and perlite are among the possible coarser materials could be used to improve the air-water relationship of cocopeat (Islam, 2008; Sambo *et al*., 2008; Marianthi, 2006). Inorganic materials were found to be more suitable for rooting. However, organic materials were more effective in root growth (Ozenc and Ozenc, 2007).

The aim of this study was to test the influence of IBA concentration and rooting media on Azayesh hardwood cuttings.

MATERIALS AND METHODS

This study was conducted in Khorasan Razavi Natural Resource and Agricultural Research Center, in Mashad, Iran during 2009 and 2010. Twenty centimeter-long Hardwood cuttings were collected from Azayesh trees on February. Immediately after collection, the base of cuttings (1 cm) were treated for 10 s with (0, 1500, 2500, 3500 mg L^{-1}) IBA in a 50% ethanol solution (96% purity).

After surface drying, their bases were treated with Captan 75% (9:1 in talc). The cuttings were then placed in three different rooting media i.e. cocopeat, perlite, cocopeat+perlite (1:1 by volume) to a depth so that the third node below the terminal whorl was 1 cm below the surface of the medium. The bottom of the rooting bench was heated at 24°C and the temperature and relative humidity in the greenhouse were kept at 21±2°C and 70-75%, respectively.

Later, Azayesh apple rootstock cuttings were investigated in the following areas for a period of 60 days: rooting percentage (%), root number and root length (cm) were determined.

The percentage of rooting was calculated by the following equation:

$$\frac{\text{No. of rooted cuttings}}{\text{Total No. of cuttings}} \times 100$$

- **Measuring root length:** In this study the roots length more than 0.5 cm were measured and then the total number of roots length in each treatment was divided by the number of the cuttings of that treatment to obtain the mean of the roots length in each treatment.
- **Measuring root number:** The procedure of measuring root number is the same as measuring root length.

The different treatments consisted of four concentrations of IBA (0, 1500, 2500 and 3500 mg L^{-1}) and three media (cocopeat, perlite, cocopeat+perlite 1:1 by volume).

This experiment had a Completely Randomized Design (CRD) with factorial arrangement in three replications. Each replication consisted of 15 cuttings. Data were analyzed with SPSS (SPSS Inc., Chicago, USA). Significant differences between treatments were tested by Duncan's multiple range test at p = 0.01.

RESULTS

Successful root induction was affected by exogenously applied IBA levels and rooting media. Although some root formation was seen in 40 days, most of the rooting occurred after 60 days. The data presented in Table 1, indicate that media and IBA concentrations have a significant effect, while the interaction has not significant effect on the rooting percentage. No rooting occurred in cuttings when they were treated with 0 mg L^{-1} IBA in all media. Based on the results, the highest rooting percentage (31.48) was obtained in the concentration of 2500 mg L^{-1} IBA in cocopeat+perlite medium (Table 2). The lowest percentage of rooting after 0 mg L^{-1} was related to

Table 1: Analysis of variance (ANOVA) of rooting percentage, root length and root number of Azayesh apple cutting

		MS		
SOV	df	Rooting (%)	Root length (cm)	Root number
Media	2	93.456**	3.299**	4.750**
Concentration	3	120.370**	64.417**	50.546**
Media×Concentration	6	12.284^{NS}	0.799**	1.046**
Error	24	6.003	0.229	0.139
C.V. %		18.68	14.99	16.56

NS: Not significant. **Significant at 1% level of significant

Table 2: The effect of media, IBA concentration on rooting percentage, root length and root number of Azayesh apple cutting

Media	IBA conc. (m gL^{-1})	Rooting (%)	Rooting length (cm)	Root number
Cocopeat	0	0.00^{g}	0.00^{e}	0.00^{g}
	1500	12.96^{de}	5.50^{a}	1.33^{ef}
	2500	22.22^{bc}	3.50^{bc}	4.33^{c}
	3500	5.55^{fg}	1.83^{d}	1.00^{f}
Perlite	0	0.00^{g}	0.0e	0.00^{g}
	1500	16.66^{cd}	6.16^{a}	2.00^{e}
	2500	27.77^{ab}	4.16^{b}	5.33^{b}
	3500	9.257^{ef}	2.16^{d}	1.33^{ef}
Cocopeat+Perlite	0	0.00^{g}	0.00^{e}	0.00^{g}
	1500	20.37^{c}	6.66^{a}	3.00^{d}
	2500	31.48^{a}	5.83^{a}	7.00^{a}
	3500	11.11^{def}	2.50^{cd}	1.66^{ef}

Values followed by the same letter in the same column are not significantly different at the 99% level according to Duncan's test

3500 mg L^{-1} IBA in all media. Our findings showed that rooting decreased by increasing IBA concentration (3500 mg L^{-1}).

IBA treated cuttings gave longer roots than the control did in all medium types. Table 1 indicates that rooting media and IBA concentrations significantly affected root length ($p<0.01$). Based on the result (Table 2), the highest root length (6.66 cm) was attained in 1500 mg L^{-1} IBA and cocopeat+perlite medium.

After 1500 mg L^{-1} IBA, the highest root length (5.83 cm) was related to 2500 mg L^{-} in perlite+cocopeat medium. Although statistically, there was no significant difference with the concentration of 1500 mg L^{-1} IBA in the same medium.

Rooting media and IBA concentrations significantly affected the number of main roots per cutting. All IBA treated cuttings gave more roots than the controls ($p<0.01$) (Table 1). Based on the result (Table 2), the greatest root number (7) was acquired in the concentration of 2500 mg L^{-1} IBA and cocopeat+perlite medium. 2500 mg L^{-1} IBA was the best treatment in terms of the main root number and rooting percentage (except the root length) per cutting in all of the media. The lowest number of roots after 0 mg L^{-1} was related to 3500 mg L^{-1} IBA.

DISCUSSION

So far, apple rootstocks have been commercially propagated by stool-layering. But today this method has some disadvantages, as they are slow, labor-intensive and cumbersome (Ersoy *et al.*, 2010).

No information is available about the ability of rooting potential in Azayesh apple rootstock, therefore the current research was conducted to study the rooting ability of cuttings of Azayesh apple rootstock using different IBA concentrations and three types of media.

The results confirmed the importance of exogenous IBA application and rooting media for rooting. IBA may enhance rooting via increase of internal-free IBA, or may synergistically modify the action of IAA or the endogenous synthesis of IAA. IBA can enhance tissue sensitivity to IAA and increase rooting (Krieken *et al.*, 1993).

In the experiment, the majority of cuttings rooted in a period of 40-60 days. Propagation of the different kinds of fruit trees could be classified into easy-to-root and difficult-to-root species. Our results confirmed that Azayesh rootstock is the difficult-to-root species.

The physiological processes leading to induction of root initiation are not yet fully elucidated. The inability of cuttings to root may be attributed to the improper physiological conditions of cuttings. At moderate temperature the cambium region is divided actively and physiological condition of the cambium for root initiation and development will be suitable (Ahmed *et al.*, 2002). The effectiveness of auxin to raise rooting percentage of the cutting could be through increasing cambial activity and differentiation of root primordial or by stimulating redistribution and mobilization of some auxin cofactors towards the base of the cuttings (Mousa, 2003). An increase of free auxins was found in the bases of the cuttings before the beginning of root initiation (Nordstrom *et al.*, 1991). It has been postulated that easily rooting species, as opposed to those which were difficult to root, have an ability to hydrolyze auxin conjugates at the appropriate time to release free auxins which may promote root initiation (Epstein and Ludwing-Muller, 1993).

By increasing IBA concentration, root length decreased, but root number increased. Wagner and Oprita (1985) reached the same conclusion in sweet cherry, they showed that low auxin concentrations cause lower root number but increase its length and high concentration increases the root number and decreases its length. Also, Turkoglu and Durmus (2005) reported that the low

concentration of IBA significantly increased the root length and higher concentration increased the number of roots. The application of auxin causes the enhancement of ethylene synthesis (Hansen, 1987) which inhibits root length growth. The results of this experiment were similar to those of Iqbal *et al.* (1999) who reported that the number of roots are enhanced by increasing the IBA concentration to 3000 mg L^{-1}. The induction of maximum number of roots in the treated cuttings may be due to the fact that cambial activity involved in root initiation is stimulated by growth regulators in many species (Ullah *et al.*, 2005).

Data are consistent with a study suggesting IBA treatment with high concentration reduced rooting percentage in cuttings of MM106 (Sun and Bassuk, 1991). Moreover, Karakurt *et al.* (2009) reported that the best concentration of IBA for rooting was 1000 mg L^{-1} for MM106 apple rootstock but by increasing IBA concentration to 2000 mg L^{-1} and 4000 mg L^{-1} no rooting occurred.

Experiment showed that the lowest rooting percentage was obtained in cocopeat medium. This might be because of its phenolic compounds which cause phytotoxicity, its high salinity (Ma and Nichols, 2004) and high water holding capacity leading to low aeration (Abad *et al.*, 2002).

In perlite medium, rooting percentage was also low. It seems that it is due to its low water retention capacity. Most of the granular inorganic substrates such as perlite have low water retention capacity (Martyn and Szot, 2001). This result suggests that cocopeat medium retains more water and less air space than the 100% perlite.

Improved rooting in cocopeat+perlite medium can be due to the improvement of both aeration and water holding capacity (Islam, 2008).

CONCLUSION

In conclusion, the results confirmed the importance of exogenous IBA applications and that choosing the right concentrations of IBA for rooting was of critical importance. The application of IBA with a concentration of 2500 mg L^{-1} and cocopeat+perlite medium were found to be the most suitable for rooting of hardwood cuttings of Azayesh apple rootstock. However, apart from IBA and rooting media, other factors such as the temperature of rooting media, cutting type and the timing of cutting sampling should be taken into consideration. When this method is compared to other methods of propagation such as grafting and layering, it saves time and is a more economical way of propagation than these alternatives.

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