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Full Length Research

Effect of indole-butyric acid (IBA) and wounding on rooting ability and vegetative characteristics of apple rootstock cuttings under Nepal conditions

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ABSTRACT: Use of seedling rootstocks is widespread in Jumla and research on the evaluation of clonal rootstocks for propagation has still not started. Study on the concentration of IBA and wounding effect in cuttings is also deficit. Therefore, this investigation was carried out in the Hi-tech Greenhouse of Kalika nursery in Jumla, Nepal from February to July, 2018 to investigate the rooting response of MM111 apple rootstock to wounding (with or without) and various Indole-Butyric Acid (IBA) concentrations (0, 2000, 2500 and 3000 ppm). The experiment was laid out in a factorial Complete Randomized Design by preparing cuttings of 20 cm length and 5 to 10 mm diameter and planting them in rooting media made by mixing perlite, cocopeat, forest soil and vermicompost in 1:1:1:1 ratio after 15 second of quick dip in IBA solution. All the rooting and vegetative parameters were significantly affected by different concentration of IBA solution and wounding. The results revealed minimum number of days to first sprouting (15.50), highest rooting percentage (68.52%), highest number of roots per cutting (23.11), highest length of the longest root (22.83 cm), maximum diameter of main sprout (2.92 mm), maximum number of leaves at 86 DAT(20.06), 93 DAT(22.50) and 100 DAT(30.17) in the cuttings treated with IBA 2000 ppm. Increasing the IBA concentration beyond 2000 ppm produced negative effects. Similarly, wounding resulted in better root and shoot parameters of cuttings than with no wounding. The rooting percentage (57.4%), number of roots per cutting (21.83), length of the longest root (20.96 cm), diameter of the main sprout (2.66 mm) were significantly higher in wounded cuttings which also recorded the lower days for first sprouting. The interaction effect was significant ($p<0.01$) only on the rooting percentage while all other parameters were non-significant. The results indicated that 2000 ppm IBA with basal wounding perform better than other treatments.

Keyword: Apple, cuttings, MM111, IBA, Wound.

INTRODUCTION

Malus domestica (apple) is one of the most important and abundantly grown crop in temperate regions of the world. It is thought to have been originated in central Asia where it was domesticated centuries years ago and was later taken to the west by Europeans (Janick, 2005). The apple tree is deciduous, varying from 2 to 4.5 m in height with wide variation in shape and structure depending upon the cultivar, rootstock used and pruning methods (Lauri and Laurens, 2005). The simple, serrated leaves are arranged alternately on the stem. Flowering in apple occurs after the end of frost in spring and the fruit is ripened in late summer to autumn and usually develops red, yellow or green colour after maturation. The fruits are mainly eaten raw although they can also be processed to make fruit juice, slices, sauce and, alcoholic beverages like wine, cider or brandy (Janick et al., 1996). They are also used in making pastries, cakes, pies and tarts.

Worldwide, the total production of apple in 2018 was

86,142,197 tonnes with a productivity of 17.56T ha^{-1} (FAO, 2018) whereas, the largest producer, China, has a total area and production of 20,71,674 hectares and 39,23,5019 tonnes, respectively. In Nepal, apple is cultivated in 4133 hectares with an annual production of 28,895 tonnes (FAO, 2018) with a comparatively low productivity. Meanwhile, the Jumla district is the largest producer of apples in Nepal with 5500 Mt of apples produced from 700 hectares of cultivated area (Atreya and Kafle, 2016).

Grafting and budding have been the most popular methods of propagation of apple plants throughout the world (Collett, 2011). For grafting, clonal rootstocks are more popular than seedling rootstocks. Clonal rootstocks can offer many advantages like control in plant vigor (Clearwater et al., 2007), precocity in bearing (Comiotto et al., 2013), increased production (Yahmed et al., 2016), resistance to disease and pests (Noguera et al., 2011), increase tolerance to detrimental soil conditions (Elkins et al., 2012) and better nutrient absorption (Nawaz et al., 2016; Jimenes et al., 2018).

MM111 is a recently introduced clonal rootstock plant in Jumla through PMAMP (PMAMP, 2016) and is more suitable for the dry climate and woolly-aphid infested conditions of Jumla district because it is well adapted to dry climate, is precocious compared to seedling rootstock, semi-vigorous and resistant to woolly apple aphid (Webster, 2000).

Although cutting has been the widely used method for rapid production of clonal rootstocks, there are many endogenous and exogenous factors that affect the rooting of cuttings. Among them, the endogenous concentration or the external application of auxin like compounds in promotion of rooting is well established (Bhatt and Tomar, 2010). One of the auxins, Indole-Butyric Acid (IBA) has been used extensively throughout the world to enhance rooting in apple rootstocks (Yusnita et al., 2017). Nevertheless, the results concerning the optimum IBA concentration are widely varied (Lone and Sofi, 2007; Ersoy et al., 2010). Also, a major approach, wounding the basal end, can significantly affect the rooting percentage of cuttings, especially by improving the effect of hormonal treatment (Davies et al., 2011). Wounding results in wound response, which increases the division of cambial cells ultimately leading to adventitious root formation (King et al., 2011). Furthermore, the efficiency of auxin entry into the stem is improved by wounding (Majumder and Howard, 1973). Several studies have found a positive effect on rooting of cuttings due to wounding (Al-Salem and Karam, 2001; Tsipouridis et al., 2005).

In Jumla, majority of the farmers are still using seedling rootstocks prepared from the seeds of indigenous crab apple (*Malus baccata*) for grafting and budding (Schnelle, 2012) despite the increase in area under apple cultivation. These seedling rootstocks are not true to type due to cross pollination and thus, show variability in growth and production of scion variety (Davies et al., 2011). Also, it takes about 2 to 3 years for these seedlings to become ready for grafting purpose, which is quite a long time. Propagation through cuttings is the most common means of clonal regeneration (Sharma et al., 2013) and the adventitious root formation is a pre-requisite to successful cutting propagation (Hartmann et al., 2007). To the best of our knowledge, the IBA application and wound response in apple clonal cuttings is scanty in Nepal. Therefore, the present study was carried out to evaluate the effect of wounding and various IBA concentration along with their interaction on rooting and shooting ability of cuttings in MM111 with an objective to determine the most suitable combination for rooting of this newly introduced clonal rootstock.

MATERIALS AND METHODS

Site of the experiment

This study was carried out in the Hi-tech greenhouse of Kalika nursery in Chandanath Jumla, Nepal from February to June of 2018. Jumla lies in the mountainous region of Karnali Province and is situated between longitude 81º 28' E to 82º 18' E and latitude 28º 58' N to 29º 30' N (MoAD, 2016) having elevation range from 915 to 4679 m above sea level.

Experiment design and treatments

The research was conducted in two factor Completely Randomized Design (CRD) with 8 treatment combinations and replicated thrice. The first factor consisted of four concentrations of IBA (L), viz; 0 ppm (control) (L_1), 2000 ppm (L_2), 2500 ppm(L_3) and 3000 ppm(L_4) and the second factor consisted of wounded (W_1) or non-wounded (W_0) cuttings. So, there were a total of eight treatments with ten cuttings per treatment.

Cuttings, IBA application, wounding and planting

Hardwood cuttings of size 20 cm long and thickness of 5 to 10 mm with 4 to 6 buds was collected from the selected mother plants of 2 years age in the month of February when plants were dormant. Wounding was done with a basal cut of about 3 cm length through the diameter, extending upwards. For IBA treatment, the base of the cuttings was dipped for 15 seconds in IBA solutions of desired concentration before planting. A rooting bed of dimension (4.35 m × 1.6 m) was prepared by mixing vermicompost, cocopeat, perlite and forest soil in 1:1:1:1 ratio. The rooting bed was watered properly before planting of cuttings to remove air pockets and care was taken to avoid leaching of hormones from one treatment to the other by placing them 15 cm apart. The weekly

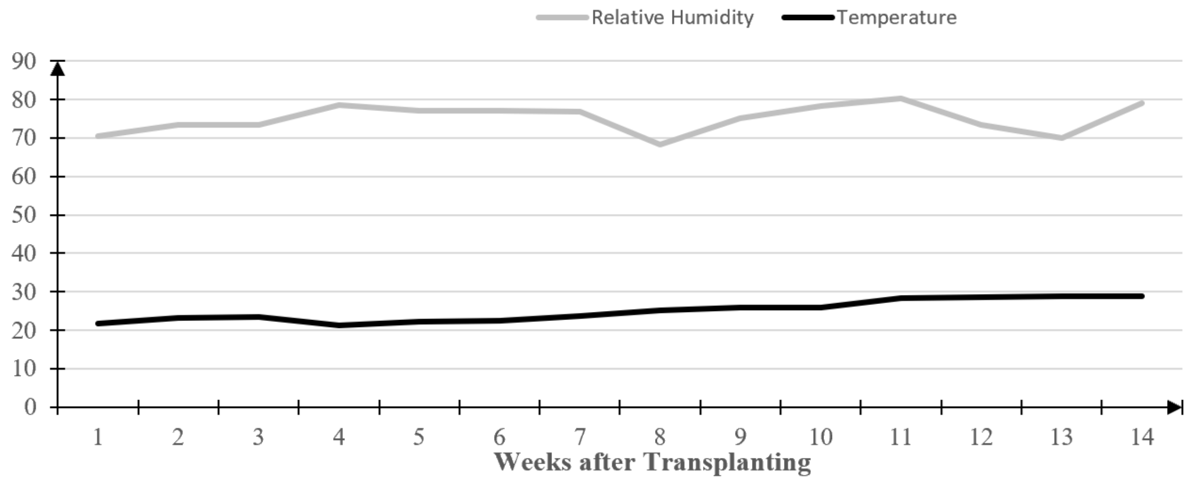

Figure 1. Average temperature (^{0}C) and average relative humidity (RH%) at 7 days interval during the research period in Jumla, 2018.

Figure 2. Sprouted cuttings in the experimental plots.

average temperature and relative humidity (RH) inside the greenhouse is illustrated in Figure 1.

Observations and data analysis

The cuttings, after planting, were allowed to root for 100 days and observations were made on the rooting percentage (%), total number of roots, length of the longest main root (cm), diameter of the longest root (mm), diameter of the main shoot (mm), days to first sprouting and the number of leaves by selecting four random cuttings from each treatment. The recorded observations were analyzed using statistical software GenStat and, MS-

Excel and mean comparison was done using DMRT.

RESULTS AND DISCUSSION

Days to first sprouting

As evident from Table 1, both IBA treatments and wounding exerted a significant effect on the days to first sprouting of cuttings. The minimum (15.50) number of days required to first sprouting was recorded in cuttings treated with IBA 2000 ppm which was better than other concentrations of IBA. On the other hand, maximum number of days (23) to first sprouting was recorded in control. These results are in line with the findings of Mohammad and Ayaz (1989) who observed earliest sprouting in cuttings of peach rootstocks Peshawar local collected in February and treated with IBA at 2000 ppm by quick dip method. Earliness in sprouting in cuttings treated with 2000 ppm IBA solution might be due to better utilization of stored carbohydrates, nitrogen and other factors with the help of growth regulators (Abebe, 2017). The delay in bud sprouting at higher concentration may be due to the increased inhibitory action of IBA with increase in its concentration (Sun and Bassuk, 1991).

In case of wounding and non-wounding treatments, the minimum number of days (18.08) to first sprouting was recorded in cuttings with wounding and maximum days (20.17) in cuttings devoid of wound. The earliness in sprouting with wounding might be due to the stress created by wounding leading to higher accumulation of auxin and increased physiological activity (Saumitro and Jha, 2014). Interestingly, the interaction effect of wounding and IBA concentration on sprouting was non-significant (Table 2)

Rooting percentage

Significantly highest rooting percentage (57.4%) was recorded in cuttings with wound as compared to non-wounded cuttings. Also, significantly, the highest percentage of rooting (68.52%) was recorded in cuttings treated with 2000 ppm IBA followed by 2500 ppm (55.56%) and lowest percentage of rooting (37.04%) was recorded in control which was at par with 3000 ppm IBA (40.74%). Similar results were reported by Fragoso et al. (2017) who reported highest rooting (80%) percentage with around 2700mg L^{-1} concentration of IBA and subsequent decline in rooting percentage with the increase in IBA application in Japanese flowering cherry. Similarly, Dvin et al. (2011) observed a decrease in rooting percentage of MM111 beyond IBA concentration of 3500 ppm. The higher rooting due to IBA application is due to its effect on increasing the cell wall plasticity and cell division, stimulation of callus development and root growth (Weaver, 1972). Van der Krieken et al. (1993) have argued that IBA can harmoniously alter the IAA action or can result in endogenous IAA synthesis which can enhance the rooting by mobilisation of reserved food materials. The decrease percentage of rooted cutting with increasing concentration of IBA might be due to hormonal toxicity.

The highest rooting percentage (85.19%) was recorded with the interaction of IBA 2000 ppm and wounding while the lowest percentage of rooting (33.33%) was noticed in control (Table 2). These results are similar to the findings of Negi et al. (2015) who reported highest rooting percentage (46.66%) when Merton 793 rootstock was treated with 2000 ppm IBA with wounding (basal split). The maximum percentage of rooted cutting with 2000 ppm IBA + wounding might be due to the fact that optimum concentration of IBA leads to mobilization and exploitation of carbohydrate and amino acids (Baghel et al., 2016), while wounding encourages cell division (Davies et al., 2011) and increases the effect of IBA in cuttings (Abdulqader et al., 2017).

Total number of roots per cutting

Both wounding and IBA concentration resulted in statistically significant ($p<0.001$) effect on total number of roots per cutting (Table 1). Application of 2000 ppm IBA produced the highest total number of roots (23.11) in cuttings which might be due to the better cambial activity in tissues involved in root initiation (Ullah et al., 2005). The reduction in root numbers beyond 2000 ppm IBA solution might be due to its inhibitory action (Shiozaki et al., 2013).

Similarly, the cuttings with wounding recorded significantly higher total number of roots (21.83) than the cuttings treated without wounding (15.56). These results are in close conformity with Howard et al. (1984) who reported that wounding at the base of M 26 winter cuttings by basal splitting increase the number of roots in cuttings. The increase in root number in wounded cuttings might be due to greater absorption of applied growth regulator by disrupting cambium tissues which facilitated the outward emergence of the new developing roots (Saumitro and Jha, 2014). The interaction effect between concentrations of IBA and wounding on the total number of roots per cutting was found to be statistically non-significant (Table 2).

Length of the longest root

In this study, IBA and wounding produced a significant effect ($p<0.001$) on the length of the longest root which is presented in Table 3. Significantly, longest root length (22.83 cm) was recorded in cuttings treated with 2000 ppm IBA and the shortest root length (14.86 cm) was recorded in control. The results are comparable to the findings of Abdulqader et al. (2017) who stated that the concentration of 2000 ppm gave the highest value of root length (20.67 cm) in olive. Increased root length may be due to early

Table 1. Effects of concentrations of IBA and wounding on days to first sprouting, rooting percentage and total number of roots of apple clonal rootstock cuttings in Jumla, Nepal, 2018.

Treatments	Days to first sprouting	Rooting percentage (%)	Total number of roots /cutting
Concentrations of IBA			
L1	23.00[a]	37.04[c]	12.00[c]
L2	15.50[c]	68.52[a]	23.11[a]
L3	19.00[b]	55.56[b]	19.44[b]
L4	19.00[b]	40.74[c]	20.22[b]
SEM (±)	0.954	2.78	0.887
LSD (0.05)	2.859 ***	8.33***	2.658***
Wounding			
W_0	20.17[a]	43.5[b]	15.56[b]
W_1	18.08[b]	57.4[a]	21.83[a]
SEM (±)	0.674	1.96	0.627
LSD (0.05)	2.022*	5.89***	1.880***
C.V (%)	12.2	13.5	11.6
Grand mean	19.12	50.5	18.69

SEM; Standard error of means, LSD; Least significant differences, C.V; Coefficients of variation, *Significance at 0.05 p level, **Significance at 0.01 p level, ***Significant at 0.001 p level.

Table 2. Interaction effect of concentrations of IBA and wounding on rooting percentage, days to first sprouting of apple clonal rootstock cuttings in Jumla, Nepal, 2018.

Treatments (Concentration of IBA × Wounding)	Days to first sprouting	Rooting percentage (%)	Total number of roots/cutting
T_1 (0 ppm + without wounding)/ Control	25.33	33.33[d]	9.33
T_2 (2000 ppm + without wounding)	17.33	51.85[b]	18.44
T_3 (2500 ppm + without wounding)	18.67	51.85[bc]	16.11
T_4 (3000 ppm + without wounding)	19.33	37.04[d]	18.33
T_5 (2000 ppm + With wounding)	13.67	85.19[a]	27.78
T_6 (2500 ppm + With wounding)	19.33	59.26[b]	22.78
T_7 (3000 ppm + With wounding)	18.67	44.44[cd]	22.11
T_8 (0 ppm + With wounding)	20.67	40.74[cd]	14.67
SEM (±)	1.35	3.93	1.25
LSD (0.05)	NS	11.78**	NS

SEM; Standard error of means, LSD; Least significant differences: *Significance at 0.05 p level **Significance at 0.01 p level, ***Significant at 0.001 p level.

initiation of roots leading to more consumption of nutrients (Jadhav, 2007). The decrease in root length above 2000 ppm IBA might be due to the enhancement of ethylene synthesis inhibiting the length of root (Pop et al., 2011). Longer root length (20.96 cm) in wounded cuttings than unwounded cuttings might be due to greater cell division and meristematic activity in wounded tissues. Moreover, interaction effect of concentrations of IBA and wounding on average length of longest root of cuttings was statistically non-significant at 5% level of significance (Table 4).

Diameter of the longest root (mm)

Statistically, non-significant result was found for the average diameter of longest root in case of concentrations of IBA, wounding and their interaction (Tables 3 and 4).

Diameter of sprouted main shoot (mm)

It is evident from data that average diameter of main sprout was significantly affected (p<0.001) by concentrations of

Table 3. Effect of the concentrations of IBA and wounding on length of the longest root (cm), diameter of the longest root (mm) and diameter of sprouted main shoot(mm) of cuttings of apple clonal rootstock in Jumla, Nepal, 2018.

Treatments	Length of the longest root (cm)	Diameter of the longest root (mm)	Average diameter of sprouted main shoot (mm)
Concentrations of IBA			
L1	14.86[c]	1.064	2.042[c]
L2	22.83[a]	1.122	2.924[a]
L3	19.00[b]	1.164	2.466[b]
L4	19.25 [b]	1.178	2.433 [b]
SEM (±)	0.985	0.1194	0.1275
LSD (0.05)	2.953***	NS	0.3823**
Wounding			
W_0	17.01[b]	1.105	2.27[b]
W_1	20.96[a]	1.160	2.66[a]
SEM (±)	0.697	0.084	0.0902
LSD (0.05)	2.088**	NS	0.2703**
C.V (%)	12.7	25.8	12.7
Grand mean	18.99	1.132	2.466

SEM; Standard error of means, LSD; Least significant differences, C.V; Coefficients of variation, *Significance at 0.05 p level, **Significance at 0.01 p level, ***Significant at 0.001 p level.

Table 4. Interaction effect of concentrations of IBA and wounding on length of the longest root (cm), diameter of the longest root(mm) and diameter of sprouted main shoot(mm) of apple clonal rootstock cuttings in Jumla, Nepal, 2018.

Treatments (Concentration of IBA × Wounding)	length of the longest root (cm)	diameter of the longest root (mm)	diameter of sprouted main shoot (mm)
T_1 (0 ppm + without wounding)/ Control	13.83	1.12	1.84
T_2 (2000 ppm + without wounding)	18.67	1.112	2.62
T_3 (2500 ppm + without wounding)	17.22	0.996	2.34
T_4 (3000 ppm + without wounding)	18.33	1.191	2.31
T_5 (2000 ppm + With wounding)	27	1.132	3.23
T_6 (2500 ppm + With wounding)	20.78	1.333	2.59
T_7 (3000 ppm + With wounding)	20.17	1.166	2.56
T_8 (0 ppm + With wounding)	15.89	1.008	2.24
SEM (±)	1.393	0.1689	0.18
LSD (0.05)	NS	NS	NS

SEM; Standard error of means, LSD; Least significant differences: Significance at 0.05 p level, **Significance at 0.01 p level, ***Significant at 0.001 p level.

IBA and wounding (Table 3). Similar to other results, maximum average sprout diameter (2.924 mm) was recorded in the cuttings treated with 2000 ppm IBA and the minimum shoot diameter (2.042 mm) was recorded under control. These results corroborated the findings of Sharma et al. (2014) who obtained maximum sprout diameter (17.92 mm) in the cuttings of M793 rootstock when treated with 2000 ppm IBA +Girdling. The increment in sprout diameter might be due to a greater number of leaves and vigorous root system (indicated by higher percentage of rooting and greater root number) which might have resulted in more carbohydrates assimilation and also enhanced the absorption of minerals and water from the soil.

The average diameter of the main sprout was maximum (2.66 mm) in wounded cuttings and minimum (2.27 mm) in cuttings devoid of wound. Wound-induced endogenous hormone Jasmonates which stunt plant growth by inhibiting mitosis (Zhang and Turner, 2008) which might have increased the diameter of shoot. The interaction effect of IBA and wounding on sprout diameter was non-significant (Table 4).

Table 5. Effect of the concentrations of IBA solution and wounding on average number of leaves of apple clonal rootstock cuttings at different DAT in Jumla, Nepal, 2018.

Treatments	Average number of leaves per cuttings		
	86 DAT	93 DAT	100 DAT
Concentrations of IBA			
L1	15.78[c]	16.94[c]	18.11[c]
L2	20.06[a]	22.50[a]	30.17 [a]
L3	18.89[ab]	20.78[ab]	24.17 [b]
L4	16.39[bc]	18.94[bc]	22.06 [b]
SEM (±)	0.986	1.054	1.290
LSD (0.05)	2.955*	3.160*	3.868**
Wounding			
W_0	16.72[b]	18.40[b]	22.19[b]
W_1	18.83[a]	20.69[a]	25.06[a]
SEM (±)	0.697	0.745	0.912
LSD (0.05)	2.089*	2.235*	2.735*
C.V (%)	13.6	13	13.4
Grand mean	17.78	19.79	23.62

SEM; Standard error of means, LSD; least significant differences, C.V; Coefficients of variation, *Significance at 0.05 p level, **Significance at 0.01 p level, ***Significant at 0.001 p level.

Number of leaves per cutting

The study revealed significant effect ($p<0.05$) of IBA concentrations and wounding treatment on the number of leaves per cutting (Table 5) wherein, significantly higher number of leaves was observed in cuttings treated with 2000 ppm IBA with recorded leaf number of 20.06, 22.5 and 30.17 at 86 DAT, 93 DAT and 100 DAT, respectively. The minimum leaf number was obtained from the control treatment during all the observations. The findings in this study are in accordance with Singh et al. (2013) who recorded maximum number of leaves (23.00) with stem cuttings of citrus Limon treated with IBA 2000 ppm among all the treatments. Similarly, Abebe (2017) also reported significantly higher number of leaves per cutting (25) on Cannonau variety of grapes at 2000 ppm IBA concentration. The IBA application at 2000 ppm produced healthier and lengthy roots causing greater absorption of water and nutrients from the growing media and might have resulted in active shoot growth which, probably led to the development of a greater number of leaves (Babaie et al., 2014).

The wounded cuttings exhibited 18.83, 20.69 and 25.06 leaf number at 86, 93 and 100 DAT respectively which was significantly higher than non-wounded cuttings. Since wounding increase the auxin entry efficiency, improving the number of roots in cuttings (Howard, 1972), this might have led to the increase in number of leaves. The findings in this study are in line with the Negi et al. (2015) who also observed highest number of leaves in M793 cuttings when treated with 2000 ppm and wounding. However, the interaction effect of IBA and wounding on average number of leaves at 86 DAT, 93 DAT and 100 DAT of cutting was statistically non-significant at 5% level of significance.

Conclusion

In conclusion, choosing the right concentration and proper technique of cutting is essential to ensure greater and successful rooting of apple cuttings. The results from this experiment indicate that the hardwood cuttings of MM111 apple rootstock can be induced to root by treating them with IBA and making a split wound at the base. Most of the rooting quality parameters like days to first sprouting, rooting percentage, total number of roots, length of the longest root, diameter of the main sprout and number of leaves can be most positively influenced by the administration of 2000 ppm IBA in wounded cuttings despite the interaction effect being non-significant except in total number of roots. Furthermore, the higher concentration of auxin was not beneficial for the rooting of MM111 cuttings. It is therefore suggested that for the rapid multiplication of MM111 apple rootstocks, it should be treated with a dose of 2000 ppm IBA along with wounding so that clonal rootstocks can be used instead of seedling rootstocks.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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