



ORIGINAL ARTICLE

Evaluation of Rooting of Stem Cuttings of *Magnolia soulangeana* Under Influence of Time and IBA Treatment

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KEYWORDS

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ABSTRACT: *Magnolia soulangeana* is a big shrub or a small tree with an irregular growth mode and a spring flowering tree whose large flowers appear before the emergence of leaves. Blossoms will be refloved in early summer. To evaluate the effect of different concentrations of indole butyric acid and the proper time of better rooting for *Magnolia soulangeana* cuttings, an experiment was conducted in 2013-2014 in Gorgan University of Agricultural Sciences and Natural Resources Research. Five prepared levels of indole butyric acid of zero, 1000, 2000, 4000, 6000 mg L⁻¹ and the bottom of cuttings were put in the above solution for five seconds, and then they were kept under mist system within the context of the greenhouse for 75 d and after that, they were removed for measuring the desired parameters. The highest percentage of rooting, the average number of roots, the average length of roots and establishment percentage related to 4000 mg L⁻¹ attendance was in late June. All traits under study were significant at the level of 1%. Cutting in late June had positive impact for all traits under study, such as rooting percentage, callusing percentage, root number, and root length. After all, the concentration of 4000 mg L⁻¹ of indole butyric acid in which the largest test number and highest percentage of survival can be seen is considered as one of the best hormonal treatments.

INTRODUCTION

Regarding the reduction of space allocated to green space, ornamental shrubs by having most benefits of the

trees, due to their smaller dimensions are economically more justifiable. The commercial production of this kind

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of ornamental plants has a satisfying effect in the economic horizon due to the existence of various weather conditions in different regions of Iran and the growing demand of the people [1]. Winter *Magnolia* with the scientific name of *Magnolia soulangeana* belongs to Magnoliaceae family. Large, purple and tulips like flowers of this tree will appear in the early Mar and decorate its bare and naked stems and its large and shiny leaves will appear in May. *M. soulangeana* will re-blossom in the early summer and its flowers appear sporadically [2, 3]. This shrub prefers rich and moist loamy soil with good drainage. In addition, it will be pruned and if needed form pruned after flowering in June [4].

M. soulangeana is suitable to plant the grass and as a single tree or in the street. It can be planted next to softwoods or as green background for flowering shrubs. The essence of the plant flowers and seed has an aromatic and oily substance that improves convulsion. This plant can be kept on the lawn surface, in the patio and the pot individually [4].

In commercial propagation of plants, the best way to reproduce the plant is selected with regard to factors such as the rate of propagation, the similarity with native plant and reasonable cost [5]. *M. soulangeana* decorative shrub can be reproduced by seed, green cutting, layering and grafting [6]. Commercial reproduction of the plant through seed is not recommended because viability seeds are low, they are limited to reproduce through cutting [7] but it is a hard rooting plant and it is not easily reproduced through cutting.

Using stem cutting is a kind of suitable vegetative reproduction for commercial reproduction of different ornamental plants and it has many benefits than sexual reproduction. This method is very simple and inexpensive and the derived plants are uniform. We can reproduce heterozygous plants in this way without changing their properties and appearance. This approach requires

a limited space and many new plants can be created from a number of native plants. In addition, the problem of seed dormancy in the sexual reproduction does not exist in the vegetative reproduction [5, 8, 9]. In cutting reproduction, we do not need particular ways of grafting and budding. Besides the problems in using grafting method, there is also a link, such as the incompatibility of stock and scion [5].

Several studies on the determination of stem exact place have been carried out from which adventitious root is derived. In general, the origin of adventitious roots, as endogenous is near the exterior of tissue vascular central core. Wooden perennial plants have one or more layers of secondary xylem and phloem vessels. Adventitious root originates from alive parenchymatous cells that are mainly available in secondary young phloem vessels, but sometimes the vessel radius, generating phloem layer, lenticel or the brain [5].

The capacity of a stem cutting for rooting is completed with interaction between genetics factors which are available in the stem cells and other factors such as the kind of native plants, the existence of leaves and buds, native plant age, type of the selected wood, rooting bed, the place of cutting, mist system, environmental conditions (the relations of water, light, temperature, photosynthesis and characteristics of rooting environment) cutting time, plant growth regulators, etc. [5, 10]. Therefore, this study was carried out twice for the sake of determining the best time for better rooting of *M. soulangeana* semi-woody cuttings.

Plant growth regulators have different effects on the plants, one of the most obvious one is the impact of rooting. These materials speed rooting and increase the percentage of rooting and number of roots as well as they make rooting more uniform. So far, the greatest impact has been observed on the rooting was by auxins. For this reason, nowadays, they have the most commercial usages and for stimulate root formation in the large areas of mist system [5, 11]. Auxin causes

the transfer of carbohydrates from the leaves to rooting area and facilitates rooting by stimulating cell division in these areas [12]. Since rooting power has a direct relationship with the available concentration in the callus tissue on the bottom of the cuttings, hard rooting plants can be forced to rooting by using this phenomenon. Naturally or artificially using auxin is the requirement of starting adventitious roots and division of first cells root developers are dependent on the existence of internal and external auxin [5].

In a research on *Erythrina crista-galli* shrub, four levels of indole butyric acid were used [13]. The concentration of 4000 mg L⁻¹ was introduced as the most appropriate level. In addition, the highest percentage of rooting (83.3%) and the greatest number of roots was observed in this treatment. The results indicated the negative effect of the high concentration of indole butyric acid (6000 mg L⁻¹) on most of the parameters.

The existence of young leaves increases root formation root in the cutting and remove the leaves will reduce the percentage of rooting. Nitrogen and sugar materials produced in leaves are probably considered as a part of rooting cofactors. Moreover, phenolic compounds such as phenolic acids by interaction with auxin will cause the beginning of rooting [14]. For cutting, at least two healthy buds were stated the upper for producing branch and leaves and the lower there must be for producing roots. Moreover, if some buds were weak and lost, a third one must be replaced [15].

Cutting time can play an important role in rooting. Cutting time is related to the physiological condition of the plant is not related to any specific time [5, 12]. The time of terminal bud, and the highest activity or reproductive development during the growing season in different species are different, so cutting in the proper time can be quite significant [16].

Native plant nutrition plays a role in the development of roots and branches of taken cutting. Usually, nitrogen and carbohydrates in this regard are more important [5,

17]. There are many studies on a number of available carbohydrates in plants and the amount of rooting cuttings. The relationship between carbohydrate metabolisms in plants is mainly controlled by hormones. The storage amount of enough carbohydrates, nitrogen compounds and the proper ratio of both, express relatively high and clear impact of carbohydrate with rooting [18, 19]. Therefore, in the current study, selected native trees to provide stem cutting, about two months before cutting in eight-day intervals were sprayed with liquid fertilizer so that the trees have no deficiency.

The aim of this study was the propagation of *M.soulangeana* through stem cutting because the intended plant is hard rooting relatively and gardeners do not root its cuttings. In addition, we aimed to determine the best level of butyric indole acid treatment in scions rooting as well as determining the most suitable time for the propagation of stem cutting of this plant.

MATERIALS AND METHODS

Place of research

One of the campuses of the Gorgan University of Agricultural Sciences and Natural Resources Research greenhouses was used. This research was carried out in a completely randomized base form design with factorial arrangement of four repetitions and in each repetition 12 samples and at the time of late June and late Aug 2013. The intended greenhouse has cultivation tables, mist system that was fog nozzle by timer in one minute in every 1:45 min. The existence of this system in addition to supplying water of cultivation beds and cuttings increased the overall humidity and reduced the temperature in warm months at the greenhouse (Figure 1).

Factors examined in the study were as follows: Rooting percentage, the percentage of callus cuttings, root length average, average number of roots, length of the longest root, root fresh weight, root dry weight, callus wet weight, the number of newly formed buds on the cut-

ting, the number of newly formed leaves on cutting and the percentage of cuttings survival after transferring to the pot.

Preparing rootingbeds

Bed used in the experiment included cocopeat, perlite at a ratio of 1: 1. Required cocopeat solid blocks were placed in the water for 24 h to absorb enough water to be softened. Then a container was selected as a unit and it was prepared by intended uniform mixed volumetric ratios and was distributed to a height of 20 cm on cultivation benches (Figure 1).

Providing various indolebutyric acid concentrations

To provide the desired concentrations, the required grams of indole butyric acid powder must be poured into a container and then some 50% ethanol will be added to it and it was kept on the shaker for 15 min. After completely dissolving the hormone and getting transparent solution, it must be reached to the desired volume through balloon by 50% ethanol and then it was completely covered by foil in order to prevent direct shining of light to the solution and loss of indole butyric acid. To make cuttings conditions equal, control cuttings were placed in distilled water solution and ethanol 50% at 50 cc volumetric ratio to neutralize possible effect of ethanol on the rooting. In this study, five concentrations (zero, 1000, 2000, 4000, 6000 mg L⁻¹) indole butyric acid were prepared.

Cuttings providing stages

In order to prepare required cuttings for this experiment, samples were prepared from shrubs in the area of Gorgan University of Agricultural Sciences and Natural Resources. Cuttings were picked from maternal stock

with equal growth conditions (grown in Gorgan climate) free of pests and pathogens, and from the same species. *Magnolia* leafy cuttings were taken from all different directions of the tree and in the approximately same conditions as much as 10 to 15 cm and an approximately same diameter and they were immediately transferred to the greenhouse.

In order to preserve moisture and prevent decay, the leaves of the lower part of cuttings were removed and the bottom part of cuttings was diagonally cut with gardening scissors. Cuttings were placed in multiple bunches, using rapid submergence method prepared specified concentrations of auxin hormone for 5 sec and they were quite unevenly distributed in rooting beds intended in the pilot project. After planting the cuttings, in order to prevent fungal contamination in the cuttings and bed, a general commercial fungicide solution was used at a concentration of 2 kg/1000 L. This procedure was performed once per month.

Greenhouse temperature

First cutting time was in late June, in which greenhouse environment temperature was kept at a moderate level using a water cooler, opening windows and mist system. The maximum and minimum temperatures during the day were about 25 to 35 °C. During the second period of cutting, which was in late Aug, the temperature was maintained by using electric heater at a moderate level, despite reducing the temperature in Sep and Oct.

Statistical Analysis

Statistical analysis of data was carried out using SAS software (Chicago, IL, USA). The results were compared using LSD test and drawing graphs was conducted using Excel 2010.



Figure 1. *Magnolia soulangeana* morphology, 1: trees Form in early spring (the first flowering)
 2: The second round of flowering trees creating leaves in summer.
 3: Coco peat and perlite mixture in a ratio of 1: 1. 4: Place the research greenhouse equipped with mist system.

RESULTS

Based on analysis table of interaction effect variance of the hormone levels and time had a significant effect on rooting percentage, callusing percentage, root numbers, root length, root fresh weight, root dry weight, callus

fresh weight, buds number, leaf number, the length of the highest root and establishment percentage at 1% and 5% (Table 1).

Table 1. Analysis of variance of some growth characteristics of cuttings *Magnolia* plant affected by the different treatments IBA and time.

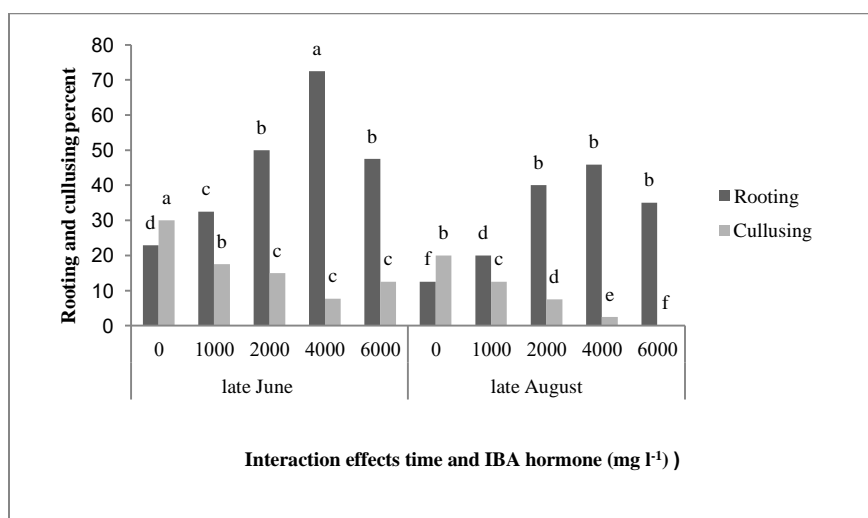
S.O.V	df	Rooting percentage	Callusing percentage	Number of roots	Root length	Root fresh weight	Root dry weight	Callus fresh weight	Number of bud	Number of leaf	Highest root length	Establishment percentage
Time	1	2078.35**	645.13**	45.09**	3.7*	0.1*	0.0009*	0.131*	0.027*	2.024**	34.22**	25660.30**
Hor-mone	4	2115.08**	516.31**	98.64**	27.66*	1.9*	0.027*	0.098*	0.465*	0.456**	154.01**	1332.49**
Time* Hor-mone	4	96.47*	20.77*	16.11*	3.075*	0.19*	0.005*	0.025*	0.796*	0.235*	38.209*	897.27*
Error	30	44.28	26.79	3.471	1.413	0.158	0.014	0.040	0.038	0.069	1.761	163.107
Cv	-	17.56	7.29	28	19.536	7.45	8/87	9.62	19.289	27.34	12.176	19.79

**Significant different at 1% level, *Significant different at 5% level, ^{ns} no significant different

Rooting percentage and callusing percentage

The maximum percentage of rooting (52.72%) is related to interaction effects of indole butyric acid 4000 mg L⁻¹ treatment and late June time. Interaction effects cutting time and rooting hormone had been significantly affected rooting percentage of cuttings. Statistically, the best percentage of rooting was obtained from cuttings

taken in late June and were treated at a concentration of 4000 mg L⁻¹ and the lowest percentage of rooting was obtained from cuttings taken in late August in the hormonal control treatment cuttings (Figure 2).



The highest amount of callusing percentage (30.02%) was related to interaction effects of hormonal control treatment and late June time.

Figure 2. Mean comparison of interaction effects IBA and different times on the rooting percentage and callusing percentage

Root numbers

The highest number of roots was related to interaction effects of indole butyric acid 4000 mg L⁻¹ treatment and late June time and the least number of roots were obtained in the hormonal control treatment in late August (Figure 3).

Root length

The maximum length of the root was related to interaction effects of indole butyric acid 4000 mg L⁻¹ treatment and late June time which were statistically significant difference with interaction effects of indole butyric acid 4000 mg L⁻¹ treatment and late August time. The least length of the root was obtained in the hormonal control cuttings in late August which statistically significant

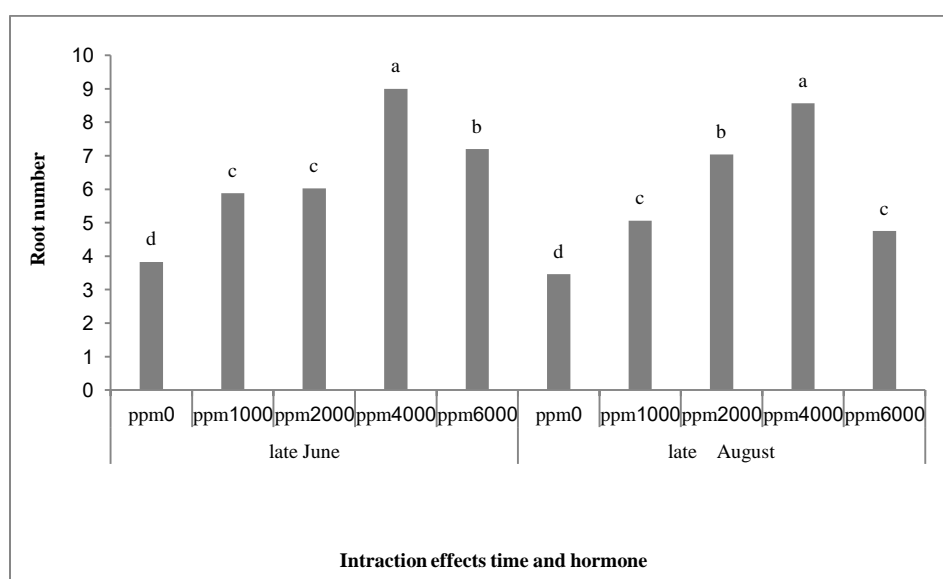


Figure 3. Mean comparison of interaction effects of IBA and different times on root number

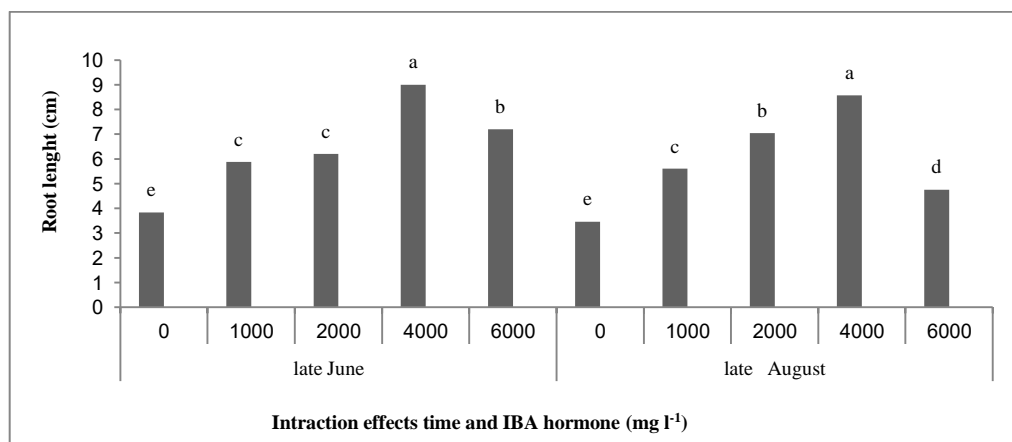


Figure 4. Mean comparison of interaction effects of IBA and different times on the root length

difference was not observed in the hormonal control treatment in late June time (Figure 4).

Root wet weight

The maximum weight of the root (1.87gr) was related to interaction effects of indole butyric acid 4000 mg L⁻¹ treatment and late Aug time and the least amount of it was related to control treatment and late August time (Figure 5).

Root dry weight and callus wetweight

The maximum amount of dry weight (0.25 gr) was related to interaction effects of indole butyric acid 4000 mg L⁻¹ treatment and late June time (Figure 6). The maximum amount of wet weight of callus (0.43 gr) was related to interaction effects of indole butyric acid 4000 mg L⁻¹ treatment and late Aug time and the least amount of it was related to control treatment and late Aug time (Figure 6).

The number of new buds and new leaves

The highest number of newly formed buds (1/8 pieces) was related to interaction effects of indole butyric acid 6000 mg L⁻¹ treatment and late June time and the least amount of it (0.47 pieces) was related to control treatment and late Junetime (Figure 7). The maximum amount of new leaves (1.53 pieces) was related to interaction effects of indole butyric acid 2000 mg L⁻¹ treatment and late Aug time which statistically did not have significant difference with indole butyric acid 1000 mg L⁻¹ treatment and late Aug time (Figure 7).

The length of the longest root

The longest root length (16.27 cm) was related to interaction effects of indole butyric acid 6000 mg L⁻¹ treatment

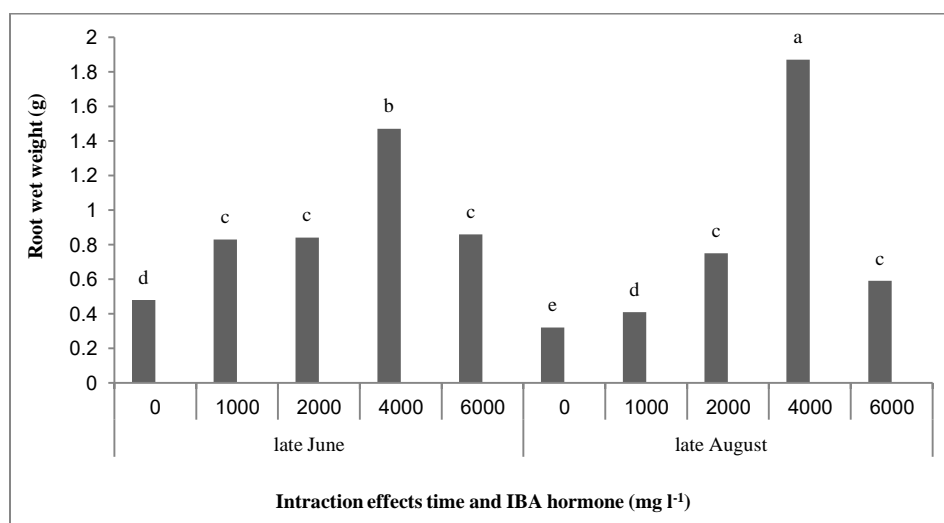


Figure 5. Mean comparison of interaction effects of IBA and different times on the root wet weight

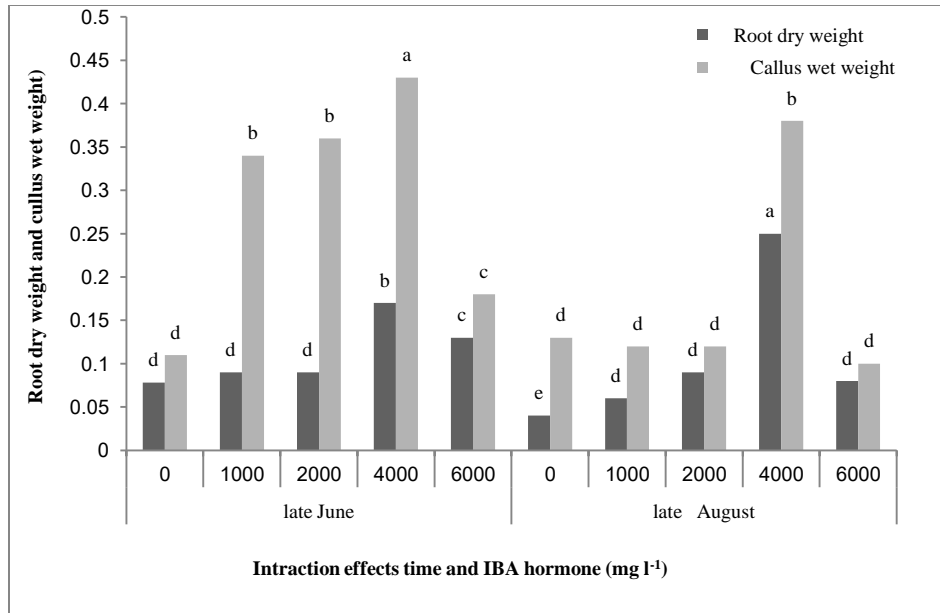


Figure 6. Means comparison of interaction effects of IBA and different time on the root dryweight and calluswet weight

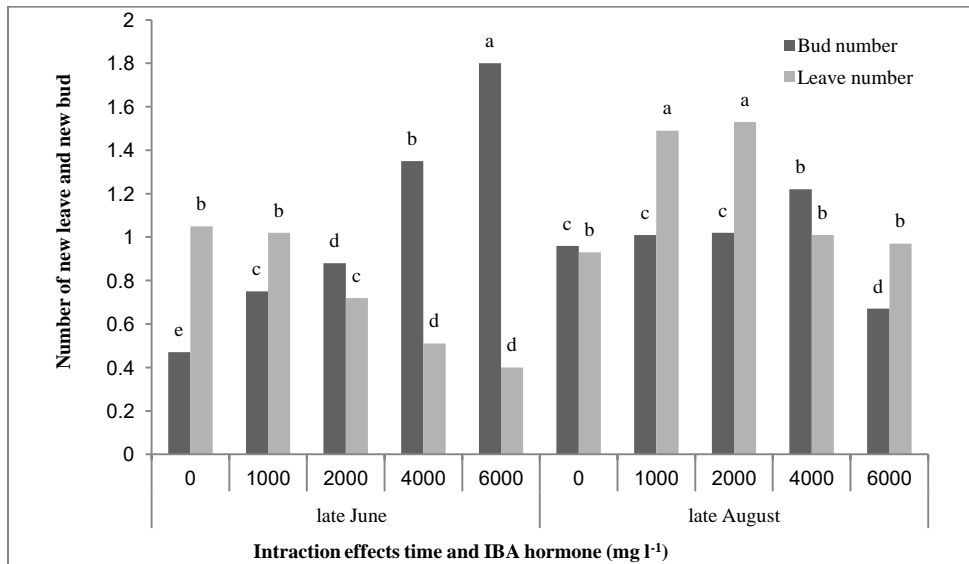


Figure 7. Means comparison of interaction effects of IBA and different time on the number of new bud and new leave

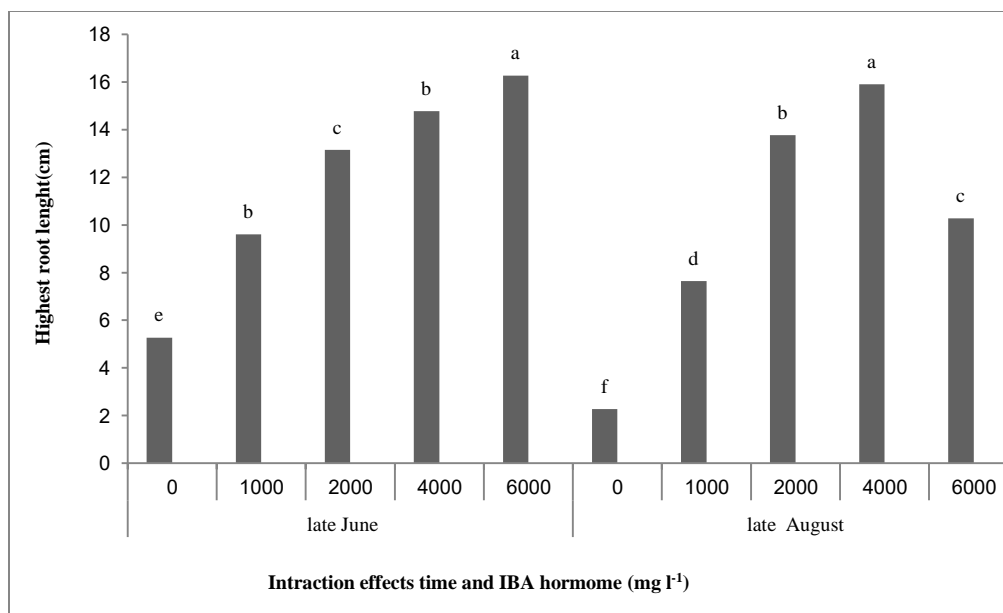


Figure 8. Means comparison of interaction effects of IBA and different time on the length of the longest root

and late June time which statistically did not have significant difference with indole butyric acid 4000 mg L⁻¹ treatment and late Aug time. The minimum amount of it (2.27 cm) was related to interaction effects of hormonal control treatment and late Aug time (Figure 8).

Establishment percentage

The highest rate of establishment percentage (100%) was related to interaction effects of indole butyric acid 4000 mg L⁻¹ treatment and late June time which statistically did not have significant difference with hormonal control treatment and late June time. The lowest rate of establishment percentage (8.35%) was related to interaction effects of control and late June time (Figure 9). An

example of the establishment of the cuttings is shown in Figures 10 and 11.

DISCUSSION

In this study, the best percentage of rooting was in cuttings taken in late June and were treated at the concentration of 4000 mg L⁻¹ and the lowest percentage of rooting were obtained from hormonal control treatment cuttings in late Aug. Significant differences between hormone and control treatments in both times show that if at the beginning of rooting, there was some auxin hormone in the environment, it can help to an increase of rooted cuttings percentage and play an effective role in accelerating rooting or cuttings mortality reduction. In cuttings taken in late June and cuttings treated at the

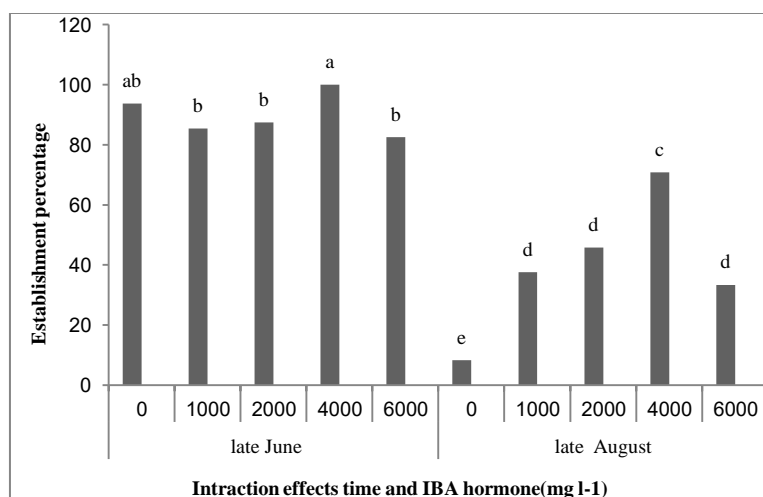


Figure 9. Means comparison of interaction effects of IBA and different time on the establishment percentage

concentration of 4000 mg L⁻¹ had a higher rooting percentage than other hormone treatments at this time. Late June taken cuttings show better response to auxin application and rooting percentage was increased compared to cuttings that did not apply auxin. Except for late August case in which hormone control treatment treated by 6000 mg L⁻¹ treatment at the same time that statistically did not have a significant difference whose possible reason may be the inhibitory effects of high concentrations of auxin hormone and hormone imbalance within cuttings[5].

The cuttings treatment with auxin hormones group along with other factors affecting cuttings rooting through its ability in stimulating root starters and increasing the transport of carbohydrates to the bottom of the cuttings root plays an important role in increasing the percentage of rooted cuttings, accelerating root generating and uniform rooting[5, 10]. Clearly, root induction in the cutting depends on high rates of the initial auxin in the plant that had a synergistic effect with the applied auxin, led to the synthesis of ribonucleic acid, and therefore will induce the production of root primordia. Using auxin has been seen in increasing rooting countless plants[18, 20]. There was no significant difference between hormonal treatments, but significant differences were observed between hormonal

and control treatments[21]. In this study, the highest percentage of rooting was in line with study on *Callistemon citrinus*[16]. Decreasing effect of higher concentrations of auxin optimal concentration may be attributed to its toxic effects. Similar results have also been proven in many studies[14, 22].

In examining the effect of different hormonal concentrations on cuttings rootings of some hard rooting kinds such as *C. citrinus* had the highest rooting percentage equivalent to 80% at a concentration of 6000 mg L⁻¹ as the superior concentration to lower concentrations of indole butyric acid [23]. In a similar research on *C. citrinus*, the interaction effect of auxin concentration and cutting time on rooting percentage was significant [24]. In this study, the positive effect of high concentrations of auxin in late June developmental stage (with higher amount of carbohydrates than late Aug) caused it to get the highest percentage of rooting that corresponded with the results of the present study.

As the first time was the proper time to obtain the highest root length average, all-hormonal treatments applied during late June have statistically significant difference with hormonal treatments applied in late Aug.

Increasing the concentration of auxin reduced the root length. This could possibly be attributed to the inhibitory effect of auxin at higher concentrations due to

the more production of ethylene [5, 25], since auxin plays an important role in increasing the number and length of root and the quality of produced roots. The high root length average or the longest root length in late June can be justified by high rates of auxin, internal cofactors or higher carbohydrate production in the plant that look suitable for rooting. Root length was reduced by an increase of auxin concentration in late June and late August that represents the inhibitory effect of auxin in higher concentrations.

The lowest percentage of establishment (8.35%) is related to the interaction effects of hormonal control treatment and late August time. Semi-hard wooden cutting is leafy cutting of deciduous or evergreen wood plants

semi-ripped of broadleaf and narrow-leaf trees usually taken in the summer from new branches and immediately after a period of growth in which branches are semi-ripped [17, 26]. Semi-hard wooden cuttings can be made at any time of year, but late spring is more suitable for rooting cuttings [27], which corresponded with the results of this research.



Figure 10. Establishment percentage of cuttings after one month



Figure 11. Establishment cuttings after seven month

The reduction of leaf temperature and high humidity of the upper side of cuttings are the main factors of rooting in using mist systems[19]. In the research on *Bougainvillea glabra* cuttings, using mist system with an increase of relative humidity and evaporation and transpiration reduction have significantly increased the water potential of *B. glabra* cuttings, therefore, the cuttings were cultivated under mist system[28].

CONCLUSION

In the research in which five different concentrations of indole butyric acid were investigated in two cutting times. Cutting in late June regarding all traits under study such as rooting percentage, the percentage of callusing, the number of roots, root length, root wet weight, root dry weight, callus wet weight, the number of obtained young shoots, the number of obtained leaves and length of the longest root had a clear positive impact. In the case of hormonal treatments, using auxin treatment in many positive parameters showed an absolutely certain impact than the time auxin was not used. Lower concentration can be considered appropriate. There is the possibility of losing many rooted cuttings, after transferring to soil because of breaking the root or its poor development, factors such as the number of roots and above all, the percentage of survival after transferring to the pot must be considered. After all, the concentration of 4000 mg L⁻¹ of indole butyric acid in which the largest test number and highest percentage of survival can be seen is considered as one of the best hormonal treatments.

Conflict of interests

The authors declare that there is no conflict of interests.

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