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Effects of IBA, NAA and GA3 on rooting and morphological features of *Ginkgo biloba* Linn. stem cuttings

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Abstract
This study analyzed the potential of production *Ginkgo biloba* Linn. using stem cuttings. Three different Hormones (IBA, NAA, and GA_3) along with control condition were applied to the cuttings, with and without buds, in three doses (1000mg/L, 2000 mg/L and 3000 mg/L) and after one growing season, Eight morphological characteristics of newly generated plants were detected, and a statistical analysis was carried out. All the application developed more number of roots than that of the control group, while all the applications developed longer roots compared to the control group, and the roots of the saplings developed with the 2000 mg/L IBA application were twice longer than those of the control group indicates that the hormone application have a great effect on root development.

Keywords: morphological, rooting, *Ginkgo biloba*

Introduction
Ginkgo biloba Linn. commonly known as Maiden hair tree belongs to family Ginkgoaceae. It is the only surviving species of the Ginkgoaceae family. Ginkgo is world's oldest tree mostly known as living fossils and only surviving member of seed plant groups. Ginkgo fossils were found from the Permian period which are identical to the living tree, called a living fossil. The tree is widely cultivated and introduced, since an early period in human history. Gingko naturally in very limited localities in the central Himalayan Mountain at an elevation of 6000 ft. (Bitencourt *et. al*, 2010) [3]. Despite of having huge medicinal properties and ornamental value, this species still has not received much attention as far as conservation is concerned particularly in India.

It has also been commonly cultivated in North America for over 200 years, but during that time it has never become significantly naturalized. In central Himalayan Mountains of India, there are very few spots in Kalimpong and Sikkim, where the individuals of this species are found growing naturally and require immediate conservation measures. Countries like China, Europe, France, and Germany are planting in large area. In China the nuts of the tree were most commonly recommended and used to treat respiratory tract ailments. A tea of the leaves was occasionally used for elderly persons experiencing memory loss. Ginkgo has wide application for treating various forms of vascular and neurological disease. It is also used in Vertigo, headache, tinnitus, inner ear disturbances including partial deafness impairment of memory and ability to concentrate. It is also used in anxiety, depression, neurological disorders complications of stroke and skull injuries, diminished sight and hearing ability due to vascular insufficiency. It also used for treating sclerosis of cerebral arteries with and without mental manifestations.

Due to poor regeneration, only few individuals exists in the nature particularly in diverse climatic conditions in different places and facing serious threat of extinction from central Himalayan mountains of India. Since this is a rare species and has got the status of oldest living tree fossils and therefore these species require urgent propagation protocol for large-scale multiplication. Rooting of stem cuttings provides the advantage of greater genetic uniformity and availability of superior stock in a short period of time for afforestation works (Ansari *et. al*, 1995) [1]. Many researchers used the growth regulators and growth hormones for propagating the plants. Plant hormones affect gene expression and transcription levels, cellular division, and growth.

Materials and Methods
Ginkgo biloba cuttings used in this research were collected from the forest of Sikkim. The cuttings were collected on October, moisturized, and stored in germination turf. The

The applications on the cuttings brought to the nursery are as explained below:

a) Two-thirds of the 3 x 3 x 15cm polyethylene tubes were filled with sand, soil and FMY at the ratio of 1:1:1.
b) The cuttings were cut to 2.5 cm long pieces using sterile lancets and grouped as cuttings with and without buds.
c) Solutions of three different hormones at different doses (1000 mg/L, 2000 mg/L and 3000 mg/L) were applied to the cuttings, respectively, and nine application groups were created, consisting of eight hormones application groups and a control group. The applications were carried out by imbruing the cuttings in hormone for 4 to 5 minutes. This application was composed of 3 replications and 30 cuttings in each replicate.
d) Measurements were carried out after one growing season. The roots were cautiously cleaned and the number of the roots was defined. The average length of the roots was measured using a digital microcompass. After this procedure, stems length were measured. Ten morphological characteristics for each cutting were defined at the end of the study, including rooting percentage (RP), seedling length (SL), number of roots (RN), and length of root (RL), Fresh root weight (FRW), Dry root weight (DRW), Fresh shoot weight (FSW), Dry shoot weight (DSW).

Variance analysis was applied on the data, using the SPSS 17.0 package program. The Duncan test was applied for the characteristics with at least 95% level of statistical reliance, and as a result, homogenous groups were acquired and interpreted.

Results

At the end of the study, the result implies that the cuttings with small buds must be used while producing *Ginkgo biloba* with stem cuttings. Hormone applications in different doses affect the germinated percentages, as well as the characteristics of the germinated individuals, at different levels. The data acquired with the result of the study, the result of variance analysis applied on these data, and the Duncan test are given in table 1.

When the values stated in the table were analyzed, the highest germination percentage (76.47) were acquired with 2000mg/L IBA followed by 72.33 in 2000 mg/L NAA hormone applications. The values acquired as a result of these applications are higher than the germination vales acquired in the control group; however, according to the result of Duncan test, these values show non-significant difference with all the hormone treatment as well as the control.

Table 1: Mean values of characters, results of variance analyzed, and the Duncan test**.**

Hormone	Dose (ppm)	G.P	S.L	N.R	R.L	F.R.W	D.R.W	F.S.W	D.S.W
Control	0	30.5	1.46	1.0	1.4	1.33	0.79	1.51	0.98
IBA	1000	73	1.97	3.67	2.58	1.89	1	2.46	1.23
IBA	2000	76.47	2.03	4	3.18	2.05	1.01	3.55	1.36
IBA	3000	69.27	1.97	3.33	2.38	1.56	0.9	1.92	1.14
NAA	1000	72	1.8	3.67	2.4	1.75	0.96	2.03	1.22
NAA	2000	72.33	1.67	3.33	2.39	1.6	0.95	1.94	1.14
NAA	3000	70	1.29	3	2.13	1.44	0.89	1.79	1.11
GA_3	1000	68.32	0.9	2.33	1.45	1.35	0.81	1.61	1.01
GA_3	2000	69	1.23	2.33	1.63	1.39	0.85	1.62	1.05
GA_3	3000	68.2	1.59	3	1.95	1.39	0.86	1.67	1.07
F-test		S	S	S	S	S	S	S	S
S.Ed. (±)		2.493	0.25	0.38	0.403	0.056	0.01	0.38	0.08
C.D. (P=0.05)		4.427	0.55	0.81	0.85	0.02	0.02	0.81	0.17

GP: Germination Percent S.L: Shoot Length N.R: Number of Roots F.R.W: Fresh Root Weight D.R.W: Dry Root Weight F.S.W: Fresh Shoot Weight D.S.W: Dry Shoot Weight

When the value stated in the Table 1 were analyzed, it is observed that the longest seedlings with 2.03 cm stem length were produced with the 2000 mg/L IBA application. The seedlings produced in the control group have 1.46 cm stem lengths.

Even though the stem length is an important indicator of the sapling quality, root generation is also very important for a healthy sapling. The sapling that can generate hairy roots are generally accepted to be healthier, and the saplings that can generate taproot in a short span of time reach ground water more easily in the natural environmental, and thus, their chances of survival increases.

Accordingly, roots generation in one of the most important sapling quality indicators. According to the results of the study, the saplings in the control developed 1.0 cm roots with an average length of 1.40 cm, while the saplings receiving the 2000 mg/L IBA application developed 4 roots with an average length of 3.18 cm. The saplings that received the 3000 mg/L IBA application had 3.33 roots with an average length of 2.38 cm. The saplings that received the 3000 mg/L GA3 application developed 1.59 roots with an average length of 3.00 cm.

The result of the study shows that the hormone applications have a great effect on root development. The fact all the application developed more number of roots than that of the control group, while all the applications developed longer roots compared to the control group, and the roots of the saplings developed with the 2000 mg/L IBA application were twice longer than those of the control group indicates that the hormone application have a great effect on root development.

When the effect of hormone application on Dry root weight and Fresh root weight is analyzed, it is observed that the IBA application have a high effect on both the Dry and Fresh root weight. It was observed that the root exposed to 2000 mg/L IBA has the most Dry and Fresh root weight of 1.01 and 3.55 respectively. Whereas the Dry and Fresh root weigh in the control group was observed to be 0.79 and 1.51.

The highest value of Fresh shoot weight i.e. 3.55 and Dry shoot weight i.e.1. 36 were acquired with the 2000 mg/L IBA application, whereas in the control group the Fresh shoot weight was 1.51 and Dry shoot weight was 0.98. As a result the hormone application has more effect then the control group.

Discussion

The result of the study, demonstrate that cuttings one year old shoot must be used in order to produce *Ginkgo biloba* using stem cuttings. The result of the applications show that the auxin group of hormones (IBA,GA_3 and NAA), the subject of this study, shows an apparent effect on rooting rate but it greatly have an effect on the morphological characteristic of newly generated seedlings. Root development in particular reached significantly different values in the seedlings that received the auxin group of hormones (Kerketta and Wani 2016) [7].

The process of adventitious root formation is influenced by a number of internal and external factors. Among the internal factors, the most important role is ascribed to phytohormones, especially the auxins. It is generally accepted that auxins have a certain role in the rooting initiation (Štefančič, Štampar, and Osterc, 2005) [8]. Auxins control growth and development in plants, including lateral root initiation and root gravity response. Many studies have shown that exogenous application of auxins results in increase initiation of lateral roots and that lateral root development is highly dependent on auxin and auxin transport (Chhun, *et al.*, 2003) [4].

Gibberellins are in the third place with a 17% share among the most commonly used herbal hormones within the natural plants growth regulators. The results of the study reveal that the length of seedlings receiving 2000 mg/L GA3 application is three times greater than that of the control group in terms of stem length and 71% greater in total length. Commercially the most common gibberellin is GA^3, and it is used to increase the length of the plant or to enhance plant yield (Kumlay and Eryiğit, 2011) [6].

Effect of GA^3 on rooting was also analyzed in several studies. The effectiveness of GA^3 on *Prunus avium* L. and *Prunus mahaleb* was analyzed by Hepakosy 2004 and Aygün and Dumanoğlu, 2006 [2] on *Cydonia oblongna*,. However, on appearance GA3 efficiency rooting was detected in several plants.

Conclusion

Study revealed that the potential production of *Ginkgo biloba* Linn. Using stem cuttings with three different Hormones (IBA, NAA, and GA_3) concentration along with control condition were applied to the cuttings, with and without buds, in three doses (1000mg/L, 2000 mg/L and 3000 mg/L) and after one growing season, eight morphological characteristics of newly generated plants were detected, and a statistical analysis was carried out. It was observed that the longest seedlings with 2.03 cm stem length were produced with the 2000 mg/L of IBA application. The saplings receiving the 2000 mg/L IBA application developed 4 roots with an average length of 3.18 cm. Fresh shoot weight i.e. 3.55 and Dry shoot weight i.e.1 .36 were acquired with the 2000 mg/L IBA application.

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