

Full Length Research Paper

Role of auxin on adventitious root formation and subsequent growth of cutting raised plantlets of *Ginkgo biloba* L.

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Role of some auxins, indol-3-butyric acid (IBA) and α -naphthalene acetic acid (NAA) has been examined for their stimulatory effects on adventitious root formation in stem cuttings of *Ginkgo biloba* as well as on subsequent growth and survival of these cutting raised plantlets. Lower concentration of IBA (10.0 μ M) was found to be the most effective treatment as it not only induced maximum rooting (88.89%) but also enhanced number of roots, length of roots and length of longest root to the maximum. Further, the growth performance of these cutting raised plantlets, via control and IBA treatment (best treatment), was compared. It was found that the IBA treated plantlets were morphologically healthy in terms of their shoot height, diameter of shoot, number of nodes per cutting, number of leaves/node and number of branches per cutting than the control plants. Besides this, the survival rate of IBA treated plantlets was 100% in comparison to control where it was 87.5%. Therefore it was concluded that IBA treatment not only improve the percent rooting but also improve the subsequent growth and survival rate of the plantlets of *G. biloba*.

Key words: Auxins, indol-3-butyric acid (IBA); rare species, propagation, stems cuttings, survival.

INTRODUCTION

The sole survivor of the ancient family of Ginkgoaceae; living fossil-*Ginkgo biloba* L. (common name-Maidenhair Tree) is the world's oldest living gymnosperm tree species with high medicinal properties (Seward and Gowan, 1900; Bailey, 1923; Dallimore and Jackson, 1948). In the central Himalayan Mountains, *G. biloba* has been reported at an elevation of 6000 ft (Anonymous, 1999) and there are very few spots in Uttarakhand (that is, Ranikhet, Nainital and Dehradun) where the individuals of this species are found growing naturally (Purohit et al., 2009).

A combination of resistance to diseases, insect-resistant wood and the ability to form aerial roots and sprouts makes Ginkgo a very long-lived tree with some specimens claimed to be more than 2,500 years old. Seeds are edible and highly nutritive. The extract of *Ginkgo* leaves contains flavonoids, glycosides and

terpenoids (ginkgolides, bilobalides) and has been used pharmaceutically. It has many nootropic properties and mainly used as memory and concentration enhancer, anti-vertigo agent (Mahadevan and Park 2008). Ginkgo is an effective treatment for arresting the development of vitiligo (Prasad, 2003). It is also used for treating eye problem, altitude sickness, asthma, depression, headache, high blood pressure etc. (Purohit et al., 2009). Ginkgo has a long reproductive cycle and it takes two years to complete it. Due to its high medicinal value the tree has been exploited indiscriminately so facing a high risk of extinction and was listed as a rare species in the 1997 IUCN red list of threatened plants and listed in the red list of endangered plant species.

Vegetative propagation via stem cuttings offers true-to-type plants and availability of superior individuals in a short period of time for large scale commercial plantation. This method has been tried in *Taxus baccata* (Nandi et al., 1996), *Cedrus deodara* (Nandi et al., 2002; Tamta et al., 2007) and in many other gymnosperms. The vegetative propagation studies on *G. biloba* have been reported by Doran (1954), Dirr and Heuser (1987), and

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Natalia (1994) by using auxins. In recent years, there are few more reports where IBA, bavistin, phenolic compounds either alone or in combination with auxins were applied to induce rooting in stem cuttings of *G. biloba* (Prakash et al., 2002; Gopichand et al., 2006; Purohit et al., 2009).

Due to high medicinal value and economic importance of *G. biloba*, its cultivation could not only play a major role to uplift the socio-economic status of people of this area, its propagation via stem cuttings would also help in the conservation of this rare and endangered species. In view of this, an efficient and cost effective investigation was carried out to examine the role of two auxins-indol-3 butyric acid (IBA) and α -naphthalene acetic acid (NAA), for their stimulatory effects on adventitious root formation in stem cuttings of *G. biloba* as well as the role of IBA on subsequent growth and survival of these cutting raised plantlets under poly house conditions.

MATERIALS AND METHODS

Collection of plant material

Semi-hard wood terminal cuttings were taken during the month of September (2007) from growing shoots of a well mature elite tree of *G. biloba* from snow view area (2270 m above mean sea level) in District Nainital, Uttarakhand, India. Cuttings were brought to the laboratory in perforated polythene bags and used within 2 h after collection.

Preparation of explants

The final cuttings were prepared (15.5±0.65 cm length and 0.89±0.23 mm diameter without lateral shoots) by removing leaves and leaving intact leaf buds in each cutting.

Explant treatment

The cuttings were treated with test compounds by dipping their basal 3.0 cm portions in various test solutions for 24 h at room temperature (20±1°C). The solutions included indol-3-butyric acid (IBA; 10.0 and 100.0 μ M), and α -naphthalene acetic acid (NAA; 10.0 and 100.0 μ M). IBA and NAA were obtained from Himedia Laboratory Pvt. Ltd, Mumbai, India. One untreated set of cuttings served as control. Chemicals were dissolved in 1.5% (v/v) aqueous ethanol; control cuttings were dipped in distilled water. Each treatment consisted of 27 cuttings.

Rooting conditions

Treated cuttings were planted vertically in polythene bags (16.0 cm height \times 8.0 cm diameter; one cutting per bag) containing sieved oak forest soil and kept inside the polyhouse, made up of semi-transparent polyethylene sheet (thickness: 162.5 μ m, UV stabilized), established at Department of Botany, Kumaun University, Nainital, Uttarakhand, India between 2921' to 2924' N and 7925' to 79 20' E; 2150 m amsl. The mean temperature and relative humidity inside the polyhouse were 25±2°C and 60 to 75%, respectively during the experiment. The cuttings were watered regularly.

Data recording

Based on random observations, root initiation in majority of cuttings was first noticed after about 12 weeks, and therefore, the final data were recorded at 16 weeks after planting of cuttings. Cuttings with roots \geq 1 mm were considered to have rooted and included for calculating percent rooting. Root initials \geq 1 cm were considered for calculating mean number of roots. After data recording, these plantlets were re-transplanted in polythene bags (20 cm height \times 12 cm diameter) containing same sieved oak forest soil and kept inside the polyhouse to monitor the growth and survival of rooted cuttings.

Growth and survival of rooted cuttings

All the rooted cuttings (from September 2007 experiment) were kept inside the polyhouse until the end of October 2009. To observe the growth data were recorded at 48 and 96 weeks after planting the rooted cuttings. For this percent survival, shoot height, diameter of shoot, number of nodes per cutting, number of leaves/node, and number of branches per cutting were recorded. To see the effect of auxin treatment we were concentrated only on those plantlets which were raised via IBA (10.0 μ M) treatment (best treatment) and then compared these with the rooted plantlets raised via control. After that the plantlets were shifted from polythene bags to earthen pots (24 cm height \times 24 cm diameter) containing the same sieved oak forest soil and kept inside the mist chamber (25±2°C mean temperature and 80% RH) until the end of May 2010. Then they were transferred to their natural habitat.

Statistical analysis

Each treatment was replicated three times (nine explants making one replicate). Standard error (SE) was calculated following the methods described by Snedecor and Cochran (1967) and the data were subjected to analysis of variance ANOVA by using SYSTAT of SPSS Inc., Chicago, USA.

RESULTS

The effect of treatments on rooting ability of stem cuttings of *G. biloba* has been observed and results on adventitious root formation are presented in Table 1. Although rooting was observed in all the treatments including control also but data revealed the maximum rooting response by lower concentration of IBA (10.0 μ M) followed by higher concentration of NAA (100.0 μ M) (Table 1). Root initiation in majority of cuttings is depicted in Figure 1A which was first noticed in about 12 weeks. Table 1 shows the rooting response of cuttings 16 weeks after treatment. The lower concentration of IBA (10.0 μ M) was more effective (88.89% rooting) than the higher concentration (100.0 μ M) of IBA (62.97% rooting). In the case of NAA, both the concentrations (10.0 and 100.0 μ M) also stimulated rooting (74.07 and 77.78%, respectively), however, it was slightly more effective at higher concentration (Table 1). Rooting was also observed in control (59.26%) but the response was very poor as the values for average number of roots, average length of roots and average length of longest root were very less in comparison to other treatments (Table 1).

Table 1. The effect of auxins on rooting of *G. biloba* stem cuttings 16 weeks after treatment and planting under poly house condition.

No.	Treatment	% rooting ^a ±SE	Average root no. per rooted cutting ^b ±SE	Average root length (cm) per rooted cutting ^b ±SE	Average length of longest root (cm) ±SE
1	Control	59.26±7.41	2.10±0.26	1.97±0.12	2.60±0.21
2	NAA (10.0 µM)	74.07±3.70	4.41±0.42	3.10±0.53	4.90±0.88
3	NAA (100.0 µM)	77.78±6.41	5.53±0.07	3.38±0.07	4.97±0.38
4	IBA (10.0 µM)	88.89±6.41	6.96±0.58	5.89±0.48	8.38±0.70
5	IBA (100.0 µM)	62.97±3.70	4.03±0.67	3.32±0.18	4.78±0.43
	f-value	4.30**	15.72*	18.0*	12.94*

^a: Cuttings with roots ≥ 1 mm were considered to have rooted and included for calculating percent rooting percent. ^b: Root initials ≥ 1 cm were considered for calculating average number of roots and average root length. SE: Standard error, *: level of significance at 1%, **: level of significance at 5%, All values are an average of 27 replicates.



Figure 1. Propagation of *G. biloba*: (A) Root initiation in majority of cuttings, 12 weeks after treatments; (B) Rooting in IBA (10.0 µM) treated stem cuttings after 16 weeks (C showing control cutting); (C) Cutting raised plantlets via IBA (10.0 µM) treatment 48 weeks after planting of cuttings; (D) Comparison of cutting raised plantlets via IBA (10.0 µM) and control, 96 weeks after planting of cuttings; (E) Cutting raised plantlets via IBA treatment planted in earthen pots 100 weeks after planting of cuttings (F) Well grown plantlet in the field.

Table 2. The effect of IBA on subsequent growth and survival of *G. biloba* cutting raised plantlets under poly house condition 48 and 96 weeks after planting of rooted cuttings.

Cutting-raised plants via	Time (weeks)	Shoot height (cm) \pm SE	Diameter of shoot (mm) \pm SE	No. of nodes per cutting \pm SE	No. of leaves/node \pm SE	No. of branches per cutting \pm SE	% survival
Control	0	15.5 \pm 0.65	0.89 \pm 0.23	3 \pm 1	-	-	
	48	19.2 \pm 0.85	0.83 \pm 0.01	2.2 \pm .133	11.6 \pm 1.80	1.8 \pm 0.2	87.5
	96	22.5 \pm 0.93	0.9 \pm 0.03	3.0 \pm .33	15.5 \pm 2.78	2.5 \pm 0.22	87.5
IBA (10.0 μ M)	0	15.5 \pm 0.65	0.89 \pm 0.23	3 \pm 1	-	-	
	48	45.3 \pm 4.45	0.97 \pm 0.02	3.9 \pm 0.48	45.8 \pm 4.35	2.8 \pm 0.33	100
	96	65.1 \pm 5.18	1.2 \pm 0.07	9.5 \pm 1.14	69.8 \pm 10.09	3.6 \pm 0.48	100

SE: Standard error, In control, all values for 48 and 96 weeks are an average of 16 cuttings; in IBA (10.0 μ M) all values for 48 and 96 weeks are an average of 24 cuttings. In zero weeks all values are an average of 27 cuttings.

Good root formation; indicated by average number of roots per cutting (6.96), average length of roots (5.89 cm) and average length of longest root (8.38 cm) was detected in IBA (10.0 μ M) treatment. Rooting in stem cuttings, 16 weeks after IBA treatment could be seen in Figure 1B. Therefore, on the basis of data it was concluded that the IBA (10.0 μ M) was the most effective treatment for induction of rooting in *G. biloba* stem cuttings. Figure 1C shows the growth of cutting raised plantlets via IBA treatment 48 weeks after planting of rooted cuttings. Significant variations in root parameters were noted among the treatments (Table 1).

The growth rate, measured in terms of increase in shoot height, diameter of shoot, number of nodes per cutting, number of leaves/node, number of branches per cutting, and percent survival of cutting-raised plantlets kept inside the polyhouse are summarized in Table 2. For this only control and IBA (10.0 μ M) treated (best treatment) plantlets were used. There was a tremendous difference between the growth of these two types of plantlets.

The average shoot height of plantlets raised via IBA treatment after 48 and 96 weeks of root induction was 45.3 and 65.1 cm, respectively in comparison to shoot height of plantlets raised via control where it was only 19.2 and 22.5 cm after 12 and 24 months of root induction, respectively. Similarly, the values for shoot parameters are also high for those plantlets which were raised via IBA treatment in comparison to control plantlets (Table 2). The difference in growth of these plantlets is clearly visible in Figure 1D. In this figure, one can easily see the difference in the roots also. However, the percent survival of control plantlets was good (87.5%) but it was 100% in case of plantlets which were raised via IBA treatment (Table 2). Figure 1E reveals the growth of cutting raised plantlets via IBA treatment in earthen pots 100 weeks after planting of rooted cuttings and when they were transferred to the field they grew well (Figure 1F).

DISCUSSION

In general, propagation of gymnosperms is difficult because of low rooting efficiency. However some success in rooting in stems cuttings of *Pinus* species (Smith and Thorpe, 1975), *G. biloba* (Dirr and Heuser, 1987), *T. baccata* (Nandi et al., 1996), and *C. deodara* (Nandi et al., 2002; Tamta et al. 2007) has been reported. Application of auxins enhanced rooting and root quality in many tree species (Hartman and Kester, 1983). The application of IBA may have an indirect influence by enhancing the speed of translocation and movement of sugar to the base of cuttings and consequently stimulate rooting (Haissig, 1974). In the present study, it was observed that initially almost all treatments including control were able to induce rooting in cuttings, however the percentage of rooting vary with the treatments, and the application of IBA was found very effective. IBA has also been reported to be more effective for root induction in stem cuttings of *T. baccata* (Nandi et al., 1996, 1997) and *C. deodara* (Nandi et al., 2002). Out of two concentrations, lower concentration of IBA (10.0 μ M) was found to be more effective. Similar observation was recorded by Tamta et al. (2007). Root initiation in stem cuttings of *G. biloba*, in this experiment, occurred only 12 weeks (3 months) after treatment whereas (Purohit et al., 2009) reported 6 months time for the induction of rooting in this species; therefore in our experiment it was three months earlier.

Although stem cuttings without any treatment (control cuttings) were also able to root but the rooting percentage was low in comparison to auxin treated cuttings and when the growth of cutting-raised plantlets via control and lower concentration of IBA was compared then it was found that the cutting-raised plantlets via IBA treatment were healthy and grew well with 100% survival. Their root growth was also good. Such type of reports were also available in Scarlet Oak seedlings given by Struve and Moser (1984) where they found 6 fold

increase in adventitious root regeneration by auxin treatment as compare to control seedlings. Present investigation showed that a sufficient number of healthy plantlets of *G. biloba* with 100% survival may be produced by applying IBA treatment within short period of time which could be an easy, cost effective, rapid and promising method of macro propagation.

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