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Research article

Effect of hormonal concentration on rooting behaviour of *Ginkgo biloba* L. in agro-climatic zone of Dehradun, Uttarakhand, India

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Abstract: A survey was conducted in the state of Uttarakhand for the collection of *Ginkgo biloba*. Samples of *G. biloba* were collected from the Botany Department (DSB College, Kumaon University, Glenthorn Compound, High Court Campus), Old Rajbhawan (Snow View), and Rajbhawan (Nainital) of Uttarakhand. Macro-propagation studies were conducted using stem cuttings of dioecious plants. The cuttings were thoroughly washed with running tap water to remove soil particles. One set of male and female stem cuttings was kept as control. The male and female stem cuttings were dipped in different concentrations (100, 200, 300 and 500 ppm) of Indole Butyric Acid (IBA), Indole Acetic Acid (IAA) and Napthalene Acetic Acid (NAA) for 4 h. Cuttings were then implanted in hycotrays (root trainers) containing soilrite. Indole Butyric Acid at 300 ppm was found to be the best for rooting of both male and female stem cuttings in *G. biloba*.

Keywords: *Ginkgo biloba* - Macro-propagation - Dioecious - Stem cuttings - Indole Butyric Acid.

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INTRODUCTION

Ginkgo biloba L. is a dioecious species, although occasional monoecious individual were also reported (Santamour *et al*. 1983, Kumar & Sati 2016), which belongs to the family Ginkgoaceae and is the most ancient living gymnosperm (Hsieh 1992, Wei *et al*. 2007). Mainly distributed in Europe, China, Korea, France, Germany and the United States, this species has also been reported in the central Himalayan mountains at an elevation of 6000 ft (Bitencourt *et al*. 2010), and occurs in the hilly tracts from northwest to northeastern region of the country (Singh *et al*. 2008). In India, *G. biloba* grows naturally in Uttarakhand (Dehradun, Nainital, Mussoorie and Ranikhet), Himachal Pradesh (Kalpa, Manali and Shimla), West Bengal (Darjeeling and Kalimpong), Punjab (Patiala) and Meghalaya (Shillong) (Roychoudhury & Mishra 2016). The flowering starts in the month of April to May, and the seeds ripen from October to November (Kumar & Sati 2016). In human beings, various disorders such as, Alzheimer's disease, falling memory, age-related dementias, poor cerebral blood circulation, oxygen deprivation in nerve cells, clot formation, congestive premenstrual syndrome, high altitude sickness, asthma, bronchitis, free radicals, wound and neuromotor disorders - therapeutic activity and protective properties were exhibited by the *Ginkgo* extracts. *Ginkgo* was used to carry out the primary treatment against the cerebrovascular dysfunctions and peripheral vascular disorders, as it is having potent antioxidant properties which enhances peripheral and cerebral circulation (Kleijnen & Knipschild 1992). The tree pursued extremely slow growing nature with very poor regeneration capacity, as seeds are recalcitrant and not able to maintain germinability for a long time period. The sexual reproduction shows some difficulties due to dioecy habit and leaves are in huge demand by the pharmaceutical industry. Although, the conventional propagation methods found slow and unable to meet these demands but vegetative propagation through *in vitro* techniques and other methods were found to be most appropriate (Doran 1954, Dirr & Heuser 1987, Tredici 1992, Natalia 1994, Tommasi *et al*. 2004, Bitencourt *et al*. 2010, Isah 2015). The use of tissue culture for the propagation of

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many species has gained importance during the last two decades, but the propagation through *in vitro* techniques is not enough on the medicinal and ornamental importance of this fossil plant yet. Most of the studies conducted so far have used many types of explants, which includes embryos. Meanwhile, the use of vegetative explants has not been widely investigated (Nacheva *et al*. 2017). The present investigation is conducted to survey the areas of occurrence of *G. biloba* for the reliable identification of collection sites located in the Himalayan mountains of Uttarakhand. Further, standardization work was undertaken on the multiplication of *G. biloba* through rooting of stem cuttings (Macro-propagation).

MATERIALS AND METHODS

Description of the study area

The macro-propagation study was carried out in the nursery of Tissue Culture Discipline, Genetics and Tree Improvement Division, Forest Research Institute, Dehradun. The area falls under humid subtropical climate with annual rainfall of 2073 mm.

Sample collection

A survey was conducted for the collection of plant material from the Uttarakhand Himalayas. Three female trees were located at the Botany Department of DSB College (Kumaon University), Glenthorn Compound (High Court Campus), and Old Rajbhawan (Snow View); whereas one male tree was come across in the campus of Rajbhawan, Nainital. Also, four trees was observed in the Forest Research Institute (Dehradun) having no record available about their sex, as they never saw in flowering. Intensive field work was carried out for the collection of vegetative material *viz*. stem cuttings and young shoots of *G. biloba* from the aforesaid places. Standard packing of vegetative material was practiced for bringing samples from far-flung places to FRI Dehradun.

Experimental setup

Experiments were carried out at the nursery of Tissue Culture Discipline of Genetics & Tree Improvement Division of FRI during February and March of each year onwards from 2015, 2016, 2017 and 2018 (Fig. 1, 2). The stem cuttings of male and female plants of *G. biloba* were thoroughly washed with running tap water to remove soil and dust particles adhered to the surface. The experiments were laid out in Completely Randomized Design (CRD) with five treatments, and each treatment consisted of 100 cuttings. The male and female stem cuttings were dipped in distilled water served as control. The male and female stem cuttings were also dipped in varied hormonal concentrations (100, 200, 300 and 500 ppm) of Indole 3-Butyric Acid (IBA), Indole 3-Acetic Acid (IAA) and Naphthalene Acetic Acid (NAA) for 4 hours and implanted in hycotrays containing soilrite. The data were recorded for the root growth parameters *viz.* number of adventitious roots and root length after 180 days in the month of August-September of each year. The data recorded were pooled over the years (2015–2018) and subjected to Analysis of Variance (One-Way ANOVA) with the treatment means were compared through Duncan Multiple Range Test (DMRT) using software SPSS Version 16.0 (SPSS Inc. 233 S. Wacker Drive, 11th Floor, Chicago, Il 60606-6307).

RESULTS

The analysis of variance revealed significant difference ($p < 0.05$) of various auxins concentrations for rooting in *Ginkgo biloba* (Table 1). In August-September (pooled over the years), maximum rooting (15±0.91) of male genotypes was observed at 500 ppm of IBA followed by 300 ppm (12±1.08). In case of female cuttings, maximum rooting (40±0.71) was observed at 300 ppm of IBA followed by 500 ppm (30±0.91) and 200 ppm (15±1.08). Rooting was also observed in all the other concentration of hormones used during the experiment (100 and 200 ppm for IBA; 100, 200, 300, and 500 ppm for IAA and NAA, respectively). In case of DMRT, concentrations of IBA (100, 200, 300, and 500 ppm) showed significant differences with each other for both male and female cuttings, respectively. With IAA, at 300 ppm, male and female cuttings showed significant difference with other hormonal concentration. All the concentration of NAA, except 100 ppm are at par with each other for male and female cuttings, respectively (Table 2).

The analysis of variance showed significant difference ($p < 0.05$) between various auxins concentrations on rooting between male and female individuals of *G. biloba* (Table 3). The effect of various concentrations of auxin on rooting of male and female genotypes of *G. biloba* after 6 months are presented in table 4. For male genotypes, in August–September (pooled over the years), maximum rooting (15±1.08) was observed at 300 ppm followed by 500 ppm (12±0.91) and 300 ppm (6±0.41) of IBA. Minimum (0±0.0) was recorded for 100 ppm followed by 200 ppm (2±0.41) of NAA and 100 ppm of IAA (2±0.41). In case of female cuttings, maximum

rooting (40±0.71) was observed at 300 ppm followed by 500 ppm (30±0.91) and 200 ppm (15±1.08) of IBA. Minimum (0±0.0) was observed at 100 ppm of NAA followed by 100 (4±0.41) and 200 ppm (5±0.71) of IAA.

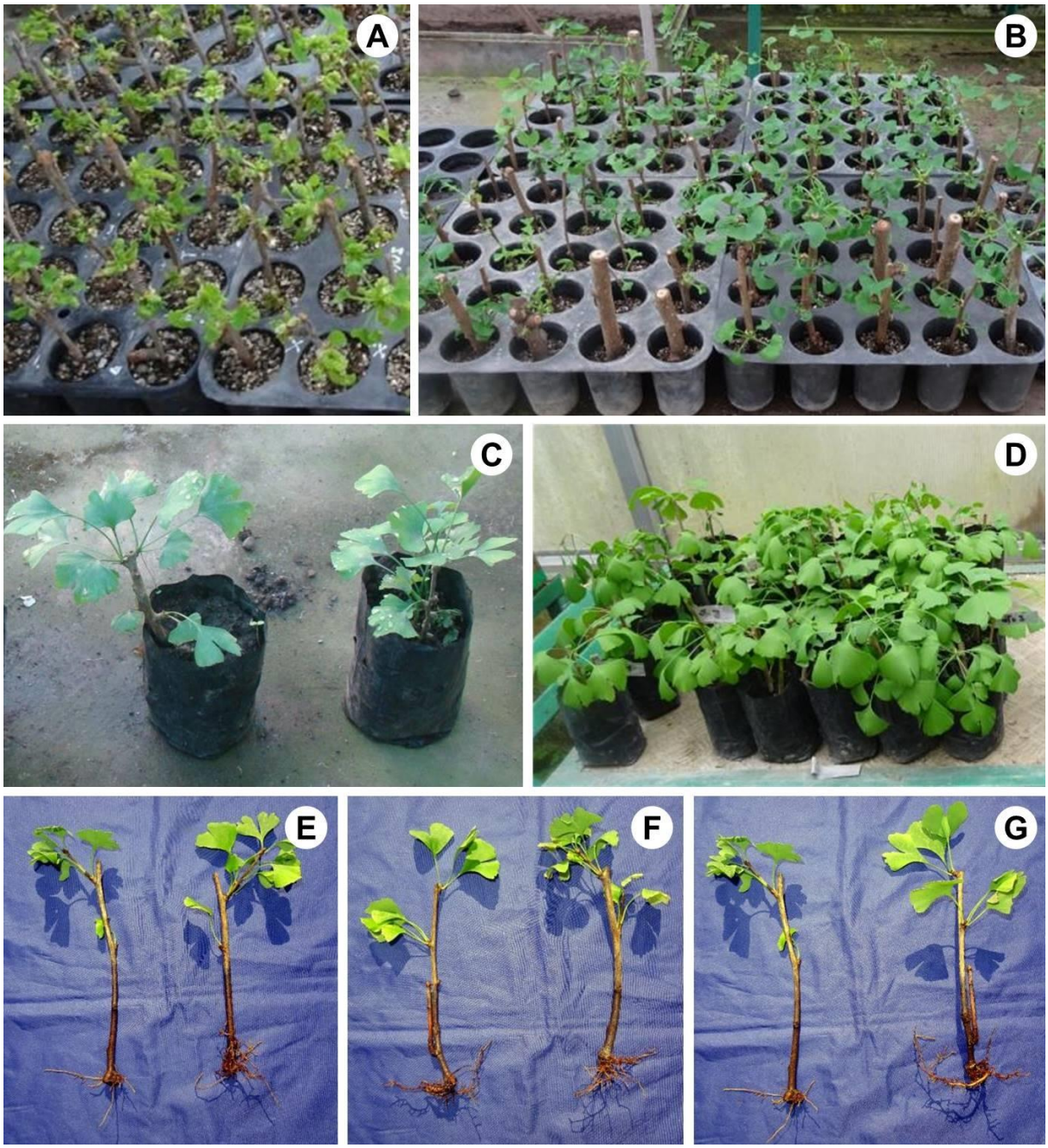

Figure 1. Macro-propagation of *Gingo biloba* L.: **A–B**, Stem cuttings in hycotrays (top view & side view); **C–D**, Stem cuttings in plantation bags (Male & Female); **E–G**, Profuse rooting after six months in stem cuttings.

Table 1. One-way ANOVA showing significance level of various auxins concentrations on rooting.

Sources of Variation	Parameter: Rooting Per cent					
	Within Auxin concentration (Year wise pooled)					
	IBA		IAA		NAA	
	Male	Female	Male	Female	Male	Female
MSS (df)	120.00* (3)	835.67* (3)	6.33* (3)	11.67* (3)	8.00* (3)	41.00* (3)
F value	51.43	313.38	4.75	7.00	16.00	30.75
P value (0.05)	0.000	0.000	0.021	0.006	0.000	0.000

DISCUSSION AND CONCLUSION

In our study, all hormonal concentration was able to proliferate roots for male and female genotypes of *Ginkgo biloba*. Earlier study revealed that this species could be propagated in the nursery from cuttings, though the rooted cuttings were slow-growing. Dirr & Heuser (1987) observed that the cuttings of 10 to 15 cm long

Table 2. Effect of various auxins concentrations on rooting of stem cuttings of *G. biloba* after 6 months.

Auxin Concentration (ppm)	Rooting Per cent (Mean ± S. Em.) August-September Pooled over the years (2015, 2016, 2017 and 2018)					
	IBA		IAA		NAA	
	Male	Female	Male	Female	Male	Female
100	6±0.41^{d}	8±0.41^{d}	2±0.41^{b}	4±0.41^{b}	0±0.0^{b}	0±0.0^{b}
200	6±0.41^{c}	15±1.08^{c}	3±0.71^{b}	5±0.71^{b}	2±0.41^{a}	6±0.41^{a}
300	12±1.08^{b}	40±0.71^{b}	5±0.71^{a}	8±0.41^{a}	3±0.41^{a}	7±0.91^{a}
500	15±0.91^{a}	30±0.91^{a}	3±0.41^{b}	6±0.91^{b}	3±0.41^{a}	6±0.58^{a}

Note: Superscript in the table indicated by the same letter (a, b, c and d) showing non-significant differences according to DMRT.

Table 3. One-way ANOVA showing significance level between various auxins concentrations on rooting between male and female individuals.

Sources of Variation	Parameter: Rooting Percentage Between Auxin concentration (Year wise pooled)							
	Male				Female			
	100	200	300	500	100	200	300	500
MSS	9.33*	17.33*	165.33*	108.00*	64.00*	121.33*	1409.33*	768.00*
(df)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
F value	21.00	15.60	67.64	69.43	144.00	49.64	704.67	288.00
P value (0.05)	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000

Table 4. Effect of various auxins concentrations on rooting of stem cuttings of *G. biloba* after 6 months between male and female individuals.

Auxin Concentration (ppm)	Rooting Per cent (Mean ± S. Em.) August-September Pooled over the years (2015, 2016, 2017 and 2018)							
	Male				Female			
	100	200	300	500	100	200	300	500
IBA	3±0.41^{a}	6±0.41^{a}	15±1.08^{a}	12±0.91^{a}	8±0.41^{a}	15±1.08^{a}	40±0.71^{a}	30±0.91^{a}
IAA	2±0.41^{a}	3±0.71^{b}	5±0.71^{b}	3±0.41^{b}	4±0.41^{b}	5±0.71^{b}	8±0.41^{b}	6±0.91^{b}
NAA	0±0.00^{b}	2±0.41^{b}	3±0.41^{b}	3±0.41^{b}	0±0.0^{c}	6±0.41^{b}	7±0.91^{b}	6±0.58^{b}

Note: Superscript in the table indicated by the same letter (a, b, c and d) showing non-significant differences according to DMRT.

should be collected from mature trees in mid-summer, and then treated with 8,000 ppm indole-butyric acid (IBA) in solution or in talc, which grows well after misted for 7 to 8 weeks. In the current study, the cuttings were also placed in March-April throughout the study period, augurs well with the root initiation in stem cuttings. In the previous study, combination of IBA (500 mg l^{-1}) and catechin (5 mg l^{-1}) enhanced the rooting upto 96.0% in *G. biloba*; besides, the applications of different phenolic compounds were applied to assess the rooting response by the Prakash *et al.* (2002). Similarly, semi-hard wood cuttings of *G. biloba* produced 53.3% and 56.7% rooting with the application of catechin and gallic acid, respectively (Gopichand *et al.* 2006). Purohit *et al.* (2009) observed that 500 mM concentration of IBA resulted into maximum rooting (50.0%) response with highest number of roots (7.5) was observed in male cuttings, while 5.0 roots per female cuttings was produced in 100 mM IBA treatment during January, 2006 at the G.B. Pant Institute of Himalayan Environment & Development (GBPIHED), Garhwal Unit, Srinagar, Garhwal (Uttarakhand, India). The role of auxins, indol-3-butyric acid (IBA) and α-naphthalene acetic acid (NAA) and their stimulatory effects on adventitious root formation in stem cuttings of *G. biloba* was evaluated by conducting the experiment in the month of September, 2007 at the polyhouse of the Department of Botany, Kumaun University, Nainital (Uttarakhand, India) by Pandey *et al.* (2011). They found that lower concentration of IBA (10.0 μM) was found to be the most effective treatment, as it induced maximum rooting (88.89%) and enhanced number of roots, length of roots and length of longest root to the maximum. Also, the growth performance of IBA treated plantlets were morphologically healthier in terms of their shoot height, diameter of shoot, number of nodes per cutting, number of leaves per node and number of branches per cutting than the control plants. Gopichand & Meena (2015) standardized the propagation and agro-techniques in *G. biloba* during 2005–2013 by taking cuttings in the last week of November to middle December at the Biodiversity farm of CSIR-IHBT Palampur (Himachal Pradesh, India), and found that the highest sprouting (%) was recorded in the catechin acid at 10 mg l^{-1}. The statistically significant rooting (%) and root length $plant^{-1}$ in the 2nd year was recorded in IBA at 250 mg l^{-1} (85.33%). Stuepp *et al.* (2017) evaluated the rooting potential of cuttings of *G. biloba* during November, 2013 and

February, 2014 at Curitiba, Brazil and found that epicormic shoots presented rooting (82.5%) and root vigor higher than those from current year shoots. Also, the efficiency of the mini-cuttings technique was proved through higher rooting (92.5%) and root vigor was obtained during the second year.

In the study conducted on other species, Shekhawat & Manokari (2016) explored the potential of exogenous auxins and significant effects on the number of shoot buds' induction and their growth were observed with 400 mg l^{-1} *α*-Naphthalene Acetic Acid (NAA) treated nodal cuttings for 5 min. of *Couroupita guianensis* Aubl. (Nagalingam), a threatened medicinal tree at the Department of Plant Science, M.G.G.A.C., Mahe, Pondicherry, India. Also, maximum 79% of stem cuttings responded to pre-treatment of 300 mg l^{-1} indole-3-butyric acid (IBA) for 5 min., and 75% of stem cuttings induced shoots with 400 mg l^{-1} indole-3-acetic acid (IAA). Thus, explaining the role of growth hormones in the root induction of stem cuttings. Seeds of *Hippophae salicifolia* D.Don successfully propagated using growth regulators IAA @ 100 and 200 ppm, along with kinetin @ 100 ppm and GA_3 @ 400 ppm (Bisht *et al*. 2008). The sexual dimorphism showed its effect on the cuttings of *Ginkgo biloba*, and species such as *Taxus wallichiana* Zucc., *Taxus brevifolia* Nutt. and *Taxus cuspidate* Siebold & Zucc., the dioecious nature showed significant variation on propagation (Davidson & Olney 1964, Nandi *et al*. 1996, Mitchell 1997, Kaul 2008). The results of our study were in concurrence with other workers (Sharma & Aier 1989, Zeng *et al.* 2005, Guo *et al*. 2009), conducted experiment on different plant species.

It can be concluded that IBA at 300 ppm was found to be the best for rooting of both male and female stem cuttings of *Ginkgo biloba* in the agro-climatic zone of Dun Valley particularly for cuttings raised in late February and early March season.

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