Rooting of Stem Cuttings of *Ginkgo* … Living Fossil Under Threat

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Abstract: A systematic study were carried out to examine the rooting ability of male and female branch cuttings of *G. biloba* under different microclimatic conditions i.e. polyhouse, polypit, and open while using plant growth hormone (IBA) and a systematic fungicide (Bavistin; containing 50% Carbendazim) for raising maximum number of planting materials. Various concentrations 100, 250 & 500 µM of IBA and 0.1, 0.5 and 1.0% of a systemic fungicide Bavistin were applied to the cuttings. The higher concentration of IBA (500 µM) resulted maximum rooting (50.0%) in male cuttings and Bavistin (1.0%) produced maximum 41.66% in male and 58.33% in female cuttings, respectively, whereas no rooting was recorded in both male & female cuttings kept in all the microclimatic conditions as control. The highest number of roots (7.5) per male cuttings was formed in 500 µM IBA while 5.0 roots per female cuttings was formed in 100 µM IBA treatment. Cuttings planted inside polyhouse and in the open conditions did not survive and died after bud sprouting. Since this is a rare species and has got the status of living fossils being known from rocks as old as 200 million years, growing naturally and only few individuals are reported to exist and therefore vegetative propagation may be viable option for its multiplication and conservation to save this species from extinction. [Journal of American Science 2009;5(5):139-144]. (ISSN: 1545-1003).

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1. Introduction

Unfortunately, due to legal and illegal exploitation of important plant species from wild, anthropogenic pressure and lack of knowledge about sustainable harvesting of useful bioresources particularly medicinal ones as many of them has been listed under the categories of rare, threatened, endangered or at the verge of extinction. Although the extinction of species is a natural process, the current speed of extinction of species through human interventions is approximately 100-1000 times faster than the natural speed of extinction. In many groups of organisms 5-20% of all species are already extinct (Chaplin et al. 2000). The species currently on earth are the result of a natural selection process over the last three billion years, which has lead to a large degree of specialization. Species diversity is therefore a prerequisite for ecosystem to function. But how important are species and ecosystems for society? This can be researched by investigating the functions and associated goods and services of ecosystems that are important for humans. A central problem is a continuing loss of biodiversity, but our knowledge about human dimension of biodiversity is still relatively limited. However, there are several plant species growing in this earth but has not still given priority for conservation due to lack of awareness about their usefulness, economic potential and applicable multiplication technology package.

The *Ginkgo biloba* Linn. (MAIDENHAIR TREE; family Ginkgoaceae) is worldôs oldest tree mostly known as living fossils and only surviving member of seed plant groups. It is found growing naturally in very limited localities in the central Himalayan mountain at an elevation of 6000 ft (Anonymous, 1999). It is a handsome, straight, up to 100 ft. high, sparsely branched when young, bearing clusters of fan shaped leaves and dioecious slow growing gymnosperm and known to have huge medicinal, spiritual and horticultural importance worldwide. The Leaves of this species is extensively used in the form of a concentrated, standardized Ginkgo biloba extract (GBE) in different countries (particularly China, Europe, France and Germany) as a source of herbal

...n kg of ... purposes ... litionally *G*inkgo is being used to treat circulatory disorders and enhance loss of memory. Its leaves contain two types of chemicals (flavonoids and terpenoids) have potent antioxident properties. It is also widely used for treating dementia, eye problems, intermittent claudicating, memory impairment, tinnitus and a variety of other ailments including altitude sickness, asthma, depression, disorientation, headaches, high blood pressure, erectile dysfunction, and vertigo (http://www.umm.edu/altmed/ConsHerbs/*GinkgoBiloba*ch.html).

In central Himalayan Mountains of India, there are very few spots in Uttarakhand (i.e. Ranikhet, Nainital and Dehradun) where the individuals of this species are found growing naturally and require immediate conservation measures. However, countries like China, Europe, France, and Germany have already undertaken initiatives for large-scale propagation and plantation for its conservation so as to maintain its status and population on one hand and to make use of it properly in the field of herbal medicine in near future. 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. 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 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. This method has been tried sufficiently in number of gymnosperms as well as angiosperms trees (Nandi et al. 1996, Tamta et al. 2000, Nandi et al. 2002, Purohit et al. 2005). The vegetative propagation studies on *Ginkgo biloba* has been reported by Dirr et al. 1987, Doran, 1954 and Natalia, 1994). Using auxins, but there are very few studies or reports available in Indian Himalayan region where phenolic compounds either alone or in combination with auxins were applied (Prakash et al. 2002 and Gopichand et al. 2006). In view of this, an experiment on vegetative propagation using rooting of branch cuttings was carried out to examine the effect of IBA and Bavistin under different microclimatic conditions.

2. Material and methods

The experiments were carried out inside the polyhouse, polypit and in the open conditions (details given below) established in the G. B. Pant Institute of Himalayan Environment & Development, Garhwal, Unit, Srinagar, Garhwal, Uttarakhand, India, (30^{o} 13' 05.8" N and 78^{o} 46' 24.5" E; 584 m amsl). Branch cuttings (semi - hardwood) were excised in the morning and brought to the laboratory in polythene bags in order to prevent desiccation, during the first week of January 2006 from mature single male and female tree of *G.biloba* (of more than 50 years old trees) growing in Kalika (1750 m amsl) and Chaubatia (1850 m amsl) areas of Ranikhet (Distt. Almora, Uttarakhand, India), respectively. The final cuttings (average length 10.52 ±0.88 cm male and 11.14 ±0.32 cm female, dia. 8.82±0.34 mm male and 9.21±0.41mm female, with atleast 3-4 nodes per cutting) were clipped from the branches. The basal 2.0 cm portion of cuttings was dipped in various concentrations of test solutions for 24 h at 22^{o}C. The treatments consisted of Indole - 3 - butyric acid, IBA 100, 250 & 500 µM; HiMedia Laboratories Pvt. Ltd, Mumbai) and Bavistin (0.1, 0.5, 1.0%; a systemic fungicide; containing 50% Carbendazim, a. i.; from BASF India Ltd., Mumbai, India); one untreated set served as control. The IBA was dissolved in 1.5% (v/v) aqueous ethanol; control cuttings were dipped in aqueous ethanol (1.5%, v/v). In each treatment only 24 cuttings were applied.

Following treatment, cuttings were planted vertically in polythene bags (16.0 cm h x 8.0 cm dia; one cutting per bag) containing sieved rooting mixture (equal parts of sand, soil and farmyard manure) and placed in following microclimatic conditions.

2.1. Polyhouse: A small polyhouse of 20 ft length x 9.0 ft width and 7.0 ft height (in the middle) was erected with the help of bamboo poles and covered with a thin semi-transparent polyethylene sheet (thickness: 162.5 µM, UV stabilized) from all the sides. The door could be opened at the front end to access the polyhouse.

2.2 Polypit: A pit dug in the ground (size 10.0 ft L x 6.0 ft W x 3.0 ft D), covered on the top with a semi ï transparent polyethylene sheet (thickness: 162.5 µM, UV stabilized) supported by a bamboo frame. On one side, a small mud wall (about 30 cm high from the ground level, sloping on the two sides) was raised. The polyethylene sheet was sealed on higher side with mud, leaving three sides could be ...ll as the ...o form of plastering (even with mud) was carried out. As a routine polyethylene covers from the top of the polypit were partially opened during the daytime after midday for a few hours (see Vyas et al. 1999 for details about its function and benefit).

2.3 Open: Open field space without any cover or modifications.

Observations were taken six months after treatment and planting for estimation of percent rooting, number of roots formed per cutting, root diameter and the length of individual roots. Only those cuttings with one or more clearly visible root initials ($\geq$ 2 mm) and/ or roots were classified as having rooted (Nandi et. al. 1996). Following this the well rooted cuttings were again planted in polybags and moved into the shade house, receiving 50% sunlight for hardening and the plants were watered periodically (Fig. 1E).

Least significant differences (LSD), standard error (SE) were calculated for comparison among the treatments following the methods as described by (Snedecor and Cochran, 1967). Analysis of variance (ANOVA) was performed using the Microsoft excel programme.

3. Results

Rooting ability of male and female branch cuttings of *G.biloba* was examined inside polyhouse, polypit and in the open conditions. Cuttings planted in all the conditions exhibited 70-80% bud sprouting (Figure 1A and B). Based on the random observations, cuttings planted in polyhouse and in the open were dried up after four months of planting, therefore, details data of both the conditions are not presented. The first root initiation was observed at six months in majority of the cuttings planted inside polypit (average temperature and relative humidity (RH) during experiment period; 25-30^{o}C, with 60 to 80%). The cuttings under control sets failed to root in both male & female even planted under polypit condition. LSD (P = 0.05) indicates significant differences in all the attributes measured for different treatments. IBA (500 µM) and Bavistin (1.0%) were found very effective for root formation. A maximum 50.0% rooting success was achieved in male cuttings (Table 1 and Figure 1C) with the application of 500µM IBA treatment on an average of 6.71 roots per cuttings and average root length of 3.27 cm, while Bavistin 1.0% treatment produce maximum rooting success 41.66% (with an average of 4.83 roots per cuttings and average root length 3.19; Table 1) in male cuttings and 58.33% (with an average of 4.66 roots per cuttings and average root length 4.0 cm) in female cuttings of *G. biloba* (Table 2 and Figure 1D). Other concentration tried 100 µM IBA was found to be slightly effective with rooting success of 33.33% in female cuttings with an average of 5.0 roots per cuttings and average root length of 3.25 cm. In male cuttings average length of longest root 7.5 cm and 7.32 cm while in female cuttings 6.83 cm and 6.72 cm was recorded with 500µM IBA and 1.0% Bavistin treatment, respectively. The maximum root diameter was recorded 1.26 mm for male (Table 1) and 1.53 mm for female cuttings (Table 2).

Table 1. Effect of IB on rooting respons male cuttings insic condition **Treatments**	**% Rooting**	**No. of roots/ cutting (mean ±SE)**	**Avg. Root length (mean ±SE, cm)**	**Length of longest root (mean±SE, cm)**	**Avg. root diameter (mean ±SE, mm)**
IBA (100 µM)	20.83	3.77±0.88	2.61±0.57	5.0±0.73	1.22±0.26
IBA (250 µM)	25.00	2.5±0.35	3.48±1.03	5.35±1.52	0.94±0.21
IBA (500 (µM)	50.00	6.71±1.30	3.27±0.36	7.5±1.02	1.26±0.06
Bavistin (0.1%)	-	-	-	-	-
Bavistin (0.5%)	29.0	5.28±0.37	2.90±0.39	5.41±0.33	1.04±0.15
Bavistin (1.0%)	41.66	4.83±0.60	3.19±0.41	7.32±1.25	1.08±0.08
LSD (p = 0.05)	**30.67**	**4.18**	**2.08**	**4.63**	**0.73**

SE = Standard error of mean. All values are an average of 24 cuttings.
A dash (-) indicates cuttings did not root.

Source of variation	DF	Mean Square	F-ratio
Treatments	5	112.6579	2.194888^{ns}
Parameters	4	742.7473	14.47077**
Error	20	51.32741	

**Level of significance at 0.05; ns: not significant

Table 2. Effect of IBA and Bavistin on rooting response of *G. biloba* female cuttings inside the polypit condition

Treatments	% Rooting	No. of roots/ cutting (mean ±SE)	Avg. Root length (mean ±SE, cm)	Length of longest root (mean±SE, cm)	Avg. root diameter (mean ±SE, mm)
IBA (100 μM)	33.33	5.0±2.0	3.25±0.76	4.96±1.56	1.46±0.12
IBA (250 μM)	20.83	2.8±0.86	3.77±1.13	4.96±1.56	1.46±0.12
IBA (500 (μM)	12.5	4.0±1.52	5.14±1.13	6.83±1.96	1.35±0.13
Bavistin (0.1%)	12.5	4.33±1.85	3.33±0.63	4.83±1.58	1.12±0.13
Bavistin (0.5%)	20.83	1.5±0.35	1.56±0.23	2.0±0.0	1.34±0.22
Bavistin (1.0%)	58.33	4.66±1.33	4.0±0.58	6.72±0.84	1.53±0.11
LSD (p = 0.05)	**30.67**	**2.35**	**1.83**	**2.94**	**0.24**

SE = Standard error of mean. All values are an average of 24 cuttings.

ANOVA Summary Table

Source of Variation	DF	Mean Square	F-Ratio
Treatments	5	72.14717	1.216312^{ns}
Parameters	4	643.7649	10.85308**
Error	20	59.31633	

**Level of significance at 0.05; ns: not significant.

Figure 1. Propagation of *Ginkgo biloba*: (A) A mature tree of *G. biloba*, (B) A sprouted male and female cuttings, (C&D) well rooted male and female cuttings and (E) Cuttings raised plants kept inside shade house for hardening.

4. Discussion

The stimulation of adventitious root formation in stem cuttings with auxins and commercial rooting mixtures is well known in many species those are difficult-to-root (Morsink and Smith, 1974, Blazich, 1998, Nandi et al. 1996, Purohit, 2002). The effect of growth hormone and fungicide on adventitious root formation in semi - hardwood cuttings of *G.biloba* in different microclimatic conditions, was examined for the first time. Although there are several studies available on the propagation of gymnosperms and angiosperms trees using stem cuttings (Nandi et al. 1996, 1997, 2002, Tamta et al. 2000, Purohit et al. 2005). The rooting efficiency observed in *G. biloba* showed satisfactory result within six months of planting inside the polypit conditions whereas reports available reveals that cuttings taking two years to root in natural conditions (Annonymous, 1999), under results te that in the present study the application of IBA (500µM) significantly improved the rooting up to 50.0% in male cuttings whereas the application of Bavistin (1.0%) was found more effective in both the cuttings (male and female) with highest rooting response was observed in female cuttings (58.33%). However, on the other hand, the application of growth regulators (catechin and gallic acid) produced 53.3% and 56.7% rooting respectively, in the semi hard woodcuttings of *G. biloba* (Gopichand et al. 2006). Besides, the applications of different phenolic compounds were applied to assess the rooting response in *G.* biloba (Prakash et al. 2002) and they reported that the combination of IBA (500 mg/l) and catechin (5 mg/l) enhanced the rooting upto 96.0%.

IBA and Bavistin have also been reported to be more effective in inducing rooting in stem cutting of *Taxus baccata* (Nandi et al. 1996, 1997) and *Cedrus deodara* (Nandi et al. 2002). The rooting percentage displayed a positive trend with increasing concentrations of chemicals in this study. Applications 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). Treatments with a systematic fungicide, Bavistin were also found effective in this study, however, the mechanism of stimulation of rooting by Bavistin is not clear. It may be related to auxin ï like activity of benomyl, which is known to be converted into carbendazim in water and in contact with plants (Thurston et al. 1979). Application of 500µM IBA and 1.0% Bavistin also enhanced the number of roots developed on each rooted cuttings as compared with another concentrations applied. This may have an advantage by enhancing good anchorage when planted in the field. Besides the effect of IBA and Bavistin, the diameter of cuttings may also have influenced the root formation on the cuttings whereas most of the thin cuttings either dried up or could not develop root even after six months.

In the present study rooting of stem cuttings was carried out in the months of January to June because of the deciduous nature of tree, best sprouting of dormant buds, active growth season which has been reported to favor rooting of cuttings (Loach, 1988). High humidity environments created by means of mist systems or plastic covers are commonly used in vegetative propagation experiments to reduce the risk of water stress (Hartmann et al.1990). Generally, spring season when the fresh flush starts is considered to be the best period for rooting.

5. Conclusions

The results of this study are important and low natural regeneration as reported in this species can be supplemented with clonally propagated plants raised through rooting of stem cuttings. Moreover, it is important to note that the cuttings were taken from mature trees from different sources; further trials in different seasons may result in better rooting efficiency and genetically specific-differences. Due to poor economic condition and tough terrain in the mountains the construction of Green houses are difficult, therefore, a low cost polypit (poor man growth chamber) can meet out the requirement of rooting of cuttings and raising maximum number of plants. Keeping in view the present status, importance and conservation value of *G. biloba* a multi-faceted efforts is required while involving local communities, scientific institutions and NGOs for its nursery raising and afforestation programmes. Further, well-rooted plants could be obtained within a short time; the method is also inexpensive and easy to perform. It is hoped that it will be acceptable to the involved in the forestry sector.

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