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Vegetative Propagation for Different Physiological Ages of *Embelia Ribes* Cuttings in Different Seasons

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Abstract

Embelia ribes Burm. f. is one of the red listed medicinal plant belongs to the family Myrsinaceae. This threatened medicinal plant valued for its thermogenic, carminative, depurative and laxative property. E.ribes is now reported as vulnerable due to over exploitation. The main threat of this plant is its unsustainable and indiscriminate harvesting for commercial purposes. The regeneration from seedling of this plant is very poor. Traditional propagation techniques are not successful in large scale production of this valuable species. Only accessible way for the conservation of this species is vegetative propagation through stem cutting. Exogenous application of IBA and NAA has a significant positive effect on the percentage of rooting. Rooting ability of cuttings was mostly influenced by the type of cutting, retention of leaves and time of the year in which cuttings were taken. Semi hardwood cuttings with two or three leaves, treated with IBA in 3000mg/l concentration in season I (January – April) appears to be a successful method for vegetative propagation (100% rooting) for producing sufficient number of propagules of this species. Significant increase in number of new root, leaves and shoots and length of roots was recorded in stem cuttings treated with 3000mg/l IBA.

Keywords: *Embelia ribes*, vegetative propagation, physiological age, Season, IBA, NAA.

Introduction

Embelia ribes commonly known as false pepper is an important medicinal plant of India having a fair demand in national and international market. *E. ribes* is a red listed large scandent climbing shrub belongs to the family Myrsinaceae. It is inhabited throughout India in hilly parts. It is extended up to 1500 m. elevation from outer Himalayas to Western Ghats. It is an endangered medicinal plant valued for its digestive, thermogenic, carminative, depurative, anthelmintic and laxative property since time immemorial. It is also used in the treatment of tumours, asthma, bronchitis, diabetes, heart related problems, nerval disorders, cancerous tumors and liver disorders[1]. *E. ribes* contain quinine derivative Embelin which has various pharmacological and medicinal properties. To bring a medicinal plants revolution in our country, which leads to good health and prosperity to people National Medicinal Plants Board selected 32 medicinal plants for large scale cultivation, *E .ribes* is one among them because of its commercial value. This plant is reported as vulnerable in Tamil Nadu and Karnataka and as threatened in Kerala[2]. The main threat of this plant is its unsustainable and indiscriminate harvesting for commercial purposes. Habitat loss, Jhum cultivation, forest fire and agriculture expansions are also some factors for its decrease in population. The regeneration from seedling of this plant is very poor[3]. The embryos of *E.ribes* are very small when present and most of the seeds are abortive. For the survival and growth of *E.ribes* specific habitat conditions are essential. Regeneration of *E.ribes* is very poor and slow[4]. *E.ribes* acquired high trade value and its demand in the local market is greater than 100 t/yr. During 1990–2000 the demand for *E. ribes* increased enormously and the export increased to 250 t/yr [5]. Due to over demand this species extensively wild harvested from the protected and conserved areas also. So the conservation of this important medicinal plant requires special attention.

Traditional propagation techniques are not successful in the large scale production of this valuable species. Although, propagation of *E.ribes* through seeds is the cheapest method, due to poor seed germination vegetative propagation methods can only cater to meet the requirements of the planting stock production on a large scale. The present paper describes the vegetative propagation studies carried out for *E.ribes* using stem cuttings in different physiological ages treated with different concentration of hormones in different seasons.

Material and Methods

Branch cuttings from young plants were collected from Ponmudi forest area in the Trivandrum district of Kerala. The collections were carried out in three different time periods *viz*; season I (January-April), season II (May-August) and season III (September- December). Hardwood and semi hardwood leafy shoot cuttings having a length 15 cm and 2 pairs of leaves intact were prepared. Semihard wood (Juvenile) cuttings taken from two months old seedling and hard wood (mature) cuttings taken from old plants. The leaf areas of the cuttings were reduced by trimming away 2/3 of the leaflets of compound leaves retaining the apical bud intact in order to minimize the transpiration rate.

Table-1
Different concentrations of IBA and NAA used for the study

Hormones	Concentrations used (mg/l)																
IBA	100	200	300	400	500	600	700	800	900	1000	2000	3000	4000	5000	6000	7000	8000
NAA	100	200	300	400	500	600	700	800	900	1000	2000	3000	4000	5000	6000	7000	8000

In order to prevent any possible fungal attack during propagation, cuttings were treated with 0.05 percent aqueous solution of Bavistin for 45 minutes. The cuttings were then treated with various concentrations (100mg/l to 8000mg/l) of indole butyric acid (IBA) and naphthalien acetic acid (NAA) prepared in talc by quick dip method (table 1). The treated cuttings were inserted immediately in the rooting medium vermiculite taken in root trainers and kept in mist chamber. Inside the mist chamber temperature maintained as 28^0C ± 2 and relative humidity as 92%. Regular misting was provided for 10 seconds at an interval of half an hour. Within a period of one week the cuttings start rooting and sprouting. After rooting was completed within a period of 8 days, they were transferred to polythene bags filled with sand and soil in equal proportions (1:1) and kept in the hardening room for about 30days. Cuttings were removed carefully from the rooting medium (vermiculite) after the root initiation. The ability to form adventitious roots were compared with mature (hard wood) and juvenile cuttings (semi hardwood). The number of rooted cuttings in each tray and number of roots per rooted cuttings were recorded. Observations were also made of the number of new leaves, number of new sprouts, root length and shoot length. For recording this data's one rooted cuttings per replicate were randomly selected from each treatment every day after hormone treatment. Statistical analyses were conducted using SPSS software.

Results and Discussion

Percentage of rooting in cuttings: Figure 1and2 gives the details of Percentage rooting of two types of Embelia cuttings in different seasons under the treatment of different concentrations of hormones, IBA and NAA. In the case of *E. ribes* semi hardwood leafy cuttings in season 1 had the highest mean percentage rooting of 100% with 3000 mg/l IBA followed by 4000mg/l IBA treated semi hardwood leafy cuttings in season 1 (70%). While the lowest mean value of 5% was obtained with hard wood cuttings in almost all treatments of IBA and NAA in all seasons. Percentages rooting for different auxin concentrations of semi hard wood cuttings were differed. IBA at 2000 mg/l had 50% rooting in season I and II while NAA at 2000 mg/l had 25% rooting in season III and 10% in season I. 3000mg/l IBA treated semi hardwood cuttings in season III showed 65% rooting whereas 3000 mg/l NAA treated semi hardwood cuttings showed 50% rooting in season I and 35% rooting in season III. Control cuttings showed no rooting. A comparison of rooting success between material taken from juvenile and mature Embelia stem cuttings revealed that physiological age affects rooting of stem cuttings. IBA treated semi hardwood leafy cuttings collected from young trees showed relatively higher success of rooting in season IandII while both types of cuttings in season II showed poor rooting percentage (≤ 35%) with all concentrations of hormones tested. Semi hardwood and hard wood cuttings of *E.ribes* did not produce roots when treated with higher and very lower concentrations of IBA and NAA (≥6000mg/l and ≤ 1000mg/l) irrespective of the season. In mature Embelia stem cuttings rooting percentage was zero in almost all treatments. Importance of leaves for rooting was revealed when no roots formed on cuttings after the senescence of leaves. In an earlier study successful rooting was reported in leafy cuttings of *Saraca asoka*[6] and in *Oroxylum indicum*[7]. Adventitious rooting in shoot cuttings of neem (*Azadirachtaindica*) and karanj (*Pongamiapinnata*) in different seasons was studied and concluded that maximum rhizogenesis coincided with the emergence of new sprouts in February (neem) and March (karanj) and IBA was the most effective auxin[8]. Auxin type, concentrations of auxins, time of the year when cuttings were taken and presence of leaf had a significant positive effect on rooting of Embelia cuttings.

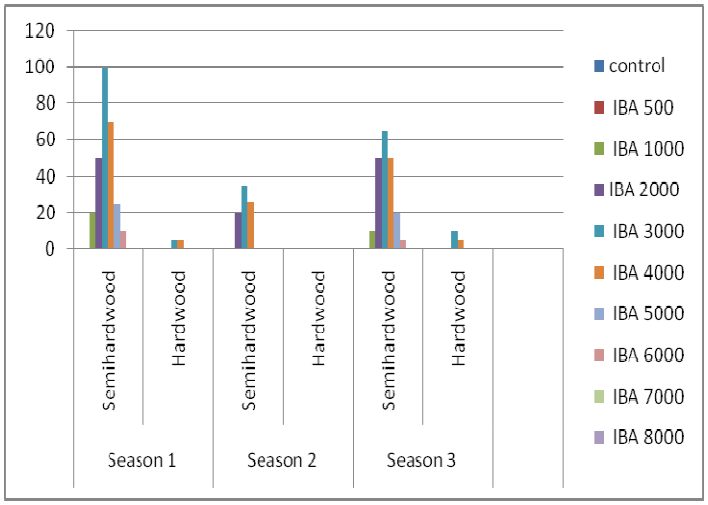

Figure-1
Percentage of rooting in *E.ribes* using hard wood and semihard wood stem cuttings treated with various concentration of IBA in different seasons

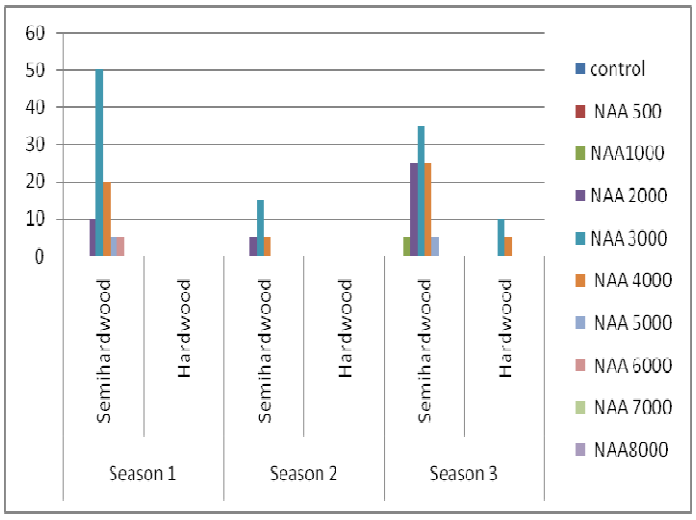

Figure-2
Percentage of rooting in *E.ribes* using hard wood and semihard wood stem cuttings treated with various concentration of NAA in different seasons

Number of new sprout, leaves, roots and length of roots and shoots per cuttings: Data on effect of different concentrations of growth hormones (IBA and NAA) on different physiological characters of semi hardwood and hardwood stem cuttings in different seasons are given in table 2-4. Physiological characters such as the number of new roots (15), the number of new leaves (2), number of new sprouts (1), length of root (33.31) and shoot length (16.20) etc were higher in 3000mg/l IBA treated cuttings in season I followed by 4000mg/l IBA treated cuttings in season I with values 9, 1, 0.01, 17.50 for number of new roots, leaves, sprout and length of root respectively. In NAA treated hard wood cuttings the physiological characters were negligible in all seasons. There is no any significant difference in shoot length before and after the hormone treatment. All new sprouted cuttings possessed roots but all rooted cuttings had no sprouts. Auxin type, concentrations of auxins, and season had a significant positive effect on physiological parameters of rooted cuttings of Embelia as in *Stevia rebaudian* where the cuttings treated with 500 mg/l IBA showed to be superior with respect to shoot length, number of branches, number of leaves and root length, survival percentage and sprouting percentage.[9]

Table-2
Effect of different concentrations of IBA on different physiological characters of semi hardwood cuttings of *E.ribes* in different seasons

Parameters	seasons	Mean ± SD			
		2000(mg/l)	3000(mg/l)	4000(mg/l)	5000(mg/l)
No:of new sprout	1	0.02±0.01	0.50±0.02	0.01±0.005	0±0
Shoot length		16.16±0.28	16.20±0.10	16.06±0.11	16.16 ±0.28
No: new leaves		0.41±0.03	1.83±0.25	0.50±0.01	0.08±0.01
No: of new roots		6.50±0.30	14.90±0.36	9.30±0.56	3.10±0.10
Length of roots		16.90±0.30	31.33±1.52	17.50±0.50	5.85±0.18
No:of new sprout	2	0±0	0±0	0±0	0±0
Shoot length		16.05±0.05	16.06±0.05	16.06±0.28	16.01±0.02
No: new leaves		0.08±0.01	0.09±0.02	0±0	0±0
No: of new roots		0.27±0.02	1.47±0.16	0.50±0.10	0.08±0.01
Length of roots		1.5±0.50	2.31±0.20	1.26±0.07	0.15±0.01
No:of new sprout	3	0±0	0.04±0.04	0.01±0.005	0±0
Shoot length		16.20±0.26	16.10±0.13	16.03±0.05	16±1
No: new leaves		0.25±0.02	0.50±0.02	0.25±0.06	0±0
No: of new roots		6.40±0.20	8.50±0.05	5.41±0.19	1.33±0.33
Length of roots		13.8±0.60	15.42±0.17	13.25±0.80	4.18±0.03

± SD- standard deviation

Table-3
Effect of different concentrations of NAA on different physiological characters of semi hardwood cuttings of *E.ribes* in different seasons

parameters	seasons	Mean ± SD			
		2000(mg/l)	3000(mg/l)	4000(mg/l)	5000(mg/l)
No:of new sprout	1	0±0	0±0	0±0	0±0
Shoot length		16.03±0.05	16.10±0.10	16.05±0.05	16.16 ±0.28
No: new leaves		0±0	0.06±0.05	0±0	0±0
No: of new roots		0.16±0.02	1.33±0.07	0.50±0.02	0.08±0.02
Length of roots		0.58±0.03	2.80±0.10	0.87±0.04	0.25±0.01
No:of new sprout	2	0±0	0±0	0±0	0±0
Shoot length		16.03±0.02	16.02±0.05	16.01±0.02	16.01±0.02
No: new leaves		0 ±0	0 ±0	0±0	0±0
No: of new roots		0.08±0.02	0.33±0.03	0.16±0.02	0.16±0.02
Length of roots		0.02±0.002	0.50±0.05	0.32±0.02	0.32±0.02
No:of new sprout	3	0±0	0±0	0.00±0.00	0±0
Shoot length		16.03±0.05	16.16±0.28	16.11±0.10	16.03±0.02
No: new leaves		0±0	0±0	0±0	0±0
No: of new roots		0.58±0.03	1.00±0.50	0.33±0.02	0±0
Length of roots		0.95±0.05	2.1±0.18	0.68±0.06	0±0

± SD- standard deviation

Table-4
Effect of different concentrations of IBA on different physiological characters of hardwood cuttings of *E.ribes* in different seasons

parameters	Seasons	Mean ± SD			
		2000(mg/l)	3000(mg/l)	4000(mg/l)	5000(mg/l)
No:of new sprout	1	0±0	0±0	0±0	0±0
Shoot length		16.10±0.10	16.08±0.10	16.08±0.10	16.05±0.05
No: new leaves		0±0	0±0	0±0	0±0
No: of new roots		0±0	0.8±0.02	0.8±0.02	0±0
Length of roots		0±0	0.33±0.09	0.33±0.09	0±0
No:of new sprout	2	0±0	0±0	0±0	0±0
Shoot length		16.03±0.02	16.00±1	16.03±0.01	16.05±0.05
No: new leaves		0±0	0±0	0±0	0±0
No: of new roots		0±0	0±0	0±0	0±0
Length of roots		0±0	0±0	0±0	0±0
No:of new sprout	3	0±0	0±0	0±0	0±0
Shoot length		16.05±0.01	16.07±0.02	16.00±0.0	16. ±0.0
No: new leaves		0±0	0±0	0±0	0±0
No: of new roots		0±0	0.25±0.05	0±0	0±0
Length of roots		0±0	0.66±0.21	0±0	0±0

± SD- standard deviation

Conclusion

It can be concluded that semi hardwood cuttings with two or three leaves, treated with IBA in 3000mg/l concentration in season I (January – April) appeared to be a successful method for vegetative propagation (100% rooting) for producing sufficient number of propagules of this species. Significant increase in number of new root, leaves and shoots and length of roots was recorded in stem cuttings treated with 3000mg/l IBA.

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