

## Standardization of seed and vegetative propagation techniques in *Embelia ribes* Burm f. : An endangered medicinal plant

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### ABSTRACT

*Embelia ribes* Burm f. is a threatened species found in Western Ghats, which is an important medicinal plant, fruits of which are used in variety of ayurvedic formulations. Natural regeneration through seeds is very difficult due to small embryo and abortive nature. Propagation through cuttings is also very difficult due to very poor rooting. Therefore, the work was initiated during 2015-16 to standardize seed and vegetative propagation of this medicinal plant. Among the different germination inducing treatments, the seed treated with GA<sub>3</sub> 750 ppm recorded early germination (30 days), highest germination rate (2.07%), seedling vigour (2258.13), seedling height (44.57 cm), number of leaves (23.33), etc. In case of vegetative propagation, hard wood cuttings were used and treated with IBA, NAA and combination of IBA and NAA. Among the treatments, IBA and combination of IBA and NAA had a significant positive effect on the percentage of rooting. Hardwood cuttings with two or three leaves, treated with IBA in 3000 mg/l concentration appeared to be a successful method for vegetative propagation (36% rooting) for producing sufficient number of propagules of this species. Significant increase in number of new root, leaves and shoots and length of roots were recorded in stem cuttings treated with 3000 mg/l IBA.

**Key words :** Acid scarification, *Embelia ribes*, indole butyric acid, seed, vegetative

### 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 belonging 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, neural disorders, cancerous tumours and liver disorders (Harish *et al.*, 2012). *E. ribes* contains quinine derivative Embelin which has various pharmacological and medicinal properties. To bring a medicinal plant 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 (Ravikumar and Ved, 2000). 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 (Shankar *et al.*, 2012). 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 (Raghu *et al.*, 2006). *E. ribes* acquired high trade value and its demand in the local market is greater than 100 t/year. During 1990-2000, the demand for *E. ribes* increased enormously and the export increased to 250 t/year (Mhaskar *et al.*, 2011). Due to over demand, this species was extensively wild harvested from the protected and conserved areas also. So, the conservation of this important medicinal plant requires special attention.

The seeds of *E. ribes* are very small; each weighing ~0.014 g. Seed germination takes a very long time and is very inefficient. The embryo is small in size and mostly abortive (Ramesh *et al.*, 2011). Natural regeneration of *E. ribes* is poor due to its over harvesting and exploitation, fragmented populations resulting in in-breeding, development of abortive embryos, and the slow germination of fertile seeds that are small in size. Artificial regeneration of this species is also difficult due to poor seed viability, low rate of germination (Annapurna *et al.*, 2013). Therefore, an attempt has been made to get good germination and healthy plants through stem cutting by treating with different growth regulators and root inducing hormone at different concentrations.

## MATERIALS AND METHODS

### Study Site

The experiment was conducted at the College of Horticulture Mudigere, Chikkamagalore during 2015-16. The experimental farm is located at hill zone of Karnataka state at 13°25' north latitude and 75°25' east longitude with an altitude of 980 m above the mean sea level.

### Seed Propagation

The required seed materials were collected from the plants grown in the Field Gene Bank of RET medicinal plants maintained at the Indian Institute of Horticultural Research (IIHR), Bengaluru. The red mucilaginous covering present in the seeds was removed. The seeds were treated with mercuric chloride 0.1% for 10 min, washed with water and shade dried for 24 h. The extracted seeds were subjected to 19 different pre-soaking treatments viz., GA<sub>3</sub>

500 ppm, GA<sub>3</sub> 750 ppm, Ethylene 25 ppm, Ethylene 50 ppm, KNO<sub>3</sub> 1.0%, KNO<sub>3</sub> 1.5%, H<sub>2</sub>SO<sub>4</sub> 1.0%, H<sub>2</sub>SO<sub>4</sub> 2.0%, HCl 1.0%, HCl 2.0% and scarified seeds were treated with GA<sub>3</sub> 500 and 700 ppm. Scarified seeds were washed in distilled water and then used for further process. The treated seeds were sown in portrays consisting of coco pith media which were collected from IIHR, Bengaluru kept in polyhouse under 50% shade and watering was done regularly. Observations were recorded for germination (Fig. 1), shoot and root growth parameters at 60, 90 and 120 days after sowing.

### Vegetative Propagation

A branch cutting from six-year old plant was collected from IIHR, Bengaluru. Hardwood cuttings having 10-15 cm long and 2 pair of leaves were prepared. 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.

In order to prevent any possible fungal attack during propagation, cuttings were treated with 0.05% aqueous solution of bavistin for 45 min. The cuttings were then treated with various concentrations of indole butyric acid (IBA) and naphthalien acetic acid (NAA) prepared by talc by quick dip method according to the treatments. 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 was maintained as 28°C±2 and relative humidity as 92%. Regular misting was provided for 10 sec at an interval of half an hour. Within a period of one week, the cuttings started rooting and sprouting. After rooting was completed within a period of eight days, they



Fig. 1. Germination of *Embelia ribes* seeds.

were transferred to polythene bags filled with sand and soil in equal proportions (1:1) and kept in the hardening room for about 30 days. The number of rooted cuttings in each tray and number of roots per rooted cutting were recorded. Observations were also made on the number of new leaves, number of new sprouts, root and shoot length. For recording the data, one rooted cutting per replicate was randomly selected from each treatment every day after hormone treatment. Statistical analyses were conducted using SPSS software.

## RESULTS AND DISCUSSION

Seed treatments can speed up germination, increase uniformity of germination and offset some environmental hazards in the seed bed. For many species, especially woody perennials, seeds must be pre-treated to overcome dormancies. Seed treatments like acid scarification, GA<sub>3</sub> treatment, etc. are commonly used for enhancing germination.

Among the different germination inducing treatments, the seeds treated with gibberellins responded well with high rate of germination and vigorous seedling growth. Early germination was also seen in GA<sub>3</sub> treatment. This may be due to instigative action of GA<sub>3</sub> for germination of seeds. GA<sub>3</sub> induces the *de-novo* synthesis of proteolytic enzymes like  $\alpha$ -amylase and ribonuclease. Amylases in turn hydrolyse starch in the endosperm, providing the essential sugars for the initiation of growth processes (Copeland and Mc-Donald, 1995). GA<sub>3</sub> treatment is also known to overrule the photo dormancy, thermo-dormancy, dormancy imposed by rudimentary embryo, mechanical barriers and presence of germination inhibitors (Diaz and Martin, 1971).

The early germination (30 days) and maximum rate of germination (2.07), vigour index (2258.13), plant height (44.57 cm), number of leaves (23.33), shoot length (20.07 cm), root length (24.50 cm), fresh weight (2055.00 mg) and dry weight (363.33 mg) of seedling were recorded in GA<sub>3</sub> 750 ppm followed by GA<sub>3</sub> 500 ppm in *E. ribes* Burm f. seeds 120 days after sowing (Tables 1 and 2). Similar results were obtained in elm (*Ulmus wallichiana*), an endangered tree species where GA<sub>3</sub> 100 ppm recorded maximum germination (71.00%) compared to control (48.00%). The

rate of germination and vigour was also higher in this treatment (Masoodi and Masoodi, 2000). Gibberellic acid at 100 ppm was found very effective for enhancing germination in *Nothapodytes foetida*. It also promotes seedling growth, survival percentage after transplanting and further performance in the field (Sharma *et al.*, 2000). Gowda *et al.* (2003) reported that GA<sub>3</sub> 400 ppm considerably improved germination (48%) than control (12%) in *Embelia tsjeriam-cottam*.

Acid scarification of *Embelia ribes* Burm f. seeds with H<sub>2</sub>SO<sub>4</sub> 2% followed by treated with GA<sub>3</sub> 750 ppm recorded highest germination per cent followed by seeds treated with GA<sub>3</sub> 750 ppm alone (Table 1). Similar results were also reported by Mawalagedera *et al.* (2013) in *Phyllanthus emblica* seeds which were scarified and treated with 1.00% gibberellins. Seed dormancy was overturned with germination per cent (43%) by the seed pre-treatment, where the seeds were scarified and treated with 1.00% gibberellins. Pipinis *et al.* (2011) revealed that 30, 60 and 90 min scarified seeds of *Paliurus spina-christi* Mill. prior to GA<sub>3</sub> application (500, 1000 and 2000 ppm), resulted in higher germination per cent compared to non-scarified seeds that were treated with GA<sub>3</sub>.

Success in propagation of *Embelia ribes* through cutting is little difficult. In this present study hard wood cutting treated with IBA 3000 ppm obtained maximum rooting per cent (36.00) followed by IBA 3500 ppm (26.00). No rooting was observed in NAA alone treatment. Combination of IBA and NAA treatment also showed better results. The cuttings took more time for root initiation up to three months and rooting per cent was moderate as compared to other easy-to-root species. There was significant influence of different treatments on parameters such as per cent rooting, number of roots, number of sprouts and number of leaves per cutting. The growth regulator treatments increased the overall root parameters as compared to control. Among different treatments, IBA 3000 ppm was found to be the best, which recorded maximum results for all parameters (Fig. 2). This may be due to the action of auxin which might have caused hydrolysis and translocation of carbohydrates and nitrogenous substances at the base of cuttings and resulted in accelerated cell elongation and cell division in suitable environment (Hartmann *et al.*, 2007). Cuttings

**Table 1.** Effect of growth regulators and chemical treatments on seed germination and seedling growth of *E. ribes* Burm f.

Treatment	Days to initiate germination	Rate of germination (%)	Germination (%)	Seedling vigour	No. of leaves	Shoot length (cm)	Root length (cm)	Height of the seedling (cm)
T <sub>1</sub> -Control	61.67	0.17	14.33 (22.23)	215.18	8.33	6.81	8.20	15.01
T <sub>2</sub> -GA <sub>3</sub> 500 ppm	31.67	2.00	44.67 (41.92)	1687.22	19.67	18.07	19.71	37.78
T <sub>3</sub> -GA <sub>3</sub> 750 ppm	30.00	2.07	50.67 (45.36)	2258.13	23.33	20.07	24.50	44.57
T <sub>4</sub> -Ethylene 25 ppm	41.33	1.07	24.67 (29.76)	418.77	9.77	7.31	9.67	16.98
T <sub>5</sub> -Ethylene 50 ppm	40.67	1.12	29.33 (32.77)	534.33	9.74	7.73	10.48	18.22
T <sub>6</sub> -KNO <sub>3</sub> 1.0%	51.00	0.82	20.67 (27.03)	314.95	9.00	6.60	8.64	15.24
T <sub>7</sub> -KNO <sub>3</sub> 1.5%	51.00	0.82	21.67 (39.40)	343.27	10.67	6.85	8.99	15.84
T <sub>8</sub> -H <sub>2</sub> SO <sub>4</sub> 1.0%	38.00	1.34	40.33 (40.76)	777.38	12.33	7.93	11.34	19.27
T <sub>9</sub> -H <sub>2</sub> SO <sub>4</sub> 2.0%	38.00	1.38	42.67 (33.40)	856.77	13.67	8.12	11.96	20.08
T <sub>10</sub> -HCl 1.0%	39.67	1.24	30.33 (34.23)	538.88	10.33	7.58	10.18	17.77
T <sub>11</sub> -HCl 2.0%	39.33	1.25	31.67 (37.64)	572.55	11.67	7.80	10.66	18.46
T <sub>12</sub> -H <sub>2</sub> SO <sub>4</sub> 1.0%+GA <sub>3</sub> 500 ppm	38.00	1.42	37.33 (39.40)	777.80	14.67	8.20	12.63	20.83
T <sub>13</sub> -H <sub>2</sub> SO <sub>4</sub> 1.0%+GA <sub>3</sub> 750 ppm	38.00	1.44	40.33 (41.53)	875.92	15.67	8.52	13.00	21.51
T <sub>14</sub> -H <sub>2</sub> SO <sub>4</sub> 2.0%+GA <sub>3</sub> 500 ppm	35.67	1.49	44.00 (53.90)	976.75	16.33	9.17	13.03	22.20
T <sub>15</sub> -H <sub>2</sub> SO <sub>4</sub> 2.0%+GA <sub>3</sub> 750 ppm	34.67	1.71	65.33 (53.90)	1641.02	17.67	9.85	15.27	25.12
T <sub>16</sub> -HCl 1.0%+GA <sub>3</sub> 500 ppm	40.00	1.33	37.67 (37.84)	789.07	14.33	8.59	12.33	20.92
T <sub>17</sub> -HCl 1.0%+GA <sub>3</sub> 750 ppm	40.00	1.40	38.67 (38.43)	827.23	15.00	8.65	12.81	21.46
T <sub>18</sub> -HCl 2.0%+GA <sub>3</sub> 500 ppm	40.00	1.39	39.67 (39.62)	877.25	15.67	8.75	13.40	22.15
T <sub>19</sub> -HCl 2.0%+GA <sub>3</sub> 750 ppm	40.00	1.44	40.33 (39.40)	943.18	17.22	8.95	14.40	23.35
Mean	40.46	1.31	36.54 (37.17)	853.98	13.95	9.24	12.69	21.93
S. Em±	0.59	0.02	0.48	15.20	0.35	0.06	0.17	0.19
C. D. (P=0.05)	1.68	0.05	1.37	43.50	0.99	0.18	0.48	0.55

Figures in parentheses indicate arcsine transformed values.

**Table 2.** Effect of different growth regulators and chemical treatments on fresh weight of root and dry weight of root in *Embelia ribes* Burm f. at different days after sowing

Treatment	Fresh weight of shoot (mg)	Dry weight of shoot (mg)	Fresh weight of root (mg)	Dry weight of root (mg)	Fresh weight of seedling (mg)	Dry weight of seedling (mg)
T <sub>1</sub> -Control	616.67	54.33	50.00	22.33	666.67	76.67
T <sub>2</sub> -GA <sub>3</sub> 500 ppm	1590.00	170.00	115.00	92.67	1705.00	262.67
T <sub>3</sub> -GA <sub>3</sub> 750 ppm	1903.33	236.67	151.67	126.67	2055.00	363.33
T <sub>4</sub> -Ethylene 25 ppm	827.33	80.00	61.67	38.33	889.00	118.33
T <sub>5</sub> -Ethylene 50 ppm	880.00	86.67	66.67	40.33	946.67	127.00
T <sub>6</sub> -KNO <sub>3</sub> 1.0%	696.67	58.33	55.00	28.33	751.67	86.67
T <sub>7</sub> -KNO <sub>3</sub> 1.5%	741.67	63.33	58.33	33.00	800.00	96.33
T <sub>8</sub> -H <sub>2</sub> SO <sub>4</sub> 1.0%	946.67	96.33	88.33	61.67	1035.00	158.00
T <sub>9</sub> -H <sub>2</sub> SO <sub>4</sub> 2.0%	963.33	99.33	90.00	66.00	1053.33	165.33
T <sub>10</sub> -HCl 1.0%	866.67	90.00	85.67	56.67	952.33	146.67
T <sub>11</sub> -HCl 2.0%	900.00	91.67	88.00	61.67	988.00	153.33
T <sub>12</sub> -H <sub>2</sub> SO <sub>4</sub> 1.0%+GA <sub>3</sub> 500 ppm	983.33	101.67	95.67	67.33	1079.00	169.00
T <sub>13</sub> -H <sub>2</sub> SO <sub>4</sub> 1.0%+GA <sub>3</sub> 750 ppm	1000.00	106.67	99.33	69.33	1099.33	176.00
T <sub>14</sub> -H <sub>2</sub> SO <sub>4</sub> 2.0%+GA <sub>3</sub> 500 ppm	1250.00	108.33	102.67	74.33	1352.67	182.67
T <sub>15</sub> -H <sub>2</sub> SO <sub>4</sub> 2.0%+GA <sub>3</sub> 750 ppm	1480.00	123.33	110.00	77.00	1590.00	200.33
T <sub>16</sub> -HCl 1.0%+GA <sub>3</sub> 500 ppm	963.33	99.00	90.00	66.67	1053.33	165.67
T <sub>17</sub> -HCl 1.0%+GA <sub>3</sub> 750 ppm	990.00	104.00	93.33	68.33	1083.33	172.33
T <sub>18</sub> -HCl 2.0%+GA <sub>3</sub> 500 ppm	996.67	112.33	95.00	70.00	1091.67	182.33
T <sub>19</sub> -HCl 2.0%+GA <sub>3</sub> 750 ppm	1080.00	114.33	97.67	75.67	1177.67	190.00
Mean	1035.56	105.07	89.16	62.96	1124.72	168.03
S. Em±	21.40	4.12	3.22	0.96	22.14	3.88
C. D. (P=0.05)	61.25	11.80	9.23	2.76	63.40	11.11



Fig. 2. Best resulted treatment (3000 ppm IBA) in vegetative propagation.

treated with combination of IBA and NAA for one minute also registered comparatively better parameters values against control. Parameters like sprouting percentage (36.00), rooting percentage (36.00), number of leaves (23.67), number of sprouts (3.00) and number of roots (24.67) were maximum at 120 days after planting was also found to be maximum in cuttings treated with IBA 3000 ppm (Table 3). Better shoot parameters in turn enhanced the rooting in cuttings and as the IBA concentration increased, rooting and sprouting per cent also increased. Similar results were obtained in *E. ribes* Burm f. cuttings treated with IBA 3000 ppm (Saumya *et al.*, 2013) and there was significant increase in rooting and sprouting

percentage against the control.

Many studies reported that combination of IBA and NAA significantly increased per cent sprouting and rooting percentage in many plant species. In this study, combined effect of IBA and NAA at 3000 ppm recorded better sprouting percentage (29.33), rooting percentage (20.00), number of sprouts (1.67), number of leaves (20.33) and number of roots (18.33) at 120 days after planting (Table 3) when compared to the untreated cuttings and NAA alone treated cuttings. This may be due to the combined effect of auxins which might have caused hydrolysis and translocation of carbohydrates and nitrogenous substances at the base of cuttings and resulted in accelerated cell

**Table 3.** Effect of growth regulators treatments on sprouting and rooting of cuttings of *E. ribes* Burm f.

Treatment	Per cent sprouting	No. of sprouts/cutting at 120 DAP	No. of leaves at 120 DAP	Per cent rooting	No. of roots/cutting at 120 DAP
T <sub>1</sub> -Control	0.00 (0.00)	0.00	0.00	0.00 (0.00)*	0.00
T <sub>2</sub> -IBA 3000 ppm	36.00 (36.85)	3.00	23.67	36.00 (36.85)	24.67
T <sub>3</sub> -IBA 3500 ppm	33.33 (35.85)	2.67	21.00	28.00 (31.93)	18.33
T <sub>4</sub> -IBA 4000 ppm	28.00 (31.93)	1.33	19.00	20.00 (26.55)	15.00
T <sub>5</sub> -NAA 3000 ppm	0.00 (0.00)	0.00	0.00	0.00 (0.00)	0.00
T <sub>6</sub> -NAA 3500 ppm	0.00 (0.00)	0.00	0.00	0.00 (0.00)	0.00
T <sub>7</sub> -NAA 4000 ppm	0.00 (0.00)	0.00	0.00	0.00 (0.00)	0.00
T <sub>8</sub> -NAA+IBA 1000 ppm	14.67 (22.51)	1.33	18.00	13.33 (21.40)	13.67
T <sub>9</sub> -NAA+IBA 2000 ppm	16.00 (23.56)	1.67	19.33	14.67 (22.51)	15.33
T <sub>10</sub> -NAA+IBA 3000 ppm	29.33 (32.77)	1.67	20.33	20.00 (26.55)	18.33
Mean	15.73 (23.35)	1.17	12.13	13.20(21.96)	10.53
S. Em±	1.46	0.24	0.45	2.02	0.54
C. D. (P=0.05)	4.31	0.70	1.32	5.97	1.59

DAP : Days after planting. Figures in parentheses indicate arcsine transformed values.

elongation and cell division in suitable environment (Hartmann *et al.*, 2007). Similar results were also obtained from Basak *et al.* (2014) in *Piper longum* L. (pippali) through leaf cuttings which showed shooting up to 83.33%. Rahdari *et al.* (2014) and Copes and Mandel (2000) also obtained similar results.

### CONCLUSION

It was found that seed propagation of *Embelia ribes* Burm f. could be effectively done by using growth regulators like gibberellic acid, ethylene, potassium nitrate, sulphuric acid and hydrochloric acid at different concentrations to get enormous number of healthy seedlings. Among different treatments tried, gibberellic acid at 750 ppm was found best to get vigorous seedling and scarification at H<sub>2</sub>SO<sub>4</sub> 2% followed by GA<sub>3</sub> 750 ppm to get highest per cent of germination. In vegetative propagation, comparatively low results were observed. It can be concluded that hardwood cuttings with two or three leaves, treated with IBA in 3000 mg/l concentration appeared to be a successful method for vegetative propagation (36% rooting). Further work on vegetative propagation has to be required to get better results when compared to seed propagation.

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