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Seed propagation studies in *Embelia ribes* burm. F.

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Abstract
Embelia ribes, commonly known as Vidanga or false pepper is a commercially important threatened medicinal plant of the Tropics belonging to the family Myrsinaceae which yields embelin, a highly valuable quinine derivative. Regeneration of the crop is poor due to over exploitation, diminishing forest area, dormant seeds, abortive embryos and hard seed coat. Seeds were subjected to several pre-soaking treatments i.e, soaking in water, aqueous solutions of GA_3 at different concentrations, acid scarification using HCl, H_2SO_4 and combination of acid scarification and GA_3. Seeds treated with GA_3 750 ppm for 24 hours and H_2SO_4 scarified seeds treated with GA_3 750 ppm for 12 hours exhibited superior results compared to the untreated control. The shoot length (8.82 cm), root length (8.03 cm), and total dry biomass (70.21mg) were maximum in seeds treated with GA_3 750 ppm whereas, germination percentage (87.5) and seedling vigour (1410.42) were maximum in H_2SO_4 scarified seeds treated with with GA_3 750 ppm.

Keywords: *Embelia ribes*, pre-sowing treatments, GA_3, HCl, H_2SO_4

Introduction
Embelia ribes is a red listed, large woody climbing shrub belonging to the family Myrsinaceae [10]. It is one of the 32 medicinal plants selected by National Medicinal Plants Board for its large scale cultivation due to its commercial value [16-17]. It is mainly distributed in warmer regions of northern and southern hemisphere. Most of the genera and species are tropical. Genus *Embelia*, represented by more than 100 species, is distributed in Tropical Asia, Africa and Australia and Pacific islands. The fruits, leaves, bark and root of *Embelia ribes* is of immense value to the Traditional systems of medicine. It is an antipyretic, anticonvulsant, antibacterial, antioestrogenic, antihelmintic, carminative, laxative, diuretic, and astringent [9]. It is used in treatment of skin fungal infections, leprosy, hemorrhoids, obesity, lung diseases, cancer, mental disorders and heart diseases [8, 2, 13]. The benzoquinone embelin obtained from berries have proven antispermatogenic effects. *Embelia ribes* is one such plant which is overexploited for commercial purpose. The threats faced by forest from humanshas eventually led to the threatened status of medicinal plants like Vidanga. Regeneration of *E. ribes* from seeds is poor, embryos 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. The propagation and conservation of this important medicinal plant requires special attention.

Materials and Methods
Seed germination studies were conducted at Division of Plant Physiology and Biochemistry and Division of Plant Genetic Resources, ICAR- Indian Institute of Horticultural Research, Hessaraghatta, and Bengaluru. The experiment was carried out in the Seed germination Chamber where the day and night temperature were maintained at an average of 20 ^{0}C /30 ^{0}C. The required seed materials were collected from the plants grown in the Field Gene Bank of RET medicinal plants maintained at Division of Plant Genetic Resources, ICAR- IIHR, Bengaluru. The fruits were subjected to floatation test, where the sinkers were collected and floaters were discarded. Then the mucilaginous seed coat present in the seeds was removed. The seeds were treated with mercuric chloride 0.1 per cent for 10 minutes, later washed with water and shade dried for 24 hours. The extracted seeds were subjected to 16 different pre-sowing treatments. Treated seeds were sown in protrays consisting of coir pith and kept in

seed germination chamber (alternate temperature of 200C /300C, 80-85% humidity). The protrays were covered with black polythene films to maintain high humidity and temperature to facilitate seed germination.

Table 1: Pre-sowing treatments given to Embelia ribes to enhance seed germination.

T_1	**Soaking in water**
T_2	GA_3 at 250 ppm (12 h)
T_3	GA_3 at 500 ppm (12 h)
T_4	GA_3 at 750 ppm (12 h)
T_5	GA_3 at 250 ppm (24 h)
T_6	GA_3 at 500 ppm (24 h)
T_7	GA_3 at 750 ppm (24 h)
T_8	HCl 35% (1 min)
T_9	HCl 35% (1 min) + GA_3 250 ppm (12 h)
T_{10}	HCl 35% (1 min) + GA_3 500 ppm (12 h)
T_{11}	HCl 35% (1 min) + GA_3 750 ppm (12 h)
T_{12}	H_2SO_4 10% (10 min)
T_{13}	H_2SO_4 10% (10 min) + GA_3 250 ppm (12 h)
T_{14}	H_2SO_4 10% (10 min) + GA_3 500 ppm (12 h)
T_{15}	H_2SO_4 10% (10 min) + GA_3 750 ppm (12 h)
T_{16}	Control

Fig1: a) Fruiting branch b) dried seeds c) ripe berries

Result and Discussion

The data pertaining to various germination parameters as influenced by different pre-sowing treatments is summarized in Table 2.

In this study the highest germination percentage (87.50) and least number of days taken for initiation of germination (26) was recorded in H2SO4 scarified seeds treated with GA3 500 ppm and H2SO4 scarified seeds treated with GA3 750 ppm followed by seeds treated with GA3 750 ppm. The seedling vigour was found to be highest in H2SO4 scarified seeds treated with GA3 750 ppm (1410.42) and similar to that of GA3 750 ppm (1372.70) followed by H2SO4 scarified seeds treated with GA3 500 ppm. Other parameters like shoot length, root length and total dry biomass of seedlings were higher in seeds treated with GA3 750 ppm followed by H2SO4 scarified seeds treated with GA3 750 ppm and H2SO4 scarified seeds treated with GA3 500 ppm.

Table 2: Effect of preconditioning of seeds on days taken for initiation of germination and germination percentage.

Treatments	Days taken for initiation of germination	Germination percentage	Shoot length 90 DAS (cm)	Root length 90 DAS (cm)	Seedling vigour	Total dry biomass (mg)
T1- Soaking in water	50.00	30.50 (33.52)	4.84	2.64	228.04	39.28
T2- GA3 at 250 ppm (12 h)	43.00	42.50 (40.68)	5.70	3.18	377.73	40.37
T3- GA3 at 500 ppm (12 h)	41.75	44.00 (41.54)	5.90	3.36	407.33	43.63
T4- GA3 at 750 ppm (12 h)	40.00	47.50 (43.56)	6.23	3.55	464.45	45.16
T5- GA3 at 250 ppm (24 h)	33.25	60.50 (51.06)	7.50	6.17	827.02	56.07
T6- GA3 at 500 ppm (24 h)	31.50	78.00 (62.03)	7.68	7.55	1187.66	58.75
T7- GA3 at 750 ppm (24 h)	30.50	81.50 (64.53)	8.82	8.03	1372.70	70.21
T8- HCl 35% (1 min)	46.25	46.50 (42.99)	5.18	3.26	392.47	40.45
T9- HCl 35% (1 min) + GA3 250 ppm (12 h)	42.00	47.00 (43.27)	6.32	3.27	450.69	46.56
T10- HCl 35% (1 min) + GA3 500 ppm (12 h)	40.25	50.00 (45.00)	6.52	3.55	504.00	49.48
T11- HCl 35% (1 min) + GA3 750 ppm (12 h)	40.50	52.50 (46.43)	6.70	3.67	545.43	53.15
T12- H2SO4 10% (10 min)	41.25	50.50 (45.28)	5.49	3.49	453.53	42.26
T13- H2SO4 10% (10 min) + GA3 250 ppm (12 h)	31.00	73.00 (58.73)	8.05	6.73	1080.99	57.75
T14- H2SO4 10% (10 min) + GA3 500 ppm (12 h)	26.00	85.00 (67.24)	8.34	7.47	1345.74	59.44
T15- H2SO4 10% (10 min) + GA3 750 ppm (12 h)	28.00	87.50 (69.30)	8.41	7.61	1410.42	63.65
T16- Control	53.00	29.50 (32.88)	3.92	2.02	175.67	38.14
S.Em ±	0.75	0.73	0.10	0.08	18.37	0.66
CD @ 5%	2.13	1.73	0.30	0.25	52.25	1.89

* Values in the parentheses are arcsine transformed value

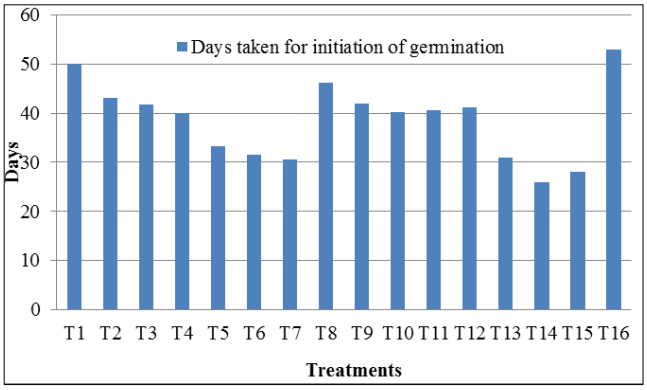

Fig 2: Effect of growth regulators and chemicals on days taken for initiation of germination in E. ribes Burm. f. seeds

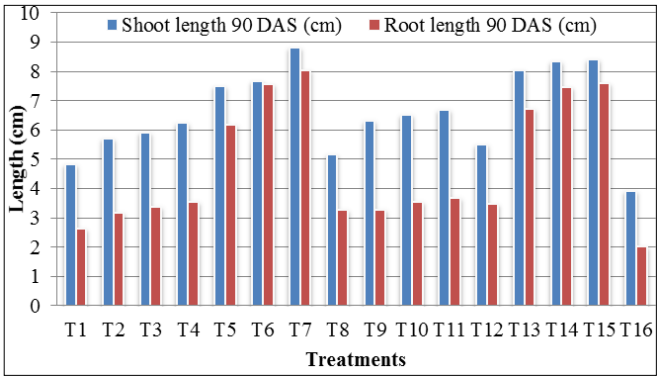

Fig 3: Effect of growth regulators and chemicals on shoot length and root length in E. ribes Burm. f. seedlings at 90 days after sowing

Similar results were obtained in Embelia ribes where seeds treated with GA3 500 ppm for 16 hours resulted in 80-85% germination in 6-7 days [1, 18]. Reported that E. tsjeriam-cottam seeds treated with 300 ppm GA3 recorded maximum germination (52%) compared to control (13.20%). Seeds of Embelia ribes when treated with GA3 500 ppm for 18 hours recorded a germination percentage of 73.33 [5]. Treatment of Vidanga seeds with GA3 750 ppm exhibited similar results in case of early seed germination, germination rate, seedling vigour, seedling height and number of leaves [19]. Gowda *et al*. (2003) [6] reported that GA 400 ppm considerably improved germination (48%) than control (12%) in E. tsjeriam-cottam. Lavandula dentate seedstreated with gibberellic acid at 1000 ppm marked a maximum germination of 67% compared to the control which did not exceed 1 per cent [3].

Among the different germination inducing treatments, the seeds treated with gibberellins responded well with high seed germination and vigorous seedling growth. Initiation of germination was also earlier in GA3 treatment at different concentrations. Paleg (1960) reported that, gibberellic acid originating from the embryo is responsible for the hydrolysis of starch reserves in the endosperm during germination of grains. GA3 induces the de-novo synthesis of proteolytic enzymes like α-amylase and ribonuclease. Amylases in turn hydrolyse starch in the endosperm, providing the essential sugars for the initiation of growth processes [4].

Low germination percentage in seeds may be due to physical or chemical barriers like hard seed coat and dormancy. When both these factors act together it's a tough job for water to penetrate and trigger germination. Acid scarification followed by treatment with growth regulators like GA3 can easily solve this problem. Here, highly appreciable results were obtained in less time compared to that of GA3 treatment alone. Acid treatment brings about softening of hard seed coat by dissolution of pectic substances, lipids and high density waxes, which is a common cause for hard seededness [7, 18]. This softens the seed coat in and makes it permeable to water and gases. Futher, the GA3 treatment induces hydrolysis of starch reserves which leads to germination of seed. Vidanga seeds scarified with H2SO4 10% and then treated with GA3 500 ppm recorded the least number of days taken for germination (26.00) with better germination (85%) whereas, highest germination (87.5 %) and seedling vigour (1410.42) was observed in H2SO4 scarified seeds treated with GA3 750 ppm.

Patwardhan *et al*. (2014) [12] reported that pre-sowing treatment of 10 percent H2SO4 for 10 minutes + GA3 4000 ppm is the best treatment for Embelia ribes with 40% germination. Pipinis *et al*. (2011) [14] observed higher germination percentage (90.83) in Paliurus spina-christi Mill. Seeds when scarified with H2SO4 (90 min) and then treated with GA3 2000 ppm. Acid scarification considerably increased the germination from 29.50 percent in control to 50 per cent. There was improvement in seedling parameters such as total dry biomass (42.26 mg) and seedling vigour (453.53). The seeds of Zanthoxylum armatum DC when treated with diluted (50 %) H2SO4 (15 min) resulted in maximum germination (93.3 %) along with mean germination time (MGT) of 149.5 days [15]. Sharma *et al*. (2011) [18] reported better germination (34%), rate of germination (0.95) and vigour index (357) in E. tsjeriam-cottam seeds when treated with concentrated H2SO4 for one minute. Though the seeds soaked in water alone did not show appreciable results in this experiment it might have aided the germination process along with acids and GA3 by converting the insoluble food into soluble form for its translocation to the embryo and by bringing dissolved oxygen. We know that seedling emergence is primarily a function of moisture availability and optimum temperature to the seed. The tropical species require high temperature and relative humidity for their germination [7]. This was achieved by covering the protrays with black polythene. The seed germination chamber was a boon for the seedlings from the harsh summer condition. From this experiment it is evident that Vidanga seeds treated with GA3 750 ppm for 24 hours and H2SO4 scarified seeds treated with with GA3 750 ppm for 12 hours exhibited nearly similar results superior to the other treatments and control. The first one reduces the cost and probable hazards from acids, but requires long duration of treatment (24 hours) meanwhile, the second one reduces the time required for seed treatment by 12 hours and slightly increases the cost involved in purchase of chemicals.

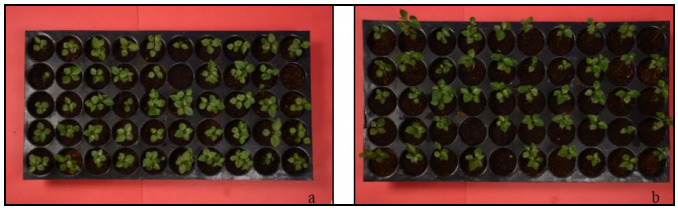

Fig 4: Seedlings raised from the best treatments 90 days after sowing a) GA3 500 ppm b) H2SO4 + GA3 500 ppm

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