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Impact of Population Structure, Growth Habit and Seedling Ecology on Regeneration of *Embelia ribes* Burm. f.—Approaches toward a Quasi *in Situ* Conservation Strategy

Dhavala Annapurna*, Ashutosh Srivastava, Trilok Singh Rathore

Tree Improvement and Genetics Division, Institute of Wood Science and Technology, Bangalore, India.
Email: *uannapurna@gmail.com

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ABSTRACT

Embelia ribes Burm f., also known as Vidanga or Baibidanga, belonging to the family of Myrsinaceae, is an important but vulnerable medicinal woody climber. Recent survey and observations of *E. ribes* in different aspects like distribution, population structure, growth habit, climate, natural regeneration and seedling ecology in Karnataka have been thoroughly discussed. This is the first report on artificial regeneration through seeds of diverse origin in *ex-situ* and *in situ* conditions and field planting of them in its natural environment. Field planting of *in vitro* and nursery raised seedlings showed better field performance in terms of survival and growth in its natural growing areas only. Current studies and observations have shown that this species has low ecological gradient and is a "habitat specialist". *E. ribes* being a threatened species with small populations coupled with low ecological gradient and virtually no natural regeneration, a modified approach of quasi *in situ* conservation where in *in-situ* raising of seedlings of diverse origin with an aim to enrich the diversity of existing population was attempted with considerable success.

Keywords: *Embelia ribes*; *Ex-Situ*; *In-Situ*; Ecological Gradient; Habitat Specialist; *Quasi in Situ*

1. Introduction

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 [1]. The fruits of *E. ribes*, a source of the drug Vidanga, are globular, dull red or brown in colour, upto 4 mm in diameter with a five partition persistent calyx and a short stalk (Sarin, 1996). Their pericarp is brittle, enclosing a single seed covered with a membrane; taste slightly astringent and aromatic [2]. The main active medicinal component in dried fruits of *E. ribes* is embelin (2, 5-dihydroxy-3-undecyl-2,5-cyclohexadiene-1,4-benzoquinone). This highly valuable medicinal plant has been shown to have antihelmintic [3], antifertility [4,5], antibacterial [6], antidiabetic [7], antitumor, analgesic, anti-inflammatory [8,9], antispermatogenic [10], chemopreventive [11] and cardioprotective [12] properties, besides antioxidant activity against methionine-induced hyperhomocysteinemia and hyperlipidemia and oxidative stress in the brain [13].

E. ribes is one of the 32 medicinal plant species identified as important for large-scale cultivation because of its commercial use by the National Medicinal Plant Board, Govt. of India, New Delhi [14]. The drug, Vidanga, is produced and used in high volumes, estimated to be at a demand of ~500 tons/yr. It was also estimated that the annual trade growth rate of Vidanga is about 23% [1]. Presently, the demand for Vidanga is being met entirely from wild collections, as its commercial cultivation is not yet practiced in India.

Western Ghats (hill chain running parallel to the west coast of India, (from 8˚N to 21˚N latitudes, 73˚E to 77˚E longitude for around 1600 km), one of the 18 globally recognized hotspots for diversity (**Figure 1**) is characterized by rich vegetation, enormous biodiversity and endemism [15]. The Western Ghats presents a whole range of gradients, both altitude and latitude wise, and in

*Corresponding author.

Figure 1. Location of Western Ghats in India.

climatic factors, such as, total annual rainfall and maximum temperature, and also in edaphic factors such as soil types and topography. It is reported that nearly 63% of the tree species of low and medium elevation in evergreen forests of Western Ghats are endemic due to unique matrix of climatic and edaphic factors [16]. Agumbe is amongst the highest rainfall receiving region in the Western Ghats, also earning itself the name, "Cherrapunji" of the South. It is felt that one of the most critical challenges of conservation of rare, threatened and endemic plants is to identify "ecological niche" and "amplitude of distribution" [17]. A species with low ecological gradient is of endemic nature because they cannot have a positive association with conditions outside their natural habitat. They are also referred to as "habitat specialist".

1.1. Distribution and Population Structure

E. ribes is found all over India in restricted patches up to an altitude of 5000 ft. The distribution of *E. ribes* in peninsular India is mainly in the states of Maharashtra, Karnataka and Kerala in the Western Ghats and in Tamil Nadu in the Eastern Ghats. The species is reported to be vulnerable in Maharashtra and Karnataka, and at a low risk status in Kerela [1]. The species grows in the Medicinal Plants Conservation Areas (MPCAs) of Karnataka viz; Agumbe, Charmadi, Devimane, Kemmanagundi, Kollur, Kudremukh, Subramanya and Telacauvery [18].

Growth habit: It is a large woody climber with smooth brown stems having distinct tubercular projected leaves, bisexual flowers and tiny globular wrinkled or warty fruits.

Flowering and fruiting habitat: The plant usually flowers during February-April and the fruits mature during May-August.

Natural regeneration: 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 [18]. Natural regeneration of *E. ribes* is poor due to its over-harvesting and exploitation, fragmented populations resulting in inbreeding, development of abortive embryos, and the slow germination of fertile seeds that are small in size [19]. Unfortunately, traditional methods of propagation are not successful in the large-scale production of *E. ribes*.

1.2. Artificial Regeneration

On the other hand, artificial regeneration of this species is also difficult due to poor seed viability, low rate of germination, and poor rooting from stem cuttings [1]. Preliminary studies conducted on seed and vegetative prorogation of *E. ribes* emphasized that specific habitat conditions are required for its survival and growth. Regeneration of *E. ribes* is also very slow [20]. Recently, direct shoot organogenesis in *E. ribes* was reported using a seedling-derived leaf [20] and hypocotyls [21] as explants. *In vitro* propagation of *E. ribes* through axillary shoot proliferation using nodal segments from mature plants has been reported [22].

However, there are no reports available on the artificial regeneration using seeds and performance of seedlings in the field on *E. ribes*. The work presented here shows our efforts and results directly in propagating *E. ribes* through conventional seed-based nursery techniques and *in vitro* propagation and field planting of them. Indirectly, this effort leads to conservation of this low adaptable, threatened species.

2. Materials and Methods

The entire study can be broadly categorized into two aspects.

1) Survey in the natural distribution areas: Extensive survey has been carried out at Agumbe, Charmadi, Devimane, Kemmanagundi, Kollur, Kudremukh, and Telacauvery during 2006-2008 to collect seeds and nodal

segments for artificial propagation. During the survey, significant differences were noted regarding their population structure, growth habit, fruiting behavior and natural regeneration.

2) Artificial regeneration:

Seed source, pre-treatment, germination and transplanting of seedlings

Wild fruits of *E. ribes* were collected from natural populations from Agumbe, Karnataka (**Figure 2A**) during July 2006. Subsequently, during the year 2007, seeds were collected from Sringeri population which was identified later (**Figure 2F**). After depulping the fruits, the seeds were dried under shade for 24 h.

With an objective to standardize the ideal pre-treatment method, the seeds were subjected to various pre-treatments and germinated on germination paper for enhanced and early germination under controlled condi--tions in a germinator (data not shown). Based on the results, the seeds were decoated manually by cracking them with a slight pressure using a pestle and then they were pre-treated with 0.05% gibberellic acid (w/v) for16 h and allowed to germinate.

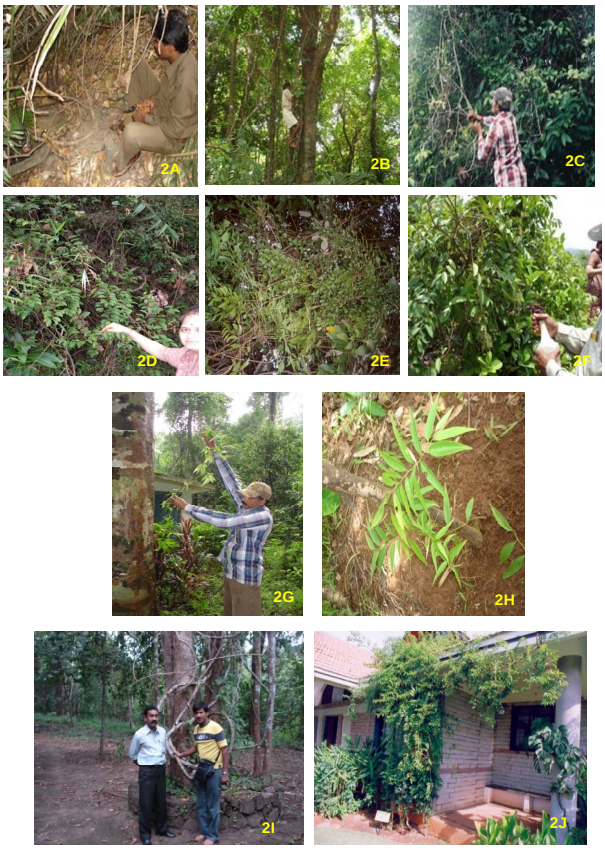

Figure 2. (A-J) Growth habit, climate and seed setting in *Embelia ribes*; (A) *E. ribes* in the form of shrub at charmadi; (B-D) Growth habit of *E. ribes* plants at Charmadi, Agumbe and Telacauvery; (E) Growth habit of *E. ribes* at Devimane MPCA; (F) Growth habit of *E. ribes* at Sringeri; (G & H) *E. ribes* at planting site 1 and 2 in Agumbe; (I) *E. ribes* at Tropical Botanical Garden Research Institute, Thiruvananthapurm, Kerala; (J) One plant grown and maintained at FRLHT, Bangalore.

2.1. Seed Germination Studies at Bangalore

In the first approach, pre-treated seeds collected from Agumbe during 2006 and Sringeri during 2007 were germinated in root trainers, each block of 12 cells and each cell of 270 cc volume consisting of potting medium combination of sand: soil: compost; brick pieces in the ratio of 25:15:50:5:5. The root trainer was maintained under controlled conditions in a mist chamber at a temperature of 28 ± 2˚C with intermittent misting @ 30 minutes during day time.

2.2. Seed Germination at Agumbe

In the second approach, the pre-treated seeds collected from Sringeri were germinated in trays consisting of sand at Karnataka Forest Department nursery in Agumbe (natural occurring area of *E. ribes*). After 30 - 35 days, the 2-leaf stage seedlings obtained from the peak germination stage were transferred to 1000 cc polybags consisting sand, soil, compost in 2:1:1 ratio.

2.3. Growth Data

The height and collar diameter of *E. ribes* seedling were recorded at the end of 6th and 12th months from sowing. Shoot length was measured with a ruler and the collar diameter of shoots at the base was measured with electronic digital Vernier callipers (Mitotoyo, Japan). Sturdiness quotient (SQ) was calculated by dividing the seedling height (cm) with the collar diameter (mm) of the same seedling [23]. The means are presented with ± SE.

2.4. Field Planting

Hardened tissue-culture raised plants of height ≥ 30 cm (n = 20) were used for planting in a nursery area of Karnataka Forest Department at Agumbe in the month of July 2008. One year old plants raised at Agumbe nursery using seeds collected from Sringeri were planted near Agumbe (n = 30) and Sirsi forest areas (n = 25), during July 2008 where *E. ribes* natural distribution is seen. Data were collected on a periodic basis to evaluate the survival and growth performance of plants.

3. Results

3.1. Survey in the Natural Distribution Areas

3.1.1. Distribution and Population Structure

Extensive survey of *E. ribes* populations through Western Ghats of Karnataka in 8 MPCAs and adjoining areas re-

vealed that, these populations existed in small and fragmented patches of 5 - 10 individuals on the fringe areas of forests. The population density of each of surveyed area is described below.

Agumbe: Four different fragmented patches of *E. ribes* were identified at Beddina Malliga, Barkana, Handsur and on the fringe of Agumbe MPCA. These populations existed in very few individuals with 5 - 10 plants per area. The individuals at Barkana and Agumbe MPCA were very old large climbers while the individuals at Handsur and Beddina Malliga were in the form of large woody shrubs. General structure of these populations was fragmented individuals occurring on a steep slopy land clinging on to nearby trees.

Sringeri: During the course of survey a new population was identified near Sringeri Narasimha Parvath which was a denuded hill top. The population size was maximum with 25 - 30 individuals occurring in a sq. Km area. These individuals occurred as shrubs as well as climbers.

Devimane MPCA: A small fragmented population of 5 individuals occurring on the edge of MPCA gate. These individuals were tall climbers clinging on to large adjoining trees.

Kolluru and Kemmangundi MPCA: In these MPCAs no individuals were found although occurrence of *E. ribes* in these areas has been reported. One of the reasons of disappearance of these individuals could be the fact that they existed on the fringe of MPCA near by road.

In the populations occurring in Agumbe and Devimane MPCA the individual climbers were so closely spaced that it was not possible for us to reach their origin (base of the stems). The individuals were intermingled with each other and their canopies also merged. It was not possible for us to ascertain if these were different individuals naturally grown or a part same mother tree growing vegetatively multiplied.

Charmadi: In one of the populations at Charmadi we observed two individuals at a distance of 5 m one in the form of tall climber and other in the form of a shrub. When we started digging from base of the climber, we reached the shrub and found that the shrub arose from the root sucker of the large climber (**Figure 2A**).

3.1.2. Growth Habit

In the climax forest areas of Agumbe, Charmadi and Telacauvery MPCAs, it was found as a large woody climber clinging on to tall adjoining trees (**Figures 2B-D**) Whereas, in Devimane MPCA, it was found on the edges of MPCA as a small woody climber (**Figure 2E**). In contrary to above habitats, the population of Sringeri (Narasimha Parvath) which was a denuded hill top, *E. ribes* was found to be growing as a shrub (**Figure 2F**) while some individuals which were growing near trees showed climber like growth pattern .

It is also worth underlying the observation that the individuals found in closed canopy sites (climax forests of Agumbe, Telacauvery and Charmadi), it tends to grow as climber in search of light where as the open site individuals grow luxuriantly in form of shrubs as there is no shortage of light. This observation was also reflexed in artificial regeneration sites at Agumbe. Seedlings planted at Agumbe nursery (closed canopy site) were growing as climber clinging onto nearby trees (**Figure 2G**) whereas the plantations established in open area the plants continued to grow as a shrub (**Figure 2H**).

3.1.3. Flowering and Fruiting Habitat

It was generally observed that *E. ribes* profusely flowered and fruited both in climber as well as shrubs. However the seeds obtained from Sringeri area (shrub habitat) were of higher viability and vigour which might be due to higher number of individuals in the close proximity and greater pollination activity (cross pollinators) at lower altitudes as compared to tall climbers.

3.1.4. Climate

Earlier attempts to plant *E. ribes* in areas outside the natural range were attempted by TBGRI (Tropical Botanical Garden Research Institute), Thiruvananthapuram, IFGTB (Institute of Forest Genetics and Tree Breeding), Coimbatore, UAS (University of Agricultural Sciences), and FRLHT (Foundation for Revitalization of local Health Tradition), Bangalore. Personal visits to these sites indicate that except in TBGRI which is in natural zone of *E. ribes* (**Figure 2I**), all other attempts did not yield positive results. Only one surviving plant exists in FRLHT (**Figure 2J**) while the Botanical Garden of UAS Bangalore contains *E. tsjeriamkottam* and not *E. ribes*. In FRLHT, though flowering was observed after 4 - 5 years of planting, but fruiting and seed setting was not observed although plant contains bisexual flowers.

3.1.5. Natural Regeneration

Extensive survey of *E. ribes* populations revealed that they exist in small patches of 5 - 10 individuals on the fringe areas of forests. The scattered populations, even though go through profuse fruiting (**Figure 2B**), they showed no virtual natural regeneration in all the survey sites (MPCAs, Agumbe, Charmadi, Devimane, Kudremukh, Subramanya and Talacauvery and in Sringeri area.

3.2. Artificial Regeneration

Pretreatment: Out of the various pre-treatments tried, we found that decoated seeds (**Figure 3A**) pretreated with GA_3 (500 ppm) for 16 h resulted in 90% - 95% ger-

Figure 3. (A-J) Germination, nursery studies and field growth performance of *Embelia ribes*; (A) Decoated seeds; (B) Albino seedlings at Bangalore nursery; (C) Normal seedlings in root trainers at Bangalore Nursery; (D) Drying of the seedlings from tip; (E) seed germination at Agumbe nursery; (F) Germinated seedlings at Agumbe nursery in trays; (G) Six-month-old seedlings at Agumbe nursery; (H) One year old seedling at Agumbe nursery; (I) One year old seedling at Agumbe forest area; (J) One year old seedling at Sirsi forest area.

mination (on germination paper) with in 6 - 7 days (emergence of radicle of 1 cm was considered as germinated; data not shown).

3.3. Raising Seedlings at IWST, Bangalore

The seeds collected from Agumbe were germinated in root trainers showed 80% - 85% germination with 20 - 25 days (emergence of 1 cm plumule above the surface of potting medium was considered germinated). They developed into two leaf stage seedlings (**Figure 3B**) within 10 days (30 - 35 days from sowing). Among these 30% - 40% seedlings showed albinism (**Figure 3C**) which died after 45 - 50 days. During 2007, we observed 80% - 85% germination in root trainers within 20 - 25 days from seeds collected from Sringeri population. They developed to green and healthy seedlings within 30 - 35 days. These seedlings showed signs of tip drying and gradually drying of entire plant (**Figure 3D**). Later on seedlings could not be raised further.

3.4. Raising of Seedlings *in Situ* at Agumbe

Failure to raise viable seedlings in Bangalore prompted us to take up the nursery work at the forest department nursery at Agumbe, India in 2007. At Agumbe, we observed 80% - 85% germination after 40 - 45 days and they developed to two leaf stage seedlings with in 60 days (**Figures 3E** and **F**). Delay in germination by 10 days at Agumbe nursery compared to Bangalore may be due to low temperatures prevailing during that period in Agumbe. At the age of 6 months the seedlings showed a height of an average 16.43 ± 5.5 cm with a collar diameter of 2.03 ± 0.55 mm and 8.32 ± 2.40 (**Table 1** and **Figure 3G**). The seedlings were maintained at the nursery for one year before being planted out in the field (**Figure 3H**). At the time of planting, the seedlings showed height 54.50 ± 25.66 cm with a collar diameter of 5.38 ± 1.23 mm and sturdiness quotient of 10.34 ± 4.44 (**Table 1**).

Field planting: *In vitro* raised plantlets which attained a height of ≥ 30 cm after 3 months of hardening were used for field planting at the State Forest Department nursery, Agumbe, Karnataka. We observed that the Survival rate of plants after 4 months of field planting was 95% resulting in new shoot growth. Even after one year the plants showed 90% survival, and they are still growing extremely well.

At four months age, nursery raised seedlings planted at two forest sites (Agumbe and Devimane sites) showed 85% and 80 % survival, respectively (**Figures 3I** and **J**). Unfortunately, after a year, the survival reduced to a mere 40%. This could be attributed to the high temperature and drought conditions after monsoon period in the area, which might have resulted in mortality of the seedlings at Agumbe and Sirsi forest areas. Whereas, at the Agumbe nursery, seedlings maintained by providing irrigation conditions once in 15 days during the summer months showed 80% survival even after one year. This calls for an intensive management of the *E. ribes* plants even after their establishment in the field.

4. Discussion

The populations near all MPCAs are represented by few

Table 1. Growth parameters of *E. ribes* seedlings at Agumbe nursery.

	Height (cm)	Collar diameter (mm)	SQ
6 months	16.43 ± 5.50	2.03 ± 0.55	8.32 ± 2.40
One year	54.50 ± 25.66	5.38 ± 1.23	10.34 ± 4.44

individual plants (5 - 10 in numbers) concentrated in a small area on the fringes of the forest, except for the new population identified at the Narasimhaparvath near Sringeri. One of the plants at Kudremukh MPCA was removed during the course of study to widen the existing road which corroborates our observations that the natural populations of *E. ribes* are under immense demographic pressures due to their spatial distribution (fringe occurrence)

This fragmented spatial distribution of *E. ribes* is the characteristic of a population under edaphic-climatic and demographic pressure. Genetic and demographic processes related to small population size play a major role in putting a species into threatened or vulnerable status. The species of *E. ribes* used in this study were of typical populations that have been under tremendous pressure of genetic stochasticity. Genetic stochasticity describes the loss of genetic diversity related to combined effects of genetic drift and inbreeding in small populations [24].

Growth habit: This growth character of *E. ribes* apparently seems to be an ecological adaptation where in the growth pattern of plant changes depending the availability of light. We feel this growing pattern of *E. ribes* could be of great use for establishing plantations in open areas adjoining the climax forest areas.

Our attempts to establish plant at IWST nursery also failed for 3 years 2006-2009. However, when we raised *E. ribes* at Agumbe (natural zone) nursery, we observed that the seeds germinated, seedlings were healthy and the planted seedlings survived in the field in its natural habitat. This indicates that *E. ribes* needs a specific habitat and does not survive outside its natural range, it could therefore be classified as "habitat specialist".

The scattered populations, even though go through profuse fruiting (**Figures 2E** and **F**), they showed no virtual natural regeneration in all the survey sites (MPCAs, Agumbe, Charmadi, Devimane, Kudremukh and Talacauvery and in Sringeri area. These populations of *E. ribes* grow on slopes in the areas of their natural occurrence where the rainfall is high, and hence making the site to be under constant threat of soil erosion. They produce copious amount of tiny seeds, which mature between May and August months of the year, and the fruit maturation time coincides with the peak rainy season of that area. Various reasons for lack of natural regeneration could be that 1) the seeds mature during the peak of rainy season and are washed away by the rain water; 2) presence of the chemical that α-amylase activity, which is required for initiation the germination process; 3) the seeds of this species are collected as Non Timber Forest product (NTFP).

However, treating the decoated seeds with GA3 500 ppm for 16 hours resulted in 80% - 85% germination in 6 - 7 days. Presence of anti α-amylase activity in seeds of *E. ribes* was reported and it has been attributed to its anti-diabetic property [25]. During the germination studies, occurrence of albino seedlings from seeds collected from Agumbe location in the nursery stock indicated the presence of inbreeding in the population resulting in the expression of recessive alleles. However, in the population identified at Narasimhaparvath near Sringeri, the number of trees was more (30 - 35 individual trees/sq·km). Inbreeding depression effects have been especially important in the context of rare and threatened species [26]. The primary genetic factor in small populations is the increased inbreeding, which can lead to reduced offspring survival, by exacerbating small population size [27,28]. Adverse effect of NTFP collections on gene flow and mating system has also been reported [24].

Growth performance of seedlings at the Agumbe nursery was very encouraging and this might be due to the micro-climatic conditions of this area and also Agumbe falls in the natural ecological niche of *E. ribes* distribution, which favored their robust growth. Successful growing of seedling at Agumbe and failure to do so at Bangalore conditions reaffirms the fact that *E. ribes* is a very strong "habitat specialist" with very low ecological gradient. Any attempt to revive this disappearing species should be seriously concentrated on their *in situ* propagation [29].

Conservation of wild plants has a major focus on preserving viability of the existing population through *in-situ* programs [30]. An endangered species is usually represented by small and isolated populations that already underwent strong effects of genetic drift and/or inbreeding [31]. Therefore, for an endangered species, creation of *ex-situ* collections and decisions about suitable material for relocation/reintroduction should take into account the potential risks of inbreeding depression, in addition to local adaptation and spatial structure of adaptive variation [32]. In species that lack local adaptation, low genetic diversity and self-incompatibility, a slightly modified approach of "forest gene bank" was suggested [33-35]. A novel approach of quasi *in situ* conservation of endangered plants was proposed by [32]. In their approach, *ex-situ* collections are maintained in natural or semi-natural environment, wherein preserving both neutral and adaptive genetic diversity is complimentary to the *in-situ*/*ex-situ* conservation strategy.

This pattern of natural distribution and habitat specificity shows the necessity for a quasi *in situ* conservation strategy as proposed [32]. Under this strategy *ex situ* collections should be maintained in a natural environment. This would ensure the preservation of both natural and adaptive (introduced) genetic diversity. Additionally, establishment of forest gene banks as suggested [35] would

go a long way in preventing this rare species from becoming extinct.

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