Comparison between traditional and conventional methods of seed storage and pretreatment in *Embelia ribes* - A threatened medicinal plant

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Abstract

Good seed storage practices ensure maximum seed viability and suitable pretreatment of seeds resulting in maximum germination percentage. The importance of using appropriate seed storage and pretreatment techniques has been well recognized since ancient times. This is evidenced by the detailed procedures that have been described in the traditional texts, both to retain viability as well as to improve germination. The raw materials used for these processes are natural and 100% organic. Conventional methods for the two processes, on the other hand, make use of inorganic chemicals, which can be harmful to both the environment and humans. A study was conducted to compare the effect of conventional practices and selected traditional methods of seed storage and pretreatment with respect to *Embelia ribes*. Seeds of the selected species were collected and subjected to monthly germination trials. Data was analysed with respect to best treatment combination and time of germination using the statistical tool ANOVA. From the observations it was concluded that with respect to storage the traditional method was on par with the conventional method; whereas with respect to pretreatment, the conventional method was better than the traditional method. The results also showed that germination of *E. ribes* was restricted to seeds sown between September and December.

Keywords: *Embelia ribes, Storage, Pretreatment, Propagation, Traditional, Vrikshayurveda*

Introduction

Seed storage and pretreatment are two important factors that affect the quality and quantity of seedlings produced. Proper storage ensures that more number of seeds remain viable for a longer duration. Similarly, best pretreatment procedure guarantees maximum germination percentage. Earlier studies have shown that factors such as light, temperature, seed moisture, concentration of O_2 and CO_2 around the seeds and other conditions of storage, time of collection, development, initial viability and physical condition of the seed at the time of harvest, *etc.* affect seed viability (Harrington, 1972; Willan, 1985; Schmidt, 2000; Mc Cormack, 2004). The main factors that affect germination of seedlings are light, water, temperature and exchange of gases (Hartmann *et al*., 2004). Seeds of different species react differently to these factors, and there is no common standard condition of storage or procedure of pretreatment that is ideal for seeds of all species.

The importance of using suitable storage and pretreatment techniques, in order to maintain seed viability and improve seed germination, has been known and appreciated since ancient times. Elaborate

procedures for these are available in ancient literature such as *Sarangdhara Samhita, Surapala's Vrikshayurveda, Vishwavallabha, Nuskha Dar Fanni Falahat, Krishiparashara, Kashyapiyakrishisukti, Lokopakara*, *etc.* (Rao, 1993; Sadhale, 1996, 1999, 2004; Akbar, 2000; Ayachit, 2002; Ayangarya, 2006). In addition to this, a number of local practices for maintaining viability of stored seeds and increasing germination are also available.

The present study was conducted to compare the effect of conventional and traditional methods of storage and pretreatment of seeds of a perennial medicinal plant species, namely *Embelia ribes*. Although, a few earlier studies have attempted to document and study effect of traditional practices for other species (Brajeshwar *et al.*, 2007; Mohan, 2008; Kumaravelu and Kadamban, 2009; Venkataramana *et al.*, 2009; Asha *et al.*, 2010; Mathad *et al.,* 2013), no attempts have been made to understand the effect of traditional techniques on seed germination of the two selected species. Moreover, so far, no attempts have been made to understand the combined effect of storage and pretreatment, as per modern scientific understanding, for any species. In the present study, germination trials were conducted to identify a combination of storage and pretreatment methods, integrating conventional and traditional techniques, to obtain maximum germination in the selected species. *E. ribes* is a woody liana that belongs to the family Primulaceae (The Plant List, 2013), and is commonly known as Vidanga. The fruit, seeds and leaves are medicinal, and are used as anthelmintic, stomachic, tonic, astringent, carminative, against skin diseases and snakebite.

Materials and Methods

Fruits of *E. ribes* were collected from Devala, Tamil Nadu in July-August in the years 2011, 2012 and 2013. They were cleaned, shade dried for 1-2 weeks, weighed and subjected to further experiments. Preliminary germination trials were carried out in the first year, followed by monthly germination trials as per the experiment design (Table 1) in the following two years.

1. Storage

Conventional storage treatment (S1): Fruits of *E. ribes*, after collection and drying, were sprinkled with water and shade dried for 3-4 days. Once dried, they were mixed thoroughly with K-vistin (a commercial form of the systemic fungicide Bavistin) @ 2 g/ kg of dried fruits, as dry mixture (Purohit *et al.,* 1996) (Figure 1).

Traditional storage treatment (S2): The traditional method chosen for this study was adopted from

Table 1: Design of experiment to understand the combined effect of storage and pretreatment on germination of seeds

Storage treatment	Pretreatment	Treatment combination	
S1	P1	S1P1	conventional* storage-conventional pretreatment
	P2	S1P2	conventional storage- traditional* pretreatment
S2	P1	S2P1	traditional storage-conventional pretreatment
	P2	S2P2	traditional storage- traditional pretreatment
S0	P0	S0P0	control (no storage or pretreatments)

**The conventional techniques referred to in this study are those methods which are currently being used for plant propagation in modern nurseries, and traditional techniques refer to the methods described in ancient Vrikshayruveda texts as well as those currently being followed by farmers who pursue traditional farming.*

Figure 1: Storage treatment of ***E. ribes*** seeds (a) sprinkling water [S1]; (b) mixing with K-vistin [S1]; (c) sprinkling milk [S2]; (d) mixing with cow dung [S2]; (e) smearing with honey [S2]; (f) smearing with Vidanga powder [S2]; (g) drying the seeds [S2]; (h) Control [S0]

Surapala's Vrikshayurveda, where it has been mentioned as an excellent method for storage of all types of seeds (Sadhale, 1996). This treatment also finds mention in Sarangadhara Samhita (Rao, 1993). According to the procedure described, dried fruits of *E. ribes* were sprinkled with raw cow's milk and smeared with fresh cow dung. Following this, they were shade dried for 4-5 days. After drying, they were profusely smeared with honey and Vidanga powder. They were once again dried, care being taken to protect them from ants.

Preparation of Vidanga powder: Vidanga (*Embelia ribes*) fruits, collected from the field, were dried and powdered whole in a grinder. A fine powder was thus prepared, which was used in the traditional storage method for storing seeds.

Control (S0): Dried fruits of *E. ribes* were also stored as such, without subjecting them to any treatment; this served as the Control.

All the seeds (S1, S2 and S0) were transferred to zip lock polythene pouches, neatly labeled and stored under ambient conditions for further studies.

2. Pretreatment

Conventional pretreatment (P1): 30 fruits in 3 replicates, subjected to S1 storage treatment, were taken and the seed coats removed. The decoated seeds were soaked in 500ppm GA_3 solution for a period of 18hrs. Similarly, 30 × 3 seeds subjected to S2 storage treatment were also decoated and the seeds were soaked in 500ppm GA_3 for a period of 18hrs (Treatment combinations: S1P1 and S2P1).

Traditional pretreatment (P2): As mentioned earlier, 30 × 3 seeds, obtained by decoating fruits subjected to S1 and S2 storage treatments, were soaked in cow dung slurry for 24hrs (Treatment combinations: S1P2 and S2P2).

In case of both conventional and traditional pretreatments, suitable treatment was identified from preliminary germination trials (not presented here).

Control (P0): 30 × 3 seeds obtained by decoating fruits of *E. ribes*, taken from Control storage, were sown without subjecting them to any pretreatment (Treatment combination: S0P0). This served as the Control.

Sand was used as medium for the germination experiment. It was sieved, washed repeatedly in running water and sterilized in a hot air oven as specified by ISTA (2004). As per the experiment design, every month *E. ribes* seeds are subjected to the treatment combinations S1P1, S1P2, S2P1 and S2P2 were sown along with the Control (S0P0). The experimental trays were placed in the nursery and regular watering was done to maintain the sand moist. Germination count was recorded daily, until no more seeds germinated. A seed was considered as germinated when the seedling was able to exist independently. At the end of the experiment, seedlings of *E. ribes* were picked out at the 5-leaf stage and transferred into polybags filled with red soil, sand and compost in the ratio 2:1:1 (Figure 2).

Meteorological data of the relevant period was collected to study the effect of various climatic factors on the germination of the species (Table 2). The results of the germination trials was analysed using one-way ANOVA, with the help of the statistical software SPSS (Version 16.0) for Windows. All data was submitted to Tukey tests ($p<0.05$). As the results from the germination trials were represented in the percentage form, they were arc sine transformed before subjecting them to analysis.

Observations

From the monthly germination trials, conducted to understand the effect of storage and pretreatment on germination percentage of *E. ribes* seeds, observations were made with respect to germination percentage of different treatment combinations and period of germination (Table 3). With regard to treatment combinations, it was observed that the germination percentage of combinations S1P1 and S2P1 were significantly higher than the combinations

Figure 2: Germination trial of ***E. ribes*** (a) & (b) sowing of seeds;
(c), (d) & (e) seeds at various stages of germination (f) bagged seedlings

S1P2, S2P2 and S0P0, in most of the months. Highest average germination was observed in S1P1, followed by S2P1, S1P2, S2P2 and S0P0. However, the treatment combination S1P1 was not significantly different from S2P1 in most of the months. The highest germination of 73.33% was recorded for the treatment combinations S1P1 and S2P1 in September in year 2013. Lowest average germination was observed for S0P0.

With regard to period of germination, it was observed that although seeds of *E. ribes* were sown every month (beginning from August in the year 2012 and from September in the year 2013), germination was found to be more or less restricted to seeds sown in the months from September to December. In both the years, more than 90% of the total germination recorded for the whole year was restricted to these four months. The rest of the germination (about 10%) was spread over the remaining eight months, the average germination varying from 0 - 2%.

Discussion

Seed is considered to be the first stage of the next generation, as it contains an embryo and adequate food reserves to sustain development of the embryo into a self-sufficient seedling (Bewley and Black, 1985). However, they begin to deteriorate from the point of their physiological maturity, slowly losing their viability and eventually dying (Justice and Bass, 1979). The process of deterioration, however, can be prolonged by various techniques, such as drying, dressing them with fungicides, fumigation, storing at low temperatures, *etc.*, thereby increasing their storability. Traditional texts such as *Kashyapiyakrishisukti*, *Krishi Parashara, Sharangadhara Paddhati, etc.* (Rao, 1993; Sadhale, 1996, 1999; Ayachit, 2002; Ayangarya, 2006) mention use of powders of *Solanum indicum*, *Sesamum indicum*, *Embelia ribes* and *Brassica juncea*, milk, ghee and cow dung for protection of seeds from various harmful agents during storage. Use of sunlight for drying the seeds is another common feature in most of the traditional texts. Traditional farmers also make use of neem leaves, cow dung and ash to increase storability of seeds.

Following storage, suitable pretreatments such as soaking in cold/hot water, mechanical/chemical scarification, application of growth hormones and weathering of seeds have been observed to improve germination in many plant species. According to Baskin and Baskin (2001), these procedures help in breaking the physical, chemical, mechanical, physiological or morphological dormancies observed in the different species. Traditional procedures, such as smearing seeds with ash of *Sesamum indicum* and *Solanum indicum*; smearing with *Alangium salviifolium* oil; fumigating with fat of animals, seeds of *Solanum indicum* and *Embelia ribes*, ghee or turmeric and soaking in water mixed with fat, marrow or meat of animals, mentioned in ancient texts are also said to increase the germination percentage. Many of the ancient pretreatment methods for good germination also make use of cow dung (smearing or rubbing the seeds) and milk of cow, sheep and goat (sprinkling or soaking the seeds).

Earlier germination studies have resulted in development of some protocols using GA_3 as pretreatment for breaking dormancy in *E. ribes* (Gupta, 2003; Kunhikannan, 2010; Annapurna *et al.*, 2013; Patwardhan and Vasudeva, Undated). In the present study, it is seen that germination in *E. ribes* is, more or less, restricted to seeds sown from September to December. Among these four months, the maximum germination was recorded in the month of September, with 73.33% for S1P1 and S2P1, and 63.33% for S2P2 (Table 3).Thus, GA_3 is observed to have a positive effect on the germination percentage of this species when seeds are sown up to four months after collection. Gibberellins are known to stimulate germination in seeds, where dormancy is imposed by incomplete embryo development, mechanically

resistant seed coats, presence of germination inhibitors or factors relating to the physiological competence of the embryo axis (Jones and Stoddart, 1977). GA_3 as an agent for breaking seed dormancy has been demonstrated earlier in other species (Tipirdamaz and Gomurgen, 2000; Lavania *et al.*, 2006; Kalidass *et al.*, 2011; Sudhakara and Veenadevi, 2013). Thus, it can be concluded that use of GA_3 promotes germination in *E. ribes* for a short period after collection.

From Table 3 it is also clear that the germination percentage of S1P1 and S2P1 are on par with each other in most of the months, which indicates that both the traditional and conventional storage techniques are equally effective in retaining viability of this species. The traditional storage treatment chosen for this study, which has been adopted from Surapala's Vrikshayurveda, makes use of raw cow's milk, fresh cow dung, honey and Vidanga powder, each of which is beneficial for storage of seeds. Cow's milk is known to possess antifungal and antiviral qualities (Balasubramanian *et al.*, 2009), while fresh cow dung possesses many beneficial microbes (Sreenivasa *et al.*, 2009), which probably helps in retaining seed viability. Vidanga is known to be antimicrobial (Radhakrishnan *et al.*, 2011), while honey probably acts as a binder. Therefore, the use of these ingredients helps in storage of seeds by protecting them from pathogens and probably also by maintaining their viability. The conventional storage treatment of using Bavistin is a common and widely accepted practice for protection of seeds from pathogens in forest nurseries. Thus, both the conventional and traditional storage techniques studied here are equally effective for maintaining seed viability in *E. ribes*.

During the monthly germination trials, when GA_3 was applied as pretreatment, it was observed that seeds sown in September and October germinated by 3-4 months, those sown in November germinated after 2 months and those sown in December after 1 month. Thus, it is observed that the seeds germinate between December and February. A comparison with the meteorological data for the relevant period shows that this period coincides with the lowest annual minimum temperature (14-17°C). Earlier studies conducted by Yanes and Segovia (1982), Bewley and Black (1985), Probert (2000), Baskin and Baskin (2001), Hartmann *et al.* (2004) and Kumar *et al*. (2011) have demonstrated the importance of temperature as a factor affecting germinability of seeds. Low temperature as a prerequisite for breaking dormancy has been reported earlier in many species (Bewley and Black, 1985; Bedell, 1998; Walck *et al.*, 2000). Thus, it can be presumed that the low temperature between December and January, combined with treatment with GA_3, results in breaking of dormancy stored seeds of *E. ribes*. Lack of germination in other months is probably because the temperature is above the required value.

Conclusion

Thus, from the present study it can be concluded that the traditional method of storage described in Surapala's *Vrikshayurveda* is as effective in storing seeds of *E. ribes* as the use of K-vistin. Also, soaking in 500ppm GA_3 for 18hrs is recommended as pretreatment of stored seeds of this species. Following the above procedures for storage and pretreatment of *E. ribes* seeds will result in production of maximum number of good quality seedlings.

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Table 2: Climatic data of Bangalore for the period 2011 to 2014

	2011						2012						2013						2014					
	Temperature °C		Sun-shine (hrs)	Rain-fall (mm)	Rain days	RH (%)	Temperature °C		Sun-shine (hrs)	Rain-fall (mm)	Raindays	RH (%)	Temperature °C		Sun-shine (hrs)	Rain-fall (mm)	Rain days	RH (%)	Temperature °C		Sun-shine (hrs)	Rain-fall (mm)	Rain days	RH (%)
	Min	Max					Min	Max					Min	Max					Min	Max				
JAN	12.75	27.79	9.07	0.0	0	41.26	14.28	27.99	9.20	0.0	0	46.34	14.81	28.77	9.59	0.0	0	43.04	14.65	27.58	9.05	0.0	0	44.82
FEB	13.62	29.47	8.93	16.6	1	39.23	15.06	30.50	9.27	0.0	0	36.13	16.36	30.49	8.85	2.2	0	39.65	16.27	29.89	8.57	0.0	0	38.69
MAR	16.38	32.88	8.98	0.0	0	33.03	18.75	33.68	8.90	0.4	0	31.80	19.23	32.74	8.00	0.0	0	36.97	18.21	32.17	8.62	10.0	2	36.91
APR	20.24	32.62	7.34	57.4	5	38.89	21.16	34.57	8.21	8.6	2	32.46	21.83	34.55	8.41	56.8	5	35.44	21.13	34.70	8.20	35.5	1	32.58
MAY	19.95	32.11	7.85	126.0	8	40.31	21.14	33.01	7.51	84.4	4	49.00	21.11	33.47	6.52	92.8	8	44.38	21.15	33.21	8.15	81.4	7	37.98
JUN	19.49	29.07	6.15	30.0	4	48.37	20.25	30.95	6.33	26.6	3	44.15	19.67	28.41	3.55	96.5	7	54.52	20.61	30.85	7.32	92.0	4	46.48
JUL	19.32	27.84	4.19	95.8	9	53.15	19.59	28.71	4.34	97.2	7	52.06	19.30	27.52	2.20	59.2	7	54.76	19.70	28.26	4.19	80.8	6	54.26
AUG	19.29	27.34	3.32	253.2	14	54.98	19.33	28.34	4.42	100.1	7	53.00	19.05	27.86	4.02	58.8	6	54.07	19.54	28.20	3.83	117.4	7	56.41
SEP	18.94	28.01	5.87	59.7	7	51.48	19.39	29.19	5.57	29.2	3	51.22	18.87	27.43	4.51	362.3	14	58.74	19.40	28.51	4.46	128.6	9	53.67
OCT	19.19	28.56	5.59	122.6	7	51.14	18.68	28.34	5.66	64.2	3	53.29	19.06	27.76	5.10	81.9	8	57.74	18.78	28.13	4.41	428.4	15	55.58
NOV	15.95	26.64	6.18	38.0	4	53.21	16.24	27.17	7.39	150.0	4	53.09	16.95	27.67	5.76	37.0	3	53.14	16.31	26.86	6.77	29.4	3	54.10
DEC	14.36	26.86	7.25	5.2	1	51.73	15.74	27.38	7.98	11.2	1	51.56	14.06	26.56	7.84	0.0	0	51.91	16.16	26.64	6.27	1.0	0	48.00

[Source of data: GKVK, Bangalore]

Table 3: Germination percentage of E. ribes seeds subjected to conventional and traditional treatments

		Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
2012	**S0P0**	0.00[a]	3.33[a]	3.33[b]	0.00[b]	0.00[a]	0.00	0.00	0.00[a]	0.00	0.00	0.00	0.00
	S1P1	6.67[a]	50.00[a]	46.67[a]	23.33[a]	6.67[a]	0.00	0.00	3.33[a]	0.00	0.00	0.00	0.00
	S1P2	0.00[a]	13.33[a]	10.00[b]	10.00[a,b]	3.33[a]	0.00	0.00	0.00[a]	0.00	0.00	0.00	0.00
	S2P1	3.33[a]	13.33[a]	53.33[a]	20.00[a,b]	6.67[a]	0.00	0.00	0.00[a]	0.00	0.00	0.00	0.00
	S2P2	0.00[a]	0.00[a]	6.67[b]	3.33[a,b]	3.33[a]	0.00	0.00	0.00[a]	0.00	0.00	0.00	0.00
	Avg	2.00	16.00	24.00	11.33	4.00	0.00	0.00	0.67	0.00	0.00	0.00	0.00
	p<0.05%	0.552	0.077	0.000	0.032	0.512	--	--	0.452	--	--	--	--
	F-value	0.801	2.916	24.478	4.107	0.875	--	--	1.000	--	--	--	--
2013	**S0P0**	--	20.00[a]	6.67[b]	23.33[a,b]	30.00[a]	0.00	0.00[a]	0.00	0.00	0.00	0.00	0.00
	S1P1	--	73.33[a]	50.00[a]	50.00[a]	36.67[a]	0.00	0.00[a]	0.00	0.00	0.00	0.00	0.00
	S1P2	--	33.33[a]	0.00[b]	23.33[a,b]	20.00[a]	0.00	0.00[a]	0.00	0.00	0.00	0.00	0.00
	S2P1	--	73.33[a]	73.33[a]	46.67[a]	43.33[a]	0.00	6.67[a]	0.00	0.00	0.00	0.00	0.00
	S2P2	--	63.33[a]	0.00[b]	13.33[b]	30.00[a]	0.00	0.00[a]	0.00	0.00	0.00	0.00	0.00
	Avg		52.67	26.00	31.33	32.00	0.00	1.33	0.00	0.00	0.00	0.00	0.00
	p<0.05%	--	0.115	0.000	0.015	0.571	--	0.034	--	--	--	--	--
	F-value	--	2.441	25.003	5.295	0.766	--	4.000	--	--	--	--	--

[Values in the same column with the same superscripts are not significantly different from each other]

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