DOI: https://doi.org/10.4038/ta.v167i3.83

Tropical Agriculturist, Vol.167 (3), 2019

Enhancing germinability of Veralu (*Elaeocarpus serratus* L.) by seed pre-treatments and germination variability of different accessions

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Abstract

Veralu (*Elaeocarpus serratus* L.) known as Ceylon Olive is one of the valuable and potential fruits. In year 2011 four superior varieties were recommended for cultivation. The major constraint in expansion of cultivation is poor degree of seed germinability and long time period taken for germination. With the objectives of increasing efficiency of germination and studying the variation in germinability a series of experiments were conducted at the Fruit Research and Development Institute, Horana during 2018-2019. None of the hot and cold temperature scarification treatments evaluated was effective. Both cracked seeds and seeds without endocarp inhibited germination, suggesting that the non-existence of seed coat imposed dormancy. However, seeds packed in sealed black colour polythene (300 gauge) and kept them for 15 days, stimulated germination giving 50% success after 115 ± 6 days. Similarly, seeds embedded in sand substrate with 1% moisture content in sealed opaque polythene bag (150 gauge) enclosed in another black color bag (300 gauge) and sun dried for 8 hrs regularly, promoted germination showing a success rate of 58% by 80 days of sun drying. When germination behavior of two different seed weight categories concerned, small seeds (0.5 – 1.0 g mean/seed) germinated faster achieving 50% success rate by 68 ± 2 days. Whereas, for large seeds (1.0 – 1.5 g mean/seed) number of days taken for 50% germination was 110 ± 10 days. One of the promising outcomes reveal from the current study was identification of two accessions having short dormancy periods of 52 days and 75 days in ACC 2 and ACC 5 respectively. Thus, these accessions are recommended for overcoming seed dormancy of *Elaeocarpus serratus* and thereby enhancing the planting material production profitably.

Key words: *Elaeocarpus serratus* L. (Ceylon Olive), Genetic variability, Germinability, Pre-treatments, Seed dormancy

Introduction

Veralu (*Eleocarpus serratus* L.) known as Ceylon Olive a native to Sri Lanka (Jayasuriya and Rajapaksha, 2004) and grown in many tropical countries as a fruit, an ornamental fruit and a medicinal plant. This is rich in vitamins, minerals, fiber and antioxidants (Rajapaksha, 1998). In other countries, many fruit products made out of both ripe and unripe forms of Veralu such as pickle, snack, jam, jelly, squash and beverage are available. However, in Sri Lanka the fruit is famous for pickle as a street food and for beverage only, as it is mostly confined to the home gardens and forests at lower altitude in the Wet Zone and Intermediate Zone (Meijer, 1995).

It has been reported that a vast, variation exits in productivity and fruit quality characteristics of *Eleocarpus serratus*. Furthermore, a gradual depletion of such germplasm was prominently observed (Anonymous, 2005). Upon exploitation, conservation and evaluation of several accessions, four high yielding varieties of superior quality were identified at the Fruit Research and Development Institute (FRDI), Horana and recommended by the Department of Agriculture in 2011. However, spread of these varieties is inadequate due to the absence of appropriate propagation techniques in raising the root stocks. Extremely low degrees of seed germinability along with prolonged time taken to germinate are the main drawbacks for vegetative propagation of veralu (Khan *et al.,* 2003; Bhuyan *et al.,* 2002). In India it has also been recognized that thirty-eight *Eleocarpus* spp. are subjected to danger of extinction due to non existence of proper regeneration process (IUCN, 2007). Hence, numerous attempts have been made to break down dormancy and stimulate germination of different *Eleocarpus* spp. with varying success. Among which, Khan *et al*. (2003) found that germinability of *E. ganitrus* (Blue Olive) was 40% with mechanically cracked seeds. Further, they stated that the time taken to commence germination reduced by immersing in Gibberellic Acid (GA) solution (500 mg/l) or a mixture of GA and Potassium Nitrate. Matakiadis *et al.* (2009); Hilhorst and Karssen (1992) reported that the presence of Abscisic Acid (ABA) in seeds - inhibits germination. It has been proven that application of nitrate ions reduces the levels of ABA promoting germination (Baskin and Baskin, 1998). Irwin *et al*. (2013) found that germination percentage of *E. venustus* grown in silt and sand media was 40% : when an incorporation of cow dung in to the same soil media showed poor degree of

germination (8%). In *E. serratus*, germinability of 15% was observed when seeds treated with 50% nitric acid (Dahanayaka *et al.*, 2003), It was reported that *Veralu* seeds soaked in 5% Sulphuric acid for 20 minutes induced germination capacity upto - 28% at 14 months after sowing and treating with 10% Sulphuric acid, a success rate of 20% was obtained at 4 months after planting (Anonymous, 2005). Raji and Siril (2018) indicated that cracking of seeds before planting enhanced the germination ability which was 49%. Many researches stated that hard seed coat was responsible for low level of germination (Hamilton *et al*., 2009; Ramasubbu and Irubhyaraj, 2016; Raji and Siril, 2018).

Beniwal and Singh (1989) reported that physiological dormancy has been found in *Elaeocarpus* spp. *(E. floribiindus, E. petiolation* and *E. stipularis)*. Iralu and Upadhaya (2018) found that *E. prunifolius* consisted of both physical and physiological dormancy. Therefore, with the objective of enhancing the efficiency of seed germination, the present study was conducted to determine the dormancy breaking technique of *E. serratus*. The experiment was also concentrated to study the variation in germinability of different accessions of *Veralu* found in the Wet Zone of Sri Lanka.

Materials and Methods

The experiments were conducted at the Fruit Research and Development Institute (FRDI) Horana, Sri Lanka during October 2018 - April 2019.

Seed collection and processing

Fallen riped fruits collected from selected accessions available at the FRDI, Horana and from different locations in the Low Country Wet Zone were taken for the experiments. Fruits were de-pulped and washed thoroughly in running water to remove any adhering pulp material. Damaged or floating seeds in water were removed and sun dried for 24 hours to get rid of moisture.

Pre treatments for dormancy breaking

Experiment 1. Temperature and mechanical scarification

The experiment was laid out in a CRD with 3 replications (30 seeds / treatment). Seeds (Accession S-5) were subjected to 11 treatments as mentioned below.

T_1 and T_2 were formed using deep frozen seeds for 12 hrs and immersed in water at 80 ^{0}C temperature for 30 sec. and at 100 ^{0}C temperature for 30 sec respectively. T_3 and T_4 were started with unfrozen seeds treated with the same way. T_5 and T_6 were mechanical scarified treatments, introduced for longitudinally cracked seeds without damaging kernals, immersed in water for 48 hrs and for extracted undamaged kernals of seeds. Seeds packed in air tight black colour polythene bag of gauge 150 and of size 15 cm x 6 cm were used for the four darkness treatments and bags were sealed. The bags were kept for 15 days (T_7), 30 days (T_8), 45 days (T_9) and 60 days (T_{10}) separately. Seeds without any treatment (T_{11}) were taken as the untreated control.

All seeds of 11 treatments were planted in sand filled nursery beds (10 x 3 m). The beds were watered at 2 day intervals (or when necessary) and monitored for germination during a period of 6 months.

Experiment 2. Exposure to sunlight

Sixty seeds (ACC.S5) in each were embedded in three opaque polythene bags of gauge 300 filled with sand containing moisture levels 0.1%, 1% and 10%. The bags were sealed and enclosed with another sealed black polythene bags of gauge 150. Then these packs were exposed to sunlight for 8 hrs and 16 hrs in ambient temperature regularly. Three weeks after treatment, temperature of sand bags was measured using a probe at three days interval and observations were made until radical emergence at weekly intervals by opening the outer poly bag. Sprouted seeds were established in sand nursery bed (3 x 10 m) - for seedling development.

Experiment 3. Germinability of different accessions

Seed from ten accessions were randomly selected with three recommended varieties (HoW-5, HoW-9 and HoW-13) for the study. Based on the fresh weight of extracted seeds, were categorized into two groups, large and small. Seeds of weight range 1.0 -1.5 g were considered as large while that of 0.50 – 1.0 g taken as small. Under two weight classes 30 seeds per accession were assessed for seed dimensions using a digital venire caliper for length, middle circumference, thickness of endocarp and seeds were established and maintained in sand nursery beds. Experimental design was a CRD having two replications. Observation on germination was made daily. The seeds were taken as

germinated when plumule protruded out of the soil surface. Germinated seedlings at four leaf stage (10 per each category including 3 replications) were transplanted into 15 x 22 cm poly pots and maintained by watering every other day. Growth characteristics of shoot length and collar circumference were determined at seven day intervals up to eight weeks. The Germination Index (GI) was determined by using the equation of; $a_1=(10xn_1)+(9xn_2)+\ldots(10xn10)$, where $n_1,n_2\ldots n_{10}$ are the number of germinated seeds on the 1^{st} ,2^{nd} and subsequent days until the 10^{th} day; 10,9…. and 1 are weights given to the number of germinated seeds on the 1^{st}, 2^{nd} and sub sequent days respectively (Bench *et al* ., 1991).

Statistical analysis

Data on germination was transformed to logarithmic transformation before analysis and two sample t-test was carried out using SAS programme. For mean separation new DMRT test was conducted. Pearson co-relation co-efficient test was done for seed parameters.

Results and Discussion

Darkness treatment

Of different pretreatments evaluated seeds packed in black polythene bag (T_7, T_8, T_9 and T_{10}) for 15 days were effective in enhancing 50% germination by 115 ± 6 days (Table 1). Retention of heat absorbed by black polythene may have accelerated the physiological process in embryo growth and development resulted in stimulation of germination. However, keeping seeds in black packs more than 15 days inhibited germination (Table 1). Longer periods of storage in sealed poly packs, seeds may deprive of getting moisture to terminate dormancy. No research information related to such darkness treatments on germinability of *Elaeocarpus* spp. was reported. Yet Danthu *et al*. (1992) found that seeds of *Ziziphus jujube* (Bber) treated under 4 temperature regimes (25 ^{0}C ,30 ^{0}C ,35 ^{0}C and 40 ^{0}C) in darkness, induced germination 85-90% after 7 days at 25 ^{0}C and 30 ^{0}C.

Subhashinidevi *et al*. (2012) observed that in seed dormancy study of *Sterculia urens* providing 15 hrs light and 8 hrs dark periods at ambient temperature of 30 ^{0}C ± 2 improved germination (60%) when compared with continued dark (53%) or light (48%)

conditions. Results of the darkness treatment in the current experiment are in support of their findings.

Mechanical scarification

In relation to the mechanical stratification treatments (T_5 and T_6) evaluated, cracked seeds followed by soaking in water for 48 hours, inhibited germination (Table 1). Similar observations made by Iralu and Upadhaya (2018) indicating that pre cracked or un-cracked treatments for seeds of *E. prunifolius* were unsuccessful in enhancing germination. Thus, their findings have proven that imbibition by seed cracking had not facilitated the dormancy termination. Similarly, extracted seed kernels failed to terminate dormancy and suggesting that physical dormancy caused by hard endocarp may not be the major factor associated in delaying the process of germination. On contrary to such findings, Raji and Siril (2018) reported that making cracks in seeds of *E. serratus* using a seed cutter promoted germination by 48%.

Thermal scarification

Findings of the current experiment indicated that any of the hot and cold water dip treatment (T_1 and T_2) was not effective in stimulating germination ability during the experimental period of 6 months (Table 1). Raji and Siril (2018) also found that when immersed seeds in water at 40 ^{0}C- 45 ^{0}C for 60 hours, a low success rate (11-13%) was obtained at 6 months after treatment. Similar results have been obtained by Iralu and Upadhaya (2018) indicating that no germination was shown in *E. prunifolius* treated either in hot water at 80 ^{0}C for 24 hours or 100 ^{0}C for 5 minutes followed by immediately transferred to cold water (0 ^{0}C) for 24 hours. They further indicated that cold water dip at 20 ^{0}C for 24 hours impeded germination (10.67%) in comparison with the control (24%). Thus, both prolong hot and cold temperature dip treatments applied for *E. prunifolius* in their study and short period dipping in present experiment provided negative results in germination of *E. serratus* (Table 1).

Sun drying on dormancy breaking

Sun drying of 8 hours and leaving at ambient temperature, packets of seeds embedded in sand having 1% moisture content induced radical emergence with a success rate of 58% at 80 days of the treatment (Table 2). Whereas, seeds sun dried in 0.1%

and 10% moisture levels containing sand beds inhibited germination indicating that low moisture level may not be sufficient to activate the physiological reactions taken place in the germination process. On the other hand, excessive moisture content of the sand medium may prevent from getting oxygen to initiate germination. Thus results indicated that temperature and ideal moisture conditions are important prerequisite for stimulation of germination.

Seeds of untreated control treatment were capable of germinating after a long time period of 167 ± 12 days, indicating its natural dormancy phase (Table 1).

Table 1. Germination behavior of seeds (mean of 60) of *E. serratus* subjected to thermal and mechanical scarification

	Treatment	Germination (%)	Days taken for 50% germination
1	12 hr deep frozen followed by dipping in water at 100 ^{0}C for 30 sec	0	-
2	30 sec dipping in water at 100 ^{0}C	0	-
3	12 hr deep frozen followed by 30 sec dipping at 80 ^{0}C	0	-
4	30 sec dipping in water at 80 ^{0}C	0	-
5	Cracked seeds soaking in water for 48 hrs	0	-
6	Extracted kernels	0	-
	Seeds in sealed black polythene bag kept for		-
7	15 days	55 ± 7	115 ± 6
8	30 days	0	
9	45 days	0	
10	60 days	0	
11	Untreated control	56 ± 5	167 ± 12

Table 2. Effects of sun drying (8 hrs) of seeds of E. serratus embedded in sand substrate at 3 different moisture levels

Percentage moisture content of sand substrate (mean)	Mean temperature in poly bags	Total no .of seeds embedded/ pack	No. of seeds radical emerged	Percentage germination	Mean time taken for germination
1	49.42^0C ± 4.74	125	73 ± 1.41	58.4 ± 1.13	80 ± 1.41
0.1	48.78^0C ±3.24	125	0	0	0
10	47.96^0C ± 4.61	125	0	0	0

Seed packs kept under the sun, gets absorbed heat through outer black colour poly bag and transformed heat to the attached seed packs. This may lead to promote germination (Table 2), even though, temperature regimes found in seed packs are different. Our observations are in line with the findings of Chien *et al*. (1998) where seeds were treated with warm and cold stratification of 30 ^{0}C/ 20 ^{0}C for 12 weeks enhanced germination. Heenkenda (2017) mentioned that seed dormancy of Veralu can overcome during a period of 1-2 month by adopting the sand pack technique that evaluated in the current experiment which are in support of the findings.

Physical characteristics and germination variability of seeds of two different weight groups of *E . serratus*

Evaluation of seed parameters indicated that weight of the large seeds and the thickness of seed coat was not co-related, while in small seeds it was negatively co-related (p=0.05). All the dimensions (diameter, length and length:diameter ratio) determined in seeds of both weight classes were positively co-related with the seed weight (Table 3).

When germinability of two different weight categories of seeds are concerned, small seeds were shown early emergence and less time period for 50% germination (Table 3). Non-significant differences were found in percentage germination of two weight groups that in contrary to the findings of Iralu and Upadhya (2018) who reported that the weight and percentage germination of seeds of E. prunifolius are co-related.

Owoh *et al*. (2011) determined that there was no effect on seed size for germination of *Gmelina arborea* which is in conformity with the observations made on the present study.

In small seeds, size of the seed embryo may have a tendency to develop into less surface area or volume in comparison with that of large sized seeds. Observations made in the experiment 1 of the present study revealed that non-existence of seed coat imposed dormancy of *E. serratus* suggesting that poorly developed embryo of the seed is mainly responsible for prolong time period taken to break dormancy. Consequently, seeds with small dimensions may require lesser time period for the growth development of embryo.

Similar observations have been made by Offiong (2008) who explained that seeds of *Gmelina arborea* with large dimensions are likely to have large embryo which enhances good germinability.

Table 3. Effects of physical parameters and germination characteristics of seeds of *E. serratus* of two different weight category and co- relation co-efficient between weight and seed parameters

Physical parameter of seeds (mean)	Seed weight category			
	Light (0.5-1.0 g)	**Co-relation coefficient**	**Heavy (1.0 – 15 g)**	**Co-relation coefficient**
Length (mm)	19.05 ±1.49	0.79 P=0.034	22.78 ±1.40	-0.86 P=0.0013
Diameter (mm)	9.92 ±1.06	0.95 P=0.001	11.38 ±0.83	0.79 P=0.007
Length: Diameter ratio	1.93 ± 0.08	0.85 P=0.014	2.01 ±0.27	0.07 P=0.0010
Thickness of endocarp (mm)	1.611429 ± 0.20	0.77 P=0.042	2.147 ±0.29	0.58 P=0.08
Days taken to 50% germination from seed sown	68.22 ± 2.36		110.33 ±10.50 P= 0.01	
% germinationns	57.33 ± 2.51		55.11 ±5.28	
			P=0.52	

ns-not significantly different (p<0.05)

Growth parameters of seed plants

Assessing of growth rate of plants raised from two different size of seeds indicated that non-significant differences (p=0.05) in both dimensions of plant height and stem diameter were found at 30 days after transplanting (Figure 2). Similarly irrespective of the seed size, by 60 day of transplanting, significantly (p=0.05) similar increase in plant height of both types of plants was observed. Whereas, a significantly (p=0.05) higher increment in plant girth was shown in light seeds compared with other seed group. Contrasting observations made by Owoh *et al*. (2011) in *Gmelia arborea*, over the medium and small seeds, large sized seeds performed better with higher success of germination, associated with greater food and energy reserve in a shorter period of time. They stated that though there were significant variations in seedling growth in early stages of development, no difference was found at later stages. Iralu and Upadhaya (2018) also showed that heavy seeds of E. prunifolius survived better. Thus, findings of current study are not in agreement with those observations made on different types of plants and species of Eleaeocarpus. Nevertheless, by 60 days of transplanting - plants grown from both types of seed weight categories have attained the required dimensions of stem height (30 cm) and stem girth (2-3 cm) for shoot grafting described by Raj and Siril (2016).

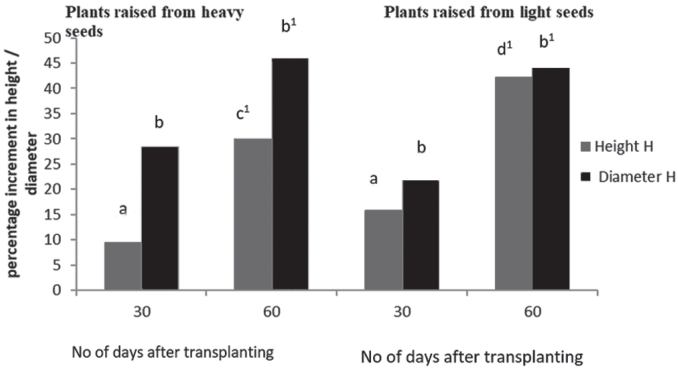


Figure 1. Growth characteristics of seed plants of *E.serratus* raised from heavy and light seeds

**Bars denoted by the same letter are not significantly different (p=0.05)*

Germination variability of different accessions

Of all seed germination parameters, Germination Index (GI) is best described the percentage and speed or rate of germination (Kader, 2005). Concerning GI, ACC 2 had obtained a significantly (p=0.05) highest value and ACC 1, ACC 3 and H13 moderate GI value while the remaining accessions showed low index value (Figure 2). Seven out of 13 accessions and varieties assessed, attained 50% germination by 52 ± 0.7 days to 92±2.82 days after nursery establishment (Figure 2). According to the number of days taken for termination of dormancy, 3 types of seed categories can be identified as early, moderate and late germinators having low, moderate and high degree of dormancy phase.

Among which ACC2 and ACC5 showed the significantly (p=0.05) early seed emergence over the others (Figure 3). Meanwhile no germination occurred in most other varieties indicating inherent variability in germinability among different individuals (Figure 2). Raji and Siril (2018) also reported the varied response of *E. serratus* collected from different locations. Joshi and Dhar (2003) explained that variability in germination possibly associated with the climatic and altitudinal differences. Furthermore, it was reported that the genetic variability of perennial crops such as *Sapium scbiferum* (Siril *et al*., 1998); *Erica australis* (Cruz *et al*., 2003); *Bixaorellana* (Joseph *et al*., 2011). It was reported that seeds of *E. sylvestris* collected from different elevations of Taiwan showed varied potential of germination having different degree of dormancy periods and stratification requirements in order to stimulate germination (Chien *et al*.,1998). Thus, findings of the current experiment are in accordance with those of previous researchers.

One of the critical problems encountered in experiment 2 was incorporation of appropriate moisture level (1%) into the sand substrate of sealed sand bag technique. However, sand nursery bed used for seed germination in experiment 2, problem of maintaining required soil moisture content was shown to be corrected naturally by draining off excessed moisture received from rainfall and watering, unlike in sealed sand bag technique. Thus, outcome of the investigations revealed that establishment of identified accessions of *E. serratus* seeds in nursery beds containing sand is the most promising technique for enhancement of seed germination.

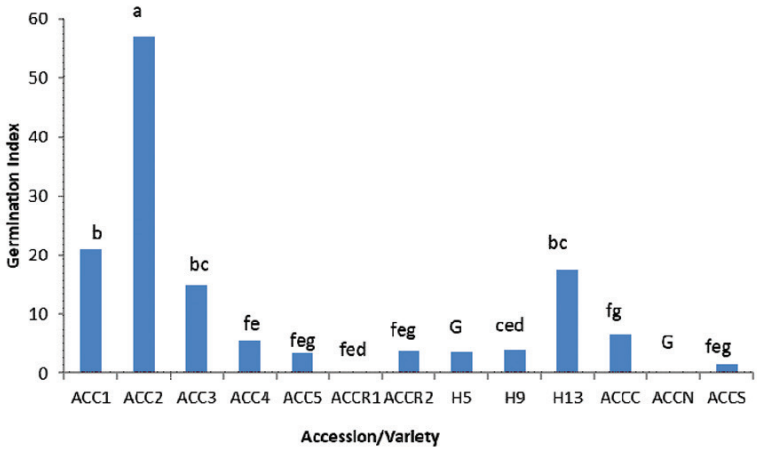
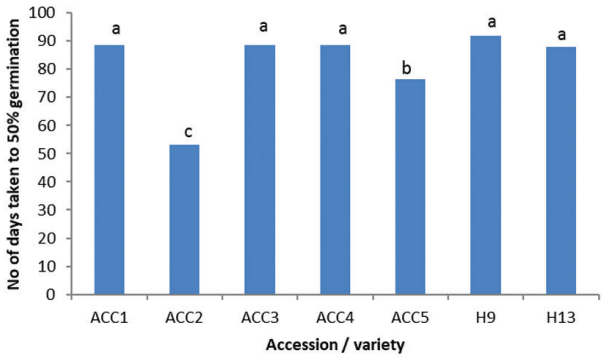


Figure 2. Germination index of 10 accessions and 3 varieties of *E. serratus*

**Bars denoted by the same letter are not significantly different (p=0.05)*



Figuer 3. Number of days taken to 50% germination of 5 accessions along with 2 recommended varieties of *E. serratus*

**Bars denoted by the same letter are not significantly different (p=0.05)*

Conclusion

Sun drying (8 hours per day) of embedded seeds in sand substrate (1% moisture content) in sealed opaque polythene bag (150 gauge) enclosed in another black color bag (300 gauge) promoted germination showing a success rate of 58% at 80 days.

Studies also revealed that light weight (0.5-1.0 g) seeds germinated (50%) faster (68±2 days) over those of heavy weight (1.0-1.5 g) seeds (110 ± 10 days). One of the promising outcomes found was identification of 2 accessions ACC 2 and ACC5 having short dormancy periods of 52 days and 75 days respectively which are more feasible over the adoption of labour intensive pretreatments for termination of dormancy. Thus, these accessions are recommended for overcoming seed dormancy in *E. serratus* to ensure rapid germination and thereby enhancing planting material production profitably.

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