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Influence of fruit colour variation on physical, physiological, biochemical and nursery characters of endangered medicinal tree bael *(Aegle marmelos* (L.) Corr.,)

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Bael (*Aegle marmelos* (L.) Corr.) is an endangered medicinal tree which is mostly propagated through seeds. Seed is the basic unit of mass multiplication and require quality characters, namely, germination and vigour. Seed quality is guaranteed through the adoption on proper post-harvest seed handling techniques. In perennial crops, selection of fruits is an important management techniques adopted after fruit harvest as it plays an important role in production of high quality seedlings in nursery. Hence, studies were formulated to evaluate the influence of fruit colour variation on seed quality characters. Fresh bael fruits were collected from Coimbatore district of Tamil Nadu, India. The fruits were categorized based on the colour into three different groups as green, greenish yellow and yellow. The recovery of yellow and greenish yellow fruits were 41 and 33%, respectively from bulk. Evaluation of physiological quality characters after extraction revealed that germination of seeds from yellow fruits were higher (86%) followed by seeds from greenish yellow (74%), bulk (66%) and green fruits (52%). The seedling vigour measured through root (7.5 cm) and shoot length (10.2 cm), dry matter production (294 mg) and vigour index (1522) also exposed the superiority of seeds from yellow fruits. The evaluation of biochemical characters such as oil content (38.9%) and seed protein (18.2%) were higher, while the electrical conductivity (0.221) was lower with seeds of yellow fruits than other coloured fruits. The seeds sown in polybag nursery also expressed that seeds of yellow fruits produced vigorous seedlings.

Key words: Bael, fruit colour, seed quality, oil and protein content, nursery.

INTRODUCTION

Bael (*Aegle marmelos* (L.) Corr.) is a medicinal tree belonging to the family Rutaceae and its various parts are used in Ayurvedic and Siddha medicine to treat a variety of ailments. It is highly habitated to tropical and subtropical climate of India, Burma, Pakistan, Bangladesh, Sri Lanka, Northern Malaya, Java and Philippines (Islam et al., 1995). Purohit and Vyas (2005) reported that bael is a medium sized tree having profuse dimorphic branches, greenish white flowers, large and globose fruits. In Tamil Nadu (13°05′N 80°16′E), a state of India, it flowers between May to July and Mazumder et al. (2006) revealed that approximately 200 to 250 kg of fruits could be obtained per tree. The roots are useful for treating diarrhea, dysentery and dyspepsia. The aqueous stem

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and root bark extracts are used as medicine for malaria, fever, jaundice, cancer, ulcer, urticaria and eczema (Nadkarni, 1954). The fruit and root of the plant have antiamoebic and hypoglycaemic activities (Ponnachan et al., 1993). Goel et al. (1997) revealed that crop is rich in alkaloids aegline, marmesin, marmin and marmelosin. Rana et al. (1997) revealed that the seed is rich in luvangetin and pyranocoumarin compounds, which has antiulcer activity. The foundation for revitalization of local health traditions (FRLHT), Banglore, India listed bael *(A. marmelos*) as rare, endangered and threatened (RET) species, specifically endangered species. Hence, more importance is being given for mass multiplication through afforestration. The tree is normally propagated through seeds (Nayak and Sen, 1999) and seed requires specific quality characters for its better performance. In perennial crops, where collection of matured fruit is not possible due to their structure and thorny nature of the tree, selecting of fruits from the collected bulk serves as harvest index for obtaining quality seeds. Hence, studies were initiated to identify the harvest index, the harvesting criteria for selection of good fruits for selection of good seed. Kaushik et al. (2001) and Kathiravan (2004) in *Jatropha curcas* also revealed that fruit colour serve as harvest index for collection of good seeds.

Fruit colour is considered as an index of seed maturation particularly in forestry species (Khullar et al., 1991; Srimathi, 1997). Sekar (2005) in Simaruba also observed similar fruit colour variation due to seed maturity. Standardization of fruit colour for each of the forestry species would be of immense help to forest tree seed collectors, as collection is a laborious process in these species owing to their inaccessibility for manual collection and longer duration of harvesting period. Willan (1985) also revealed that fruit colour would serve as a tool for collection of good quality seeds in forestry as the persons involved in the collection process, mostly lack in technical skill.

There were difficulties in collecting matured fruits from the ground and selection of which type of fruit is collected from the bulk fruits. Hence studies were initiated to identify the variation in fruit colour based on maturation of the bulk fruit by evaluating the influence of fruit colour on seed quality characters as dry weight of seed, germination and vigour which serve as a maturity index for good production in next generation at laboratory and nursery.

MATERIALS AND METHODS

The fresh fruits of bael (*A. marmelos)* were collected from Coimbatore district (76°57 E, 11°8 N and 320 MSL) of Tamil Nadu state, India (Figure 1).

The fruits were collected from five different trees situated in the same place and same age group of 8 to 10 years. Fruits were collected in same season (November, 2012). Fruits were classified into various categories based on colours as green, greenish yellow and yellow. The sorted fruits were recorded for their recovery, the quantum of fruits available in each category based on total weight

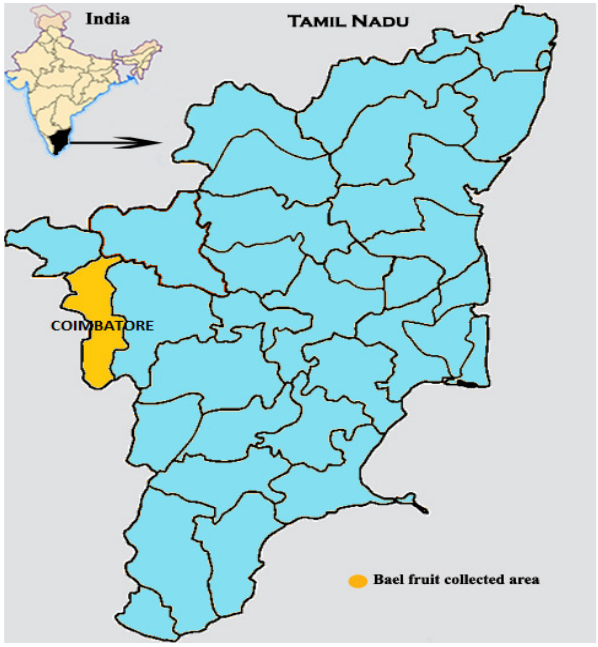

Figure 1. Study area (Coimbatore district of Tamil Nadu state, India).

of fruits (weight of green or greenish yellow or yellow fruits/total weight of fruits × 100). The physical characters of the fruit were evaluated with 5 replications of 10 fruits each. The seeds were then extracted by wet extraction as the pulp are semisolid and the seeds are embedded in that from each of the colour groups as the quality of seed of each fruit is extracted to vary with one another. The seeds were dried to 9 to 10% moisture content and evaluated for physical, physiological and biochemical characters as described subsequently along with bulk (mixture of fruits with different colours).

Physical characters of fruit

The fruits of each category were measured for fruit length (the length between the stalk end to style end of flower was measured using vernier calipers and the mean was expressed in centimeter), fruit girth (by running a thread around the fruits at the part of maximum width and stretching the thread on the measuring scale, having the mean expressed in centimeter) and fresh weight of fruit^{-1} (weighed in a electronic top pan balance and the mean expressed grams).

Physical characters of seed

The seeds of each category of fruits were counted as fresh after extraction for the number of seeds per fruit. Then the seeds were dried under shade for one week to reduce to uniform moisture content and hundred seeds in each category of the fruits were weighed separately as eight replications (ISTA, 2010) and the mean weight were determined.

Physiological characters of seed

The seeds of each of the colour category of fruits were evaluated for germination in sand media in a germination room maintained at

Table 1. Influence of fruit colour variation on fruit and seed characters of Bael (*Aegle marmelos*).

Grades (Based on colour)	Fruit characters				Seed characters		
	Recovery (%)	Fresh weight fruit^{-1} (g)	Length fruit^{-1} (cm)	Diameter fruit^{-1} (cm)	Number of seeds fruit^{-1} (Nos)	Seed weight fruit^{-1} (g)	100 seed weight (g)
Green	26.0	128	7.9	19.6	56	11.1	16.8
Greenish yellow	33.0	141	8.2	19.9	69	13.4	17.1
Yellow	41.0	154	8.6	20.5	72	15.3	19.5
Bulk	-	145	8.1	19.8	68	12.9	16.2
SEd	2.648	10.669	0.098	0.234	5.125	1.058	1.536
CD(P=0.05)	5.769	23.247	0.209	0.497	11.168	2.306	3.348

25 ± 1°C and 90 ± 3% relative humidity (RH) using 100 seeds in four replications (ISTA, 2010). Germination was observed every day to determine the days to first and 50% germination. After the germination period of 23 days, the test was terminated and evaluated for the occurrence of normal seedlings the seedlings (seedling which shows the capacity for continued development into normal plant), abnormal seedlings (Seedling which does not show the capacity for continued development into normal plant, it may be deformed, decayed and diseased) and dead seeds (collapse and milky paste comes out when pressed at the end of the test) based on the extent of exhibition as normal seedlings, the germination percentage of each category were recorded in percentage as per the standards of ISTA (2010). Ten normal seedlings were selected at random and measured for their root and shoot length and dry matter production in each of the replications. Using the data, the vigour index values were adopting the formula germination (%) × total seedling length (cm) (Abdul-Baki and Anderson, 1973).

Biochemical characters of seed

The seeds of each category were also evaluated for electrical conductivity as per Presley (1958) soaking the seeds for16 h duration. The seed oil and protein content were also measured using standard procedures, respectively (Sadasivam and Manickam, 1995; Ali-khan and Youngs, 1973).

Nursery performance

The seeds of each fruit grade, based on colour were sown in 50 bags of three replications each (comprising of 150 bags/treatment). The polybags were filled with potting mixture containing Soil:Sand:FYM in 2:1:1 ratio and the seedlings were maintained in the nursery with required management practices. After 30 days after sowing the nursery emergence was observed based on the number of seed put forth as in the nursery and reported as percentage to the total seed sown in nursery. After three months of sowing, the seedlings were evaluated for survival percentage (surviving seedlings after three months/total number of seeds sown × 100) and seedling quality characters, namely, root and shoot length, dry matter production and vigour index values) were calculated as mentioned earlier.

Statistical analysis

The statistical analysis design adopted for laboratory and nursery experiments were completely randomized design. The data gathered for each of the aforementioned parameters were subjected to analysis of variance and tested for significance as per Panse and Sukhatme (1995). The percentage values were arcsine transformed prior to statistical analysis.

RESULTS AND DISCUSSION

Fresh fruit colour variations observed for fruit and seed characters, seed quality characters and for their performance at nursery were highly significant. In colour grading, the highest recovery of 41% was obtained from yellow fruits followed by greenish yellow (33%) and green (26) fruits (Table 1). The fresh weight of the fruits was higher in yellow fruits (154 g) (Table 1). Length and diameter of greenish yellow fruits (8.2 and 19.9 cm, respectively), green fruits (7.9 and 19.6 cm, respectively), and yellow fruits (8.6 and 20.5 cm, respectively). The green fruits expressed the least physical expressions. The increase in fruit weight of yellow fruits might be due to more uptake of water, nutrients and also due to the accumulation of photosynthates from source to sink (Patel et al., 1977) with progress in maturation period. The results were also in accordance with the results of Dutta et al. (1963) in *Rauvolfia serpentina,* Kalavathi (1996) and Ramasamy (2006) in *Catharanthus roseus* and *Cassia angustifolia*, Gurunathan (2006) in *Jatropha curcas*, Venudevan et al. (2010) in *Gloriosa superba* and Sumathi (2010) in *Psoralea corylifolia.*

According to Harrington (1972), fresh weight of seed is an important factor that determines the quality of seed. Abdul-Baki and Anderson (1973) also used the fresh weight of seed for differentiating seed development and maturation. In the present study, the fresh weight of seed was maximum in yellow fruits (15.3 g) followed by greenish yellow (13.4 g), bulk (12.9 g) and green fruits (11.4 g) (Table 1), which could be attributed to the steady accumulation of dry matter during seed maturation phase in this orthodox species beyond fruit development also supported by Patel et al. (1977). Similar results were also reported by Bharathi (1999) in neem, Gunasekaran (2003) in *Solanum nigrum* and Ponnuswamy et al. (2005) in Punnai.

The seed germination recorded in the fruits collected

Table 2. Influence of fruit colour variation on physiological characters of Bael (*Aegle marmelos*).

Fruit grades (Based on colour)	Physiological characters						
	Days to first germination	Days to 50% germination	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (mg seedlings^{-10})	Vigour index
Green	16	19	52 (46.14)	5.6	6.9	193	650
Greenish yellow	13	16	74 (59.34)	6.1	7.4	221	999
Yellow	12	14	86 (68.02)	7.5	10.2	294	1522
Bulk	14	17	66 (54.33)	6.3	7.9	212	937
SEd	0.975	1.226	5.175	0.515	0.677	19.314	93.164
CD (P=0.05)	2.125	2.672	11.276	1.122	1.476	42.083	202.990

Figures in parentheses indicate arc sine transformed values.

Table 3. Influence of fruit colour variation on biochemical characters of Bael (*Aegle marmelos*).

Fruit grades (Based on colour)	Biochemical characters		
	Electrical conductivity (dSm-^{1})	Oil content (%)	Protein content (%)
Green	0.086	28.5	12.3
Greenish yellow	0.221	33.6	16.6
Yellow	0.138	38.9	18.2
Bulk	0.187	31.7	14.9
SEd	0.013	2.786	1.244
CD (P=0.05)	0.029	6.071	2.710

based on various colours revealed that the seeds of green, greenish yellow, yellow coloured and bulk fruits recorded germination of 52, 74, 86 and 66%, respectively suggesting the collection of yellow fruits for obtaining seeds with maximum germination capacity. Yellow fruits also presented higher seed vigour index (1522), given the greater root length (7.5 cm), shoot length (10.2 cm) and dry matter production (294 mg) (Table 2) were also higher in seeds obtained from yellow fruits followed by seeds from greenish yellow, bulk and green fruits. Srimathi et al. (2001) in Jamun also observed that black purple fruits were better in seed and seedling quality characters than reddish and green fruits and they considered blackish purple the colour of the mature Jamun fruit. In neem, Bharathi et al. (1996) and in *Bixa orellana*, Nelsonnavamaniraj (2005) also reported similar seed quality variations due to fresh fruit colour.

The oil/protein content in green and greenish yellow fruits (28.5/12.3 and 33.6/16.6%, respectively) was lower in comparsion with yellow fruits (38.9/18.2%) (Table 3). Maithani et al. (1989), Sacande et al. (1996) and Manimohan (2008) observed in *Andrographis paniculata* that collection of fruit based on colour could serve as a good index for quality seed collection. Lott et al. (1984) reported that protein and oil are subcellular structures commonly deposited in the cells of seed during seed development. Abdul-Baki and Baker (1973) also supported the results of the study indicating that seed dry weight, nucleic acid, lipid and protein accumulation followed a sigmoidal pattern in developing seeds as indication of maturation.

Electrical conductivity of seed leachate of the seed leachate which are the index of seed vigour were green fruit low (0.086 dSm^{-1}) and then increased towards maturity to reach maximum in greenish yellow (0.221 dSm^{-1}) then slightly reduced to yellow fruits (0.138 dSm^{-1}). Bhojwani and Bhatnagar (2005) also supported that at initial stage the seed size and the biochemical components were minimum, which increased during maturation. During the full maturation stage, the seed coat become thick walled which might have reduced the solutes to leach out.

Nursery studies conducted with polypots also indicated that seeds of yellow fruits produced elite seedling with 83% emergence. It was followed by seeds of greenish yellow fruits (74%). The green fruits (60%) performed poorer than bulk (69%) in the nursery (Table 4). This nursery performance agrees with the one found by Manonmani et al. (1996) working in Pungam. Seedling quality characters and survival percentage recorded after six months in nursery also revealed better performance of yellow fruits in nursery compared to other colour categories and may be rejected (green colour fruits) during selection of fruits for collection of seeds. Gurunathan et al. (2009) in their research with Jatropha also reported similar positive association in nursery with

Table 4. Influence of fruit colour variation on seedling quality characters of Bael (*Aegle marmelos*) at nursery.

Fruit grades (Based on colour)	Initial nursery emergence (%)	Seedling quality characters after three months of nursery period				
		Seedling survival (%)	Root length (cm)	Shoot length (cm)	Dry matter production (mg seedlings^{-10})	Vigour index
Green	60 (50.76)	57 (49.02)	15.7	12.1	1386	1585
Greenish yellow	74 (59.34)	70 (56.79)	20.6	16.6	1948	2492
Yellow	83 (65.65)	79 (62.72)	25.5	19.8	2065	3579
Bulk	69 (56.16)	67 (54.94)	19.9	15.1	1796	2450
SEd	(5.590)	(5.426)	1.685	1.312	142.281	222.842
CD (P=0.05)	(12.181)	(11.822)	3.672	2.859	310.006	485.536

Figures in parentheses indicate arc sine transformed values.

fruit size and seedling quality characters.

Conclusion

Bael being an underutilized crop, the initial propagation material of the seed should be of higher quality. But collection of matured fruit is difficult due to be thorny nature. Hence ground collection is practiced. Selection of fruits from ground collection as bulk will be highly helpful in collection of good fruits for good seeds. The study also emphasized the need for colour grading of fruits. As the demand for seed is comparatively lesser, adopting stringent selection for best propagative material for raising seed production area, the yellow fruits alone should be used for raising effective plantations. But on higher demand for seeds for afforestration and forestry programmes, the greenish yellow fruit can also be used for collection of quality seeds as their performance was better than the standard method of collecting fruits in bulk from ground and extracting the seeds.

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