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Influence of seed polymorphism on physical, physiological and biochemical seed quality characters of endangered medicinal tree Bael (*Aegle marmelos* (l.) corr.)

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Bael is a medicinal tree with multipurpose utility propagated through seeds. Bulk seeds of Bael exhibit polymorphism in seed characteristics. Hence, a study was undertaken to evaluate the influence of seed polymorphism on physical, physiological and biochemical seed quality characters. Polymorphic seeds based on size were separated by size grading the seeds using round perforated metal sieves of sizes 6.4, 6.0 and 5.5 mm retained and 5.5 mm passed (hole width), and the recovery of seeds retained on these sieves were 40, 23, 31 and 6% respectively. The physiological seed quality characters measured in terms of germination was higher with 6.4 mm sieve retained seeds (77%) and was followed by seeds retained on 6.0 mm (68%), 5.5 mm (55%) sieve; the seeds passed through 5.5 mm sieve registered only 41% germination while the germination recorded with bulk seeds was 63%. The seedling vigour measured through root length (11.3 cm), shoot length (9.3 cm), dry matter production (366 mg) and (1586), vigor index 2 (870) and vigour index 3 (28182) values were also higher in large seeds retained on 6.4 mm sized sieve. The biochemical characters measured as α– amylase activity (5.2 cm) and dehydrogenase (0.193) were higher in larger seeds including seed protein (17.4%) and oil content (38.1%). The study thus expressed a linear relationship between seed size and physical, physiological and biochemical seed quality characters and necessitates homogenizing the seed lot based on size.

Key words: Bael, seed polymorphism, seed germination, oil content, α–amylase, dehydrogenase activity.

INTRODUCTION

Aegle marmelos (L.) Corr., (Rutaceae) is a popular medicinal plant in the Ayurvedic and Siddha systems of medicines used to treat a wide variety of ailments. In India, this plant is known as “Bael Tree”. It is mostly found in tropical and subtropical region. The tree grows wild in dry forests on hills and plains of central and southern India, Burma, Pakistan, Bangladesh, Sri Lanka, Northern Malaya, Java and Philippine Islands (Islam et al., 1995). It is medium sized tree having profuse dimorphic branched, alternate, trifoliate, deep green leaves, membranous leaflets, large sweet scented, greenish white flowers, large and globose fruits. It flowers from May to July and yields an average of 350 to 400 fruits (200 to 250 kg) per tree (Mazumder et al., 2006).

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Bael fruits are popular due to its medicinal and nutritional properties and regarded as 'Amrit Phal' in cure of diarrhoea and dysentery, malaria, fever, jaundice, cancer, ulcers, urticaria and eczema (Nadkarni, 1954). All part of the tree (stem, bark, root, leaves and fruits) at different maturity stages has one or the other use. It is also used in the preparation of Ayurvedic medicines since ancient times (Rai et al., 1991). The foundation for revitalization of local health traditions (FRLHT), Banglore, India listed Bael (*A. marmelos*) as RET (Rare, Endangered and Threatened) species, specifically as endangered species. Hence, more importance is being given for mass multiplication through afforestration programmes. So far, no organized and systematic orcharding of this fruit crop has been taken in India (Kumar et al., 1994). It can easily be grown on eroded soil and adverse climatic conditions where most of the other fruits can not be grown. The tree is normally propagated through seeds (Nayak and Sen, 1999) and seed requires specific quality characters for its better performance with uniformity on seedling production. Quality seeds are obtained through selection of seeds from bulk.

Seed is the basic source of reproduction, though crops are multiplied through vegetative and seed propagation techniques; in tree species seed play a predominant role in mass multiplication and is much used in tree improvement programme. The reproductive material - the seed fetches much value only on procession of seed quality characters and only these quality characters can improve the nursery and planting value of the seedlings. But seeds which exhibit wider varieties such as seed morphological characters as per the influence of developmental variation as development and maturation (Abdul-Baki and Baker, 1973), position of formation (Swaminathan et al., 1991) and techniques used for harvesting (Khullar et al., 1991) only by reducing this polymorphic nature, uniformity could be brought with the seedling produced from the seed sowing.

To obtain uniform seedling growth, seeds are handled in such a way of removing the ill filled immature, undersize seed by reducing the polymorphism within the seed lot. The steps handled to reduce the polymorphism based on physical characters namely: size, shape and colour are termed as seed grading (Sekar, 2004). Seed grading is the most important seed preparation technique in crop seeds, practiced based on morphological characters of the seed. The process that reduces the seed polymorphism in seed is termed as grading and researchers like Malavasi and Malavasi (1996), Murali (1997), Mandal et al. (1997) and Girish et al. (2001) had expressed that physical grading could improve the physiological quality characters of the seed, due to the biochemical variations, which become the causes for the variation observed with physiological characters. The works in this aspect had been carried out by number of workers (Indira et al., 2000) in *Tectona grandis*; Arjunan et al. (1994) in *Pongamia pinnata* found a linear relationship between seed size and seed quality characters. Hence, an attempt was made to analyze the influence of seed size on seed quality characters of Bael through the expression of physical, physiological and biochemical characters.

MATERIALS AND METHODS

Bulk fruits collected from the seed source Dindugal, Tamil Nadu, India, were extracted manually dried for a week and graded with round perforated metal sieves of various sizes of 6.4, 6.0 and 5.5 mm based on the ability of seed to be retained on the sieve.

The **s**eeds retained on each sieve (6.4, 6.0, and 5.5 mm) that passed through 5.5 mm were weighed individually and the seed recovery percentage was calculated as below:

$$\text{Seed recovery (\%)} = \frac{\text{Weight of each of the seed size grade (g)}}{\text{Total weight of the seed}} \times 100$$

The seeds of each grade along with bulk were evaluated for the physical seed quality characters namely: 100 seed weight (g), seed length (cm) and seed breadth (cm) as follows:

Hundred seed weight (g): The seeds were dried to 8 ± 1% under shade, the approximate equilibrium moisture content of seed. These seeds of each grade were counted manually as 100 seeds in eight replicates as per ISTA, (2010) and the mean expressed as 100 seed weight in gram.

Seed length (cm): Five replications of ten seeds were randomly selected in each of the grade and the length between the micropylar end and chalazal end was measured using vernier calipers and the mean expressed as seed length in centimeter.

Seed breadth (cm): The seeds measured for seed length were measured for breadth at its largest portion using screw gauge and the mean expressed as seed breadth in centimeter.

The seeds of each of the size category were evaluated for the physiological seed quality characters of the seeds in terms of germination and vigour as follows.

Germination: Were evaluated for germination in sand media in a germination room maintained at 25 ± 1°C and 90 ± 3% Relative Humidity using 100 seeds of four replicates (ISTA, 2010). After the germination period of 23 days, days to first germination, the test was terminated and evaluated for the occurrence of normal, abnormal and dead seeds based on the extend of exhibition as normal seedlings the germination percentage of each of the category were recorded in percentage as per ISTA (2010).

Root and shoot length: Ten normal seedlings were selected at random in each of the replication and measured for their root and shoot length.

Dry matter production: Ten normal seedlings used for linear measurements as above were dried at first in shade and then in a hot air oven maintained at 85 ± 2°C for 48 h, then cooled in desiccators, weighed and expressed as dry matter production 10 seedling^{-1} in milligram.

Vigour index: The values were also computed adopting the following formulae, as these values are the totality expressions

seed quality characters.

Vigour index 1= Germination (%) × Total seedling length (cm) (Abdul – Baki and Anderson, 1973)

Vigour index 2 = Germination (%) × Root length (cm)

Vigour index 3 = Germination (%) × Dry matter production 10 seedling $^{-1}$ (mg)

The seeds of each category were also evaluated for biochemical characters as follows:

α–amylase activity (cm): Two grams of agar shreds and one gram of potato starch were mixed together in water to form a paste and the volume was made up to 100 ml. The homogenous solution of agar starch mixture after boiling was poured into sterilized petri dishes and allowed to settle in the form of gel after cooling. The seeds pre-soaked for 16 h were prepared by giving a longitudinal cut in such a way that both portions of the seeds have equal embryo portion. The prepared seeds as 8 replicates of five seeds were placed in petri dishes with agar in such a way that the cut portion remained in contact with agar starch gel. The dishes were closed and kept in dark at 30°C for 24 h. Then the dishes were uniformly poured with potassium iodide solution (0.44 g iodine crystal + 20.008 g potassium iodide in 500 ml of distilled water) and the excess solution was drained off after few minutes. The diameter of halo (clear) zone formed around the seeds were measured in millimeter and the mean value reported as α- amylase activity in centimeter (Simpson and Naylor, 1962).

Dehydrogenase activity: Representative seed samples from each of the sources were taken in four replications and were pre-conditioned by soaking them in water for 4 h at room temperature. Ten seeds of each of the replications and sources were taken at random and prepared by removing the seed coat. Then the seeds were steeped in 1% of 2, 3, 5-triphenyl tetrazolium chloride solution and kept for staining in dark at 40°C for 3 h. After staining, the stained seeds were soaked in methyl cellosolve solution @ 1 ml per seed for 4 to 6 h with occasional stirring till the complete extraction of red colour formazon. The extract was decanted and intensity of colour was read in a spectrophotometer (ELICO SL 159) at 470 nm. The mean OD values reported as dehydrogenase activity (Kittock and Law, 1968).

Protein content: Seed material of each sources were powdered using pestle and mortar and 100 mg of each of the sources were taken in 50 ml polyethylene screw cap bottle and 25 ml of 1 N sodium hydroxide was added. The mixture was shaken for 30 min at a wrist action shaker to disperse the protein. Then 10 ml of suspension was poured into a graduated test tube and used as blank to compensate for the differences in the amount of natural pigment extracted. To the remaining suspension in the bottle, 0.25 ml of 10% copper sulphate was added and the bottle was reshaken for five min. to develop the colour complex (Ali-Khan and Youngs, 1973).

The sample solution was poured into a separate test tube and left overnight to allow the dispersed material to settle down along with its blank. After centrifugation for 10 min the optical density of the clear supernatant solution was measured in an Erma photoelectric calorimeter using the red filter (620 nm) after adjusting with their respective blank. From the mean optical density value the protein content of each sample was calculated as follows:

Protein content (%) = 3.78 + (6.16 × OD value).

Oil content: The oil content of the seed was estimated using the soxhlet extraction apparatus. A known weight of finely ground seed sample of each sources were placed inside the filter paper thimble and kept inside the soxhlet funnel (butt tube). Approximately 150 ml of petroleum ether (40 to 60°C boiling points) was taken in the pre-weighed flat bottom flasks (Sadasivam and Manickam, 1995). Then the soxhlet apparatus was assembled and the flask was heated gently upto six reflux of the petroleum ether. The petroleum ether was removed from the butt tube and the flask with oil and small quantity of ether was heated to evaporate the petroleum ether completely (Sadasivam and Manickam, 1992). The flask with oil was weighed and the oil content was calculated using the following formula

$$\text{Oil content (\%)} = \frac{W_2 - W_1}{\text{Weight of sample}} \times 100$$

Where,

W_1= Weight of empty flask

M_2= Weight of flask with oil

Statistical analysis: The data obtained from different experiments were analysed for 'F' test of significance following the methods described by Panse and Sukhatme (1995) adopting FCRD for laboratory experiments and RBD for nursery studies. Wherever necessary, the per cent values were transformed to angular (Arc-sine) values before analysis. The critical differences (CD) were calculated at 5% probability level. The data were tested for statistical significance (*).

RESULTS AND DISCUSSION

In the present investigation, seeds were graded using three different metallic sieves with 6.4, 6.0 and 5.5 mm round perforations (Figure 1).

The results expressed that recovery of seeds retained on 6.4 mm sieve was higher (40%) and was followed by the seeds retained on 5.5 mm sieve (31%). The recovery of seeds retained on 6.0 mm was 23%, while the recovery seeds passed through 5.5 mm were 6% indicating the uniform seed size distribution within the lot, where larger and smaller seeds were distributed in higher order than the medium sized seed. The ill filled, immature seed developed with irregularities that passed through 5.5 mm size was also higher recording the recovery of 6% (Table 1 and Figure 2) based on weight of bulk seeds.

This variation is highly possible as the seed size is highly influenced by developmental (Venudevan et al., 2010) and managerial variation (Sumathi, 2010). The evaluated physical seed quality characters namely: 100 seed weight (9.9 g), seed length (1.1cm) and seed breadth (0.8 cm) were maximum with 6.4 mm retained seed and it reduces positively with reduction in sieve size as 6.0 and 5.5 cm.

Similar observations were also reported by Kathiravan (2004) in Jatropha. The physiological seed quality

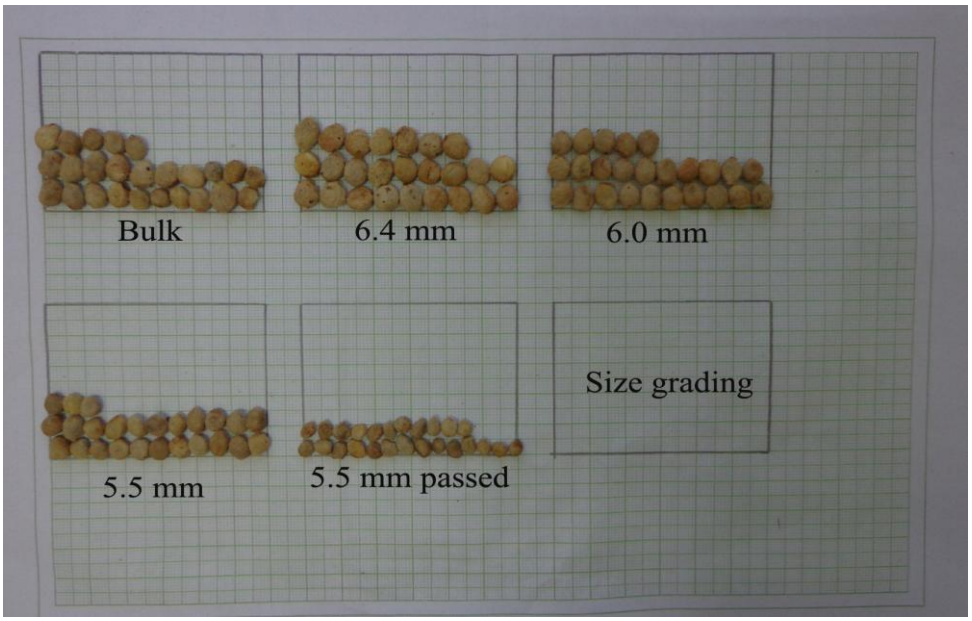

Figure 1. Arrangement of seeds based on size grading.

Table 1. Influence of seed polymorphism on recovery (%) and seed morphological characters of Bael (*Aegle marmelos*).

Seed retained on the sieve (Width of aperture)	Recovery %	100 seed weight (g)	Seed length (cm)	Seed breadth (cm)
6.4 mm	40	9.9	1.1	0.8
6.0 mm	23	8.5	0.8	0.6
5.5 mm	31	7.4	0.6	0.4
5.5 mm passed	6	5.4	0.4	0.3
Bulk	-	8.0	0.8	0.5
SEd	0.424	0.137	0.014	0.016
CD (P=0.05)	0.945	0.307	0.032	0.036

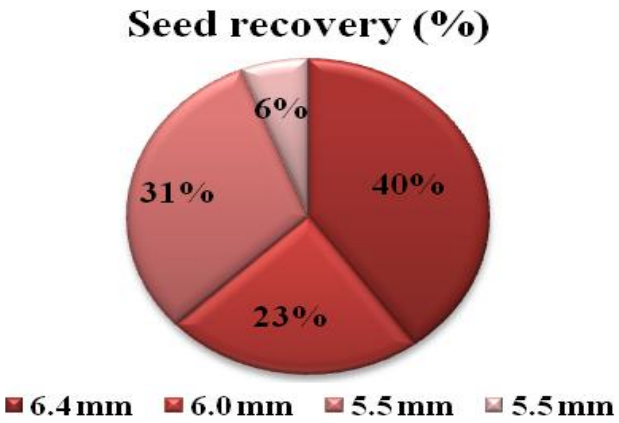

Figure 2. Influence of seed polymorphism on seed recovery percentage.

characters (Table 2 and Figure 3) such as days to first germination (11 days), germination (77%), root length (11.3 cm), shoot length (9.3 cm), dry matter production (366 mg) and vigour index values as vigour index 1 (1586), vigour index 2 (870) and vigour index 3 (28182) values were more for 6.4 mm sieve retained seeds, followed by other size grades in decreasing order (Figure 4).

Similar relationship between seed size and seed germination and seedling vigour were also reported by Hoppe et al. (1991) in *Melia azadiracta* (Srimathi, 1997) in amla (*Emblica officinalis*) and jamun (*Syzygim cuminii*) (Srimathi et al., 1998) in ber (*Zizyphus mauritiana*) and Malarkodi et al. (1999) in Punnai. The positive association was recorded with growth of seedlings from larger to smaller size seed. The better performance of

Table 2. Influence of seed polymorphism on seed and seedling quality characters of Bael (*Aegle marmelos*).

Seed retained on the sieve (Width of aperture)	Days to first germination (days)	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (mg in 10^{-1} seedlings)
6.4 mm	11	77 (61.34)	11.3	9.3	366
6.0 mm	13	68 (55.55)	10.5	8.5	298
5.5 mm	15	55(47.87)	9.4	7.9	278
5.5 mm passed	16	41(39.81)	8.0	6.2	202
Bulk	14	63(52.53)	9.7	7.9	283
SEd	0.122	(0.128)	0.080	0.087	3.741
CD (P=0.05)	0.255	(0.268)	0.166	0.183	7.803

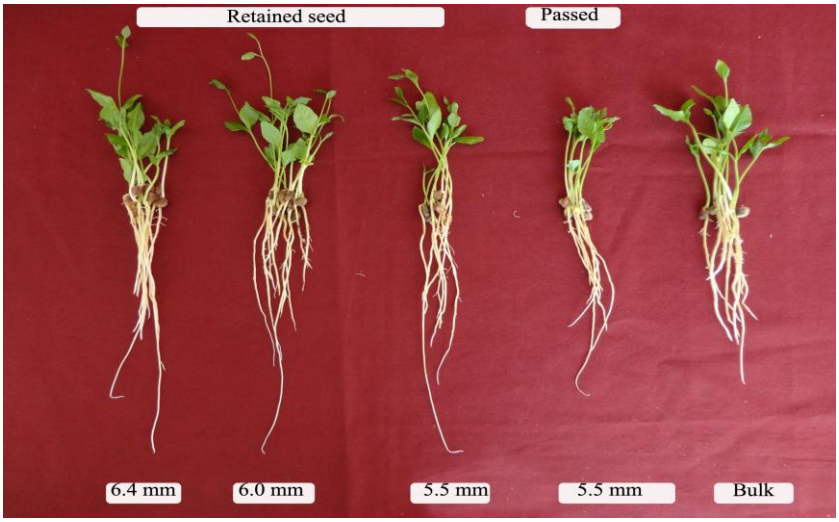

Figure 3. Influence seed polymorphism on physiological seed quality characters.

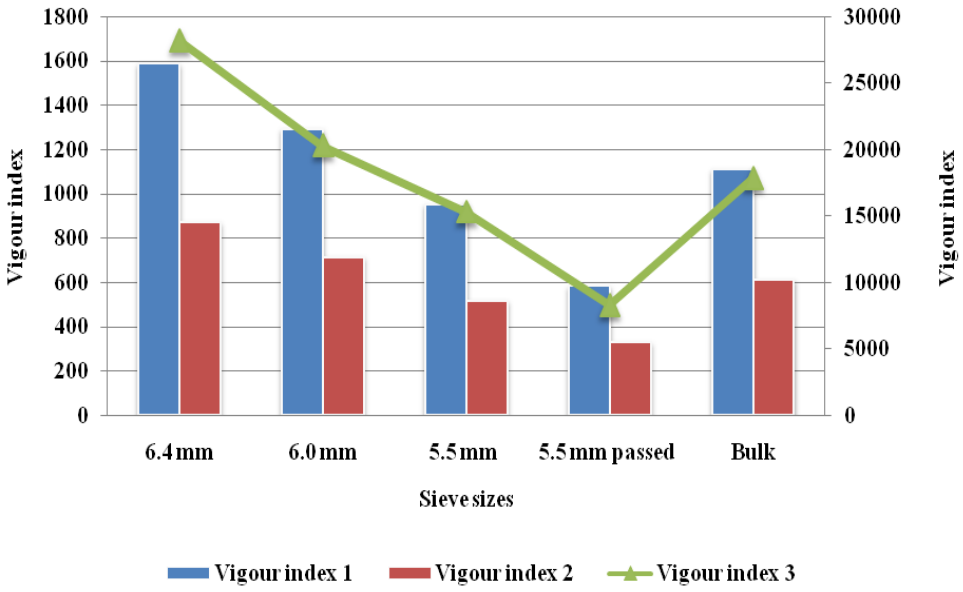

Figure 4. Influence seed polymorphism on physiological seed quality characters.

Table 3. Influence of seed polymorphism on biochemical characters of Bael (*Aegle marmelos*).

Seed retained on the sieve (Width of aperture)	α– amylase activity (cm)	Dehydrogenase activity (OD value)	Protein content (%)	Oil content (%)
6.4 mm	5.2	0.193	17.4	38.1
6.0 mm	4.8	0.084	16.9	34.2
5.5 mm	3.5	0.047	15.7	26.4
5.5 mm passed	2.3	0.035	10.1	21.5
Bulk	4.0	0.054	16.6	32.4
SEd	0.045	0.001	0.136	0.360
CD (P=0.05)	0.095	0.002	0.284	0.751

bigger seed compared to smaller seeds explained not only by the quantum of accumulated reserve of nutritional matter in them (Ashby, 1936), but also by their higher chemical composition. Gunaga et al. (2011) opined that translocation of reserve from endosperm to embryo proceeds differently in large and small seeds and the better-filled, larger seeds of *Pinus thunbergii* transferred more nitrogen from the endosperm to the embryo after sowing than the small seeds. Ponnuswamy (1993) in neem, Arjunan et al. (1994) and Manonmani et al. (1996) in Pungam and Kathiravan (2004) and Gurunathan et al. (2009) in Jatropha and Venudevan et al. (2013) in Bael also reported that seed size and seed quality characteristics were positively related to each other.

The biochemical characters are the intrinsic seed character that reveals the physiological and physical seed quality characters (Sudhirkumar, 2003). The evaluated biochemical characters (Table 3) of the study revealed that α– amylase (5.2 cm), dehydrogenase (0.193) enzyme activity, closely related to seed viability and germination (Palaniswamy et al., 1994) were higher in larger sized seed and reduced with reduction in seed size. The nutrient component of the seed evaluated in terms of protein (17.4%) and oil (38.1%) content were more in large seeds and was followed by seeds of medium (6.0 mm retained sieve), bulk and small (5.5 mm retained sieve) seeds which might be due to the developmental variation that was exerted as seed size variation as reported by Parameswari (1999) in tamarind.

Conclusion

Seed is the economic product and fetches higher money with better quality characters, hence the sieve size recommended as a standard sieve for seed processing should have higher recovery with better seed quality characters. Based on the results of the present study, recovery and seed and seedling quality characters were higher with 6.4 mm which could be selected as standard sieve for Bael on instances better selection irrespective of demand but when the demand is higher seed retained as 6.0 mm size could be selected as the seed quality characters and recovery will be higher to meet the demand and the seeds perform better than control. Thus the study indicated that in Bael.

1. Seed size and seed quality characters are positively related.
2. Based on seed demand either 6.4 mm or 6.0 mm sieve should be selected seed for production of elite seedlings at nursery.

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