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Variability Studies in Fruits and Seed Characters of *Aquilaria malaccensis*

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ABSTRACT

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Aquilaria malaccensis Lam. (Agarwood) is one of the important aromatic oil yielding tree species naturally found in the Indo-Malaesian region. In recent years, the species is being cultivated on plantation scale and it is important to understand the variation in the fruits and seed characters to support genetic improvement in the species. A study was conducted to assess the variability in the regional populations and trees within the population with respect to fruit and seed traits. The existence of variation in fruit and seed characters of different populations and trees in the population was evident. Broad sense heritability values of different fruit and seed traits were determined and the values were relatively higher. Phenotypic and genetic correlations between fruit and seed traits were also estimated and most of the fruit traits were having positive correlation with important seed traits such as seed weight, seed length and seed width.

Introduction

Aquilaria malaccensis Lam. is one of the 15 tree species in the Indo-malaesian genus *Aquilaria* belongs to family Thymelaeaceae (Ng *et al.*, 1997). The tree grows in natural forests at an altitude of a few meters above sea level to about 1000 m, and it grows best around 500 m in locations with average daily temperatures of 20 to 22°C (Keller and Sidiyasa, 1994; Wiriadinata., 1995). The tree is traded internationally for the agarwood,

which produced in its trunk to protect the tree when it is wounded and infected by fungus (Liu Y. *et al.*, 2013; Mohamed *et al.*, 2014) and has been used for incense as well as a traditional sedative, an analgesic and digestive medicine (Yagura *et al.*, 2005). In India, *A. malaccensis* grows mostly in the foothills of the North-eastern region (Assam, Meghalaya, Nagaland, Mizoram, Manipur, Arunachal Pradesh and Tripura) and West Bengal up to an altitude of 1000 m. In Assam and Meghalaya, it occurs sporadically in the

district of Sibsagar, Sadiya, Nowgong, Darrang, Goalpara, Garo Hills and Cachar (Atal and Kapoor, 1982). *Aquilaria* sp. has adapted to live in various habitats, including those that are rocky, sandy or calcareous, well-drained slopes and ridges and land near swamps (Chakrabarty *et al.*, 1994; Sumadiwangsa, 1997). Over exploitation of the species due to international demand, made the species more threatened and entered into CITES Appendix-II (CITES, 1994).

The number of trees in the natural habitats has been reduced and the species is widely grown in the home gardens of North East India to meet the demand. Considering the economic value of the agarwood, the species is introduced in the Western Ghats regions of Karnataka and Kerala and in some places of Tamil Nadu. The formation of agarwood in the tree depends on natural infection and only few trees produces agar wood. There is no successful artificial inoculation method available for the production of agarwood in the agar tree. Unfortunately, before understanding the role of genetics and environment in formation of agar wood, the trees in plantations, natural habitat and home gardens were vanished for the demand of agarwood.

The population in the natural habitat is disappearing and further, plantations are raised with unknown seed sources, leading to inbreeding. If continued at the same level as now low levels of genetic diversity within species can lead to inbreeding depression which affects growth, survival and adaptation (Kjaer, 1997) leading to genetic loss. Genetic variation within and between species is important to the long-term natural adaptation of species (Falk and Holsinger, 1991). Hence, a attempt to explore the existing variations in the species and conserving those germplasm is done in the present study.

Materials and Methods

The study was conducted in Institute of wood science and technology, Bangalore. Based on the existing literature and interaction with the local people different natural populations were identified and located on the geopolitical map of the region. Each located points were visited and reconnaissance survey was carried out in the region and totally 44 phenotypically superior trees (Candidate trees) were selected in North East India comprising Assam, Arunachal Pradesh, Meghalaya, Nagaland and Tripura. Along with these 44 trees in the natural populations two introduced populations, one each from Karnataka and Tamil Nadu were also considered for the study (Table 1). Matured fruits were collected from selected candidate trees from May to September, 2019. The collected fruits were brought within 3-4 days to the laboratory at Institute of Wood Science and Technology, Bangalore. A total of 20 fruits were taken randomly from each tree seed lot with identity and subjected to morphometric measurements of fruits and seeds. Fruits were measured for its weight, width and length. The fruits were predominantly double seeded and some of them were single seeded. Seeds were separated from the fruits and the seed weight, length and width of the seeds were recorded. All measurements were made with digital Vernier Caliper (0.01mm precision) and digital electronic balance (0.001g precision). The average of weight, length and width of seeds were taken for statistical analysis where the fruits are two seeded. In all, data from 920 fruits and seeds were considered for the study.

Data analysis

Statistical analysis of the data was carried out by using the Crop Stat for Windows Version 7.2.2007.3 software by grouping the entire population under six regions. The phenotypic and genotypic correlation and broad sense

heritability for the fruit and seed traits were determined using the Plant Breeding Tools (PBTools), Version: 1.3 software (International Rice Research Institute <http://bbi.irri.org>).

Results and Discussion

Variation in the fruit and seed traits among the populations of different regions

Important fruit and seed traits of different populations are the indicators of similarity or variability between the populations and data pertaining to fruit and seed traits of different populations are presented in Table 2. Existences of significant variation in the different populations with respect to fruit and seed traits were evident in the present study. Among the different populations, introduced population had significantly higher values for all the fruit traits. Among the natural populations, population from Assam and Meghalaya had on par values for fruit weight (1.86g each) and were significantly higher over populations from Arunachal Pradesh (1.76 g), Nagaland (1.74 g) and Tripura (1.71 g). Fruit length of different natural populations were found to be on par except Tripura population (28.09 mm) which was significantly lower over all the populations. Similar trend was observed in the fruit width of different natural populations wherein Tripura population had significantly lower value (28.09 mm) compared to other populations. Fruit thickness of different natural populations varied significantly and among them, Meghalaya (12.29 mm) had significantly higher value over Nagaland (11.75 mm), Arunachal Pradesh (11.42 mm), Assam (11.18 mm) and the least value was recorded for Tripura (10.99) which was significantly lower to all other populations,

Existence of significant variation in seed traits of different populations was evident in the

present study (Table 2). Seed weight of introduced population (0.20 g) had significantly higher value and was on par with Assam population (0.18 g). On the other hand, seed length of Arunachal Pradesh source (14.77 mm) had significantly higher values over Meghalaya (14.30 mm) Nagaland (13.48 mm) and Tripura sources (13.39 mm) and was on par with Assam (14.62 mm) and introduced populations (14.50 mm). Seed width of introduced populations was found to be significantly higher (4.54 mm) over all the natural populations. Interestingly, seed width among the different natural populations did differ significantly. The values on the number of seeds per fruit were also varied significantly and Nagaland population (1.89) had significantly higher values over other populations except introduced population (1.87). Number of seeds per fruit in other populations did vary considerably and the least number of seeds per fruit was observed in Meghalaya (1.58) and Arunachal Pradesh populations (1.55).

Variation in fruit and seed traits of candidate trees of different populations

Data on fruit and seed traits of different candidate trees of different populations are presented in Table 3 and the perusal of the data on fruit characters of candidate trees revealed the significant difference in fruit characters of different candidate trees. Among the different populations studied, maximum fruit weight of 2.16 g was recorded in tree number 19 and tree numbers 1, 8 and 21 recorded the lowest fruit weight (1.68 g). Tree number 46 from introduced population had the highest value of fruit length which was 31.89 mm whereas the lowest value of fruit length (26.24 mm) was recorded from the tree number 45 which was also from introduced population from different locality. Fruit width of different candidate trees varied significantly and values were in the range of

9.86 mm (Tree42) to 12.86 mm (Tree 33). Fruit thickness of different trees differed significantly with values ranging from 13.34 mm (Tree 42) to 18.08 mm (Tree 39).

Close perusal of the data on seed traits of candidate trees from different populations revealed the existence of significant variation in the seed traits of candidate trees from different populations except seed weight. The seed weight of different trees varied from 0.13 g to 0.19 g. Seed length of different candidate trees varied significantly with values ranging from 12.73 mm (Tree 17) to 15.37 mm (Tree 7). Seed width of different candidate trees varied from 3.37 mm (Tree 15) to 4.56 mm (Tree 6) and the values differed significantly. Number of seeds per fruit did vary considerably where the values

varied from 1.42 to 2.16 (Tree 24).

Broad sense heritability

The analysis on broad sense heritability of the fruit and seed traits indicated higher values (Table 4). In case of fruit traits the broad sense heritability values varied from 0.82 to 0.90 and values for seed traits were ranging from 0.71 to 0.96. Among the different fruit characters, the fruit thickness had relatively higher value (0.90) followed by fruit width (0.89) fruit length and fruit weight (0.82). Close perusal of the data presented on the broad sense heritability of different seed traits revealed that the values were ranging from 0.71 (seed weight, number of seeds per fruit) to 0.96 (Seed width).

Table.1 Geographical details of regions and candidate trees for the study

Sl.No.	Region	No. of Trees	Latitudinal range	Longitudinal range	Altitudinal range(MSL)
1	Assam	22	N 26°32'54.7" To N 27°59'57.0"	E 94°00'03.0" To E 95°45'28.0"	95-163
2	Arunachal Pradesh	7	N 27°06'54.3" To N 28°04'04.4"	E 93°48'20.4" To E 95°28'46.7"	137-163
3	Meghalaya	3	N 25°32'39.2" To N 25°55'59.0"	E 90°13'54.6" To E 90°39'75.0"	68-137
4	Tripura	8	N 23°33'45.2" To N 23°53'13.7"	E 91°17'03.2" To E 91°25'50.4"	31-39
5	Nagaland	4	N 26°17'20.4" To N 26°17'22.6"	E 94°23'57.1" To E 94°23'59.2"	1075-1088
6	Introduced sources				
	(i) Ponnampet, Karnataka	1	N 12°08'35.2"	E 75°56'19.3"	916
	(ii) Karumandruai, Tamil Nadu	1	N 11°48'51.4"	E 076°37'21.6"	766

Table.2 Variations in fruit and seed traits among the populations of different regions

Sl No	Populations	Fruit Weight (g)	Fruit length (mm)	Fruit width (mm)	Fruit Thickness (mm)	Seed weight (g)	Seed Length (mm)	Seed Width (mm)	Seed /fruit (#)
1	Assam	1.86	29.16	16.03	11.18	0.18	14.62	4.27	1.78 (1.33)*
2	Arunachal Pradesh	1.76	29.08	15.70	11.42	0.14	14.77	3.90	1.58 (1.26)
3	Meghalaya	1.86	29.33	15.61	12.29	0.13	14.30	3.73	1.55 (1.25)
4	Tripura	1.71	28.09	14.98	10.99	0.14	13.39	3.57	1.67 (1.29)
5	Nagaland	1.74	28.91	15.61	11.75	0.17	13.48	3.42	1.89 (1.38)
6	Introduced source	2.23	31.72	16.83	12.78	0.20	14.50	4.54	1.87 (1.37)
CD (0.05)		0.09	0.76	0.43	0.38	0.02	0.32	0.13	0.05
SEM		0.03	0.24	0.14	0.12	0.01	0.10	0.04	0.02

*Values in parenthesis are square root transformed values

Table.3 Mean fruits and seeds traits of individual candidate trees

Candidate Tree No.	Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	Fruit thickness (mm)	Seed weight (g)	Seed Length (mm)	Seed Width (mm)	Seed /fruit (#)
1	1.68	28.74	10.93	15.14	0.19	15.19	4.32	1.82 (1.35)*
2	1.78	28.90	10.83	15.54	0.18	13.84	4.04	1.88 (1.37)
3	1.97	29.34	11.51	15.92	0.16	14.57	4.28	1.72 (1.31)
4	1.96	30.29	11.92	15.91	0.15	14.54	4.28	1.56(1.25)
5	1.79	30.15	11.18	15.53	0.16	13.65	4.11	1.72(1.31)
6	1.87	30.60	11.22	16.21	0.16	14.45	4.56	1.82(1.35)
7	1.77	30.65	10.86	15.52	0.16	15.37	4.13	1.72(1.31)
8	1.68	27.58	10.71	15.47	0.14	14.20	3.89	1.61(1.27)
9	1.82	29.21	11.43	15.60	0.15	15.02	4.08	1.72(1.31)
10	1.83	29.20	11.84	15.56	0.17	14.89	4.12	1.88(1.37)
11	1.87	31.22	11.74	15.68	0.17	13.83	4.08	1.82(1.35)
12	2.06	28.85	12.17	16.67	0.15	14.24	3.99	1.66(1.29)
13	1.73	28.46	10.92	15.06	0.19	14.02	4.05	1.72(1.31)
14	1.80	28.98	11.15	15.97	0.15	14.24	3.71	1.77(1.33)
15	1.76	28.36	12.44	16.35	0.18	14.52	3.37	1.82(1.35)
16	1.76	27.61	10.85	15.58	0.16	14.35	3.73	1.82(1.35)
17	1.92	27.74	12.02	16.63	0.13	12.73	3.70	1.56(1.25)
18	1.72	29.70	10.89	15.27	0.14	14.69	4.34	1.77(1.33)
19	2.16	28.88	12.48	16.77	0.19	14.43	3.70	1.93(1.39)
20	1.79	29.00	11.16	16.28	0.19	14.22	4.01	1.77(1.33)
21	1.68	27.55	11.15	14.79	0.14	13.46	3.81	1.51(1.23)

22	1.77	28.40	10.83	15.47	0.15	14.20	3.62	1.51(1.23)
23	1.70	29.63	10.72	15.22	0.17	14.34	4.38	1.77(1.33)
24	1.89	30.12	10.81	15.95	0.19	14.14	3.97	2.16(1.47)
25	1.87	28.99	11.09	16.28	0.16	14.18	3.73	1.77(1.33)
26	1.69	27.85	11.02	15.05	0.16	14.22	3.88	1.54(1.24)
27	1.78	28.87	11.60	15.22	0.15	14.28	4.16	1.59(1.26)
28	1.94	28.70	12.15	16.26	0.15	13.79	3.83	1.59(1.26)
29	1.92	29.28	12.22	16.41	0.16	15.17	4.03	1.72(1.31)
30	1.86	28.69	11.04	16.12	0.16	14.82	4.13	1.61(1.27)
31	1.83	29.31	11.48	15.58	0.17	14.25	4.11	1.77(1.33)
32	1.79	29.19	11.60	15.60	0.16	13.84	3.75	1.77(1.33)
33	1.89	30.36	12.86	16.14	0.17	14.62	4.13	1.82(1.35)
34	1.73	28.73	10.97	14.73	0.14	14.56	3.91	1.56(1.25)
35	1.83	29.09	10.24	15.00	0.19	14.60	3.77	2.04(1.43)
36	1.88	29.32	10.16	15.04	0.17	14.59	4.07	1.82(1.35)
37	1.89	29.68	11.57	15.33	0.16	14.51	4.17	1.66(1.29)
38	1.79	28.59	11.02	16.35	0.18	14.42	4.10	1.93(1.39)
39	1.83	28.16	12.50	18.08	0.14	14.20	4.13	1.42(1.19)
40	1.77	28.59	11.67	15.50	0.15	12.90	3.69	1.61(1.27)
41	1.84	29.77	11.26	16.73	0.16	14.37	3.80	1.66(1.29)
42	1.82	29.26	9.86	13.34	0.17	14.24	3.96	1.82(1.35)
43	1.92	28.53	11.96	16.55	0.17	14.29	4.17	1.82(1.35)
44	1.72	28.70	12.42	16.45	0.15	14.31	4.06	1.61(1.27)
45	1.74	26.24	11.96	14.69	0.16	13.85	3.49	1.66(1.29)
46	1.92	31.89	10.78	16.85	0.16	14.76	4.50	1.77(1.33)
CD (0.05)	0.72	0.18	1.46	0.82	NS	0.61	0.26	0.10
SEM	0.26	0.06	0.53	0.30	0.015	0.22	0.09	0.04

*Values in parenthesis are square root transformed values

Table.4 Broad Sense Heritability of fruit and seed traits of *A. malaccensis*

Sl.No.	Traits	Broad Sense Heritability (H ²)
1	Fruit weight	0.82
2	Fruit length	0.82
3	Fruit width	0.89
4	Fruit thickness	0.90
5	Seeds per fruit	0.71
6	Seed weight	0.71
7	Seed Width	0.96
8	Seed Length	0.91

Table.5 Phenotypic Correlations between fruit and seed traits of *A. malaccensis*

	Fruit weight	Fruit length	Fruit width	Fruit thickness	Seed weight	Seed Width	Seed Length	Seed per fruit
Fruit weight	1.00							
Fruit length	0.58	1.00						
Fruit width	0.61	0.35	1.00					
Fruit thickness	0.47	0.17	0.55	1.00				
Seed weight	0.45	0.34	0.31	-0.08	1.00			
Seed Width	0.48	0.59	0.39	-0.07	0.53	1.00		
Seed Length	0.30	0.43	0.29	-0.03	0.37	0.65	1.00	
Seed per fruit	0.29	0.34	0.08	-0.16	0.76	0.20	0.11	1.00

Table.6 Genotypic Correlation between fruit and seed traits of *A. malaccensis*

	Fruit weight	Fruit length	Fruit width	Fruit thickness	Seed weight	Seed Width	Seed Length	Seed per fruit
Fruit weight	1.00							
Fruit length	0.64	1.00						
Fruit width	0.63	0.36	1.00					
Fruit thickness	0.48	0.18	0.56	1.00				
Seed weight	0.54	0.42	0.37	-0.10	1.00			
Seed Width	0.53	0.67	0.41	-0.08	0.64	1.00		
Seed Length	0.34	0.48	0.31	-0.04	0.45	0.69	1.00	
Seed per fruit	0.34	0.42	0.08	-0.21	0.86	0.25	0.16	1.00

Phenotypic and genetic correlation between traits

The data on phenotypic correlations for fruit and seed characters of *A. malaccensis* are depicted in Table 5. Perusal of the data revealed high significant positive correlation between number of seeds and total seed weight (0.76) seed width and seed length (0.65). The data also revealed that the fruit and seed traits have a positive phenotypic correlation except for fruit thickness which exhibited a negative correlation with seed traits of *A. malaccensis*. The statistical analysis for genetic correlation between fruit and seed characters revealed that there exists a positive correlation between fruit and seed characters except for fruit thickness which recorded a weak negative correlation with the seed characters (Table 6).

Variation in fruit and seed traits of different populations

The variability between trees occur due to three causes viz. differences in edaphoclimatic conditions, genetic differences among trees, and the interactions between tree genotypes and the environments in which they grow. If the variations are of genetic origin, it can be manipulated to obtain good gains in some tree characteristics. Early life history characteristics, such as seed traits, are among the most important because they strongly influence patterns of distribution and abundance of populations and species (Rees, 1996; Silvera *et al.*, 2003). Despite stabilizing selection to maintain constant seed morphological characteristics, variability is seen within species, among populations, among individual plants and fruits

(Jacquemyn *et al.*, 2001; Bañuelos and Obeso, 2003). The variation in seed traits may cause differences in reproductive success, dispersal potential (Yamada and Suzuki, 1999), germination potential (Jacquemyn *et al.*, 2001; Silvera *et al.*, 2003), and seedling establishment (Geritz *et al.*, 1999).

Present study has revealed significant variations between the populations for fruit characteristics of *A.malaccensis* which can be attributed to the variations in the environmental conditions as well as to the genetic causes (Table 2). The populations considered in this experiment experience a difference in their edaphoclimatic conditions which might have resulted in variations in fruit traits. The results were on par with the works of Sivakumar *et al.*, (2002), who reported that the fruit characters of Teak varied among the different locations. Similar variations in fruit traits between the various provenances has been recorded by the authors Ladipo *et al.*, (1996), Atangana, (2010), Tsobeng *et al.*, (2019) and Uma Shankar & Idaiarilin (2012). The results are supported by Munthali *et al.*, (2012), who reported significant variations in fruit weight, length and width between different provenances and opined that the variations are due to environmental and genetic causes.

Apart from the fruit characters, *A.malaccensis* also found to have variations in seed traits of different populations (Table 2). Variability in seed size within species has been reported for many plants by various workers. Several researchers opined that the variations in the seeds mass among the population and within the populations may result from maternal effect, variations in phenology and due to the interaction among the parent plant (Zheng and Sun, 2008). In addition, seed size variation can also result from low or variable selection pressure, resource availability, the effect of position on plants and other physiological

factors (Herrera 1990; Stearns 1992). In this study, the climatic variations and topographic factors mainly the altitude, among the populations has its greater contribution. Further, with in the population differences in the local climatic conditions and the soil fertility has a major role to play. Rawat *et al.*, (2006) reported variations in the seed size (seed length, width, test weight and seed volume) among the provenances over the natural range, which was employed as a criterion for the identification of a region in *Pinus wallichiana*. Similar study was conducted by Hegde *et al.*, (2000) in *Acacia auriculiformis* and *A. mangium*, Kumar *et al.*, (2003), Manjare *et al.*, (2003) and Patil *et al.*, (2011) in *Pongamia pinnata*.

A significant variation in fruit characters among the individual trees was found in the study which indicates a wide genetic base in the population (Table 3). These are the sources and raw materials for the tree improvement programmes which can be exploited. Since the size and weight of the fruits have great implication on the dimension of the seeds they contain which in turn results in their impact on germination makes it a great need to select the populations with higher fruit characters. Uma Shankar and Idaiarilin (2012) opined that the tree-to-tree variation in fruit and seed traits is common and may be determined both by genetic and environmental factors. However, it is also argued that the most within-species variation occurs within a plant rather than among plants or populations indicating a greater role of environment during development rather than genetic differences between mothers.

Broad sense heritability of different traits

The variations can be usefully exploited only when it is known that whether the variations are of genetic origin or are of simply environmental. The heritability values are one

such tool which separates the origin of variations and indicate how phenotype is affected by environment and genotype. The higher values of h^2 are indicative of the fact that these phenotypic traits are highly of genetic in origin rather than environmentally controlled. Heritability values for fruit and seed characters tabulated in Table 6 showed higher values which indicates the considerable portion of variance is additive. High additive genetic variance and variation between wild germplasm sources offer good scope for genetic improvement of this species. Such traits which are of genetic origin can be exploited through vegetative propagation or are useful in self pollinating species (Table 5).

Phenotypic and genetic correlations between traits

Correlations studies done for the fruit and seed traits have shown that fruit width was positively correlated with seed width. Similar positive correlation between pod width with seed width and significant positive correlation of seed width, seed length with 100 seed weight in *Pongamia pinnata* has been reported by Sahoo *et al.*, (2011). Similar to the findings in the present experiment Okwillo *et al.*, 2018 observed strong uphill positive linear relationships between seed mass and seed number ($R=0.78$), total fruit mass and fruit length ($R=0.73$), pulp mass and total fruit mass ($R=0.72$), and seed mass and total fruit mass ($R=0.72$) in *Tamarindus indica*. The positive correlation between seed length and width has also been reported by Shen *et al.*, (2013). Genotypic correlation of fruit and seed traits with each other have shown higher values than the corresponding phenotypic correlation values indicating presence of higher inherent association between these traits. Sahoo *et al.*, (2011) found higher genotypic correlation than the respective phenotypic correlation in case of pod and seed traits of *Pongamia pinnata* indicating the

presence of strong inherent association between those traits.

In conclusion the existence of variation between the populations and between the trees within the populations is evident with respect to fruit and seed characters of *Aquilaria malaccensis*. It is important to consider the variation while introducing population from one region to another region.

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