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Influence of levels of genetic diversity on fruit quality in teak (*Tectona grandis* L.f.)

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The study on the influence of genetic diversity on the fruit emptiness and seed germination (as a measure of fruit quality) of teak populations was carried out. The populations comprised three unimproved plantations, three seed-production areas and a clonal seed orchard within Karnataka. Significant variation between the populations was observed for fruit emptiness, seed germination and Jaccard's dissimilarity index of the parent population. Genetic dissimilarity of populations was positively correlated to fruit emptiness and negatively correlated to seed germination. It is inferred that higher genetic dissimilarity of individuals within the population results in higher flower asynchrony and close-related mating, thereby leading to higher inbreeding depression manifested in the form of higher emptiness and low germination percentage.

Keywords: Flower asynchrony, fruit emptiness, genetic diversity, seed germination, teak.

TEAK (*Tectona grandis* L., family Lamiaceae) has been recognized as the most valuable and premium wood in the world's timber trade. Presently, it is grown in plantations across 36 tropical countries of Asia, Africa and Latin America¹. Considering the net area of teak plantations in 1995, about 94% lay in tropical Asia, with India (44%) and Indonesia (31%) contributing the bulk of the resource². In India, teak ranks second only to *Eucalyptus* in terms of plantation area (8.67%)³, with an annual plantation rate of around 50,000 ha. Consequently, there is a great demand for quality planting materials across the country.

The most ideal source of quality seeds for the purpose of raising plantations are clonal seed orchards (CSOs). However, the seed yield among teak CSOs has been low in India⁴ and other South Asian countries⁵. As a result, much of the seed demand is met with from seed production areas (SPAs) and sometimes unimproved plantations

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(UIS). High variation in fruit emptiness and erratic germination behaviour of teak have also contributed to the scarcity of good planting material. Fruit emptiness of teak provenances in India is reported to range from 13% to 86% (ref. 6) and germination percentage ranges between 0.50 and 49.0 (ref. 7). Consequently, the average seed consumption for planting per ha in India varies with locality⁸.

The reasons for high population variation in fruit emptiness and seed germination (seed quality) of teak remain unclear; although strong association between these two characters has been established⁹. According to Banik¹⁰, fruit emptiness will always provide misleading data about the seed germination percentage, since teak fruit is sown as a single seed. Therefore, the factors controlling fruit emptiness in teak are implicative of the germination behaviour as well, although seldom emphasized. Since most of the population-based studies have given little importance to the factors responsible for fruit emptiness^{11,12}, those investigating the factors have discussed little about the population variation^{13,14}. Levels of genetic diversity in a population have been shown to influence seed fitness traits in herbaceous species like *Gentianella germanica*¹⁵ and *Dictamnus albu*¹⁶. In these species, seed traits like seed number and seed mass were higher in populations that were genetically more variable. However, association between the seed quality traits and genetic diversity has not been reported in any forest species and especially from the viewpoint of poor seed quality in teak.

In this study, variation in fruit emptiness and seed germination characters among populations of teak with different levels of improvement was studied. We also estimated the genetic diversity of all parent populations with the help of DNA-based inter simple sequence repeat (ISSR) technique. The objective of this study was to understand the association between levels of genetic diversity and the level of variation in fruit quality in different populations of teak.

Three sites, constituting the north, central and south region within Karnataka along the Western Ghats range, were chosen from the teak-growing areas. Within each site, one UIS and one SPA were considered for the study

(Table 1). Additionally in the northern region, one CSO was also included for the study. This orchard was established by the Karnataka State Forest Department using vegetative propagules of 49 plus trees representing all the three teak zones of the state. From each of the three UISs and three SPAs, 30 mother trees separated by a minimum distance of 70 m were randomly selected. Leaf samples for genetic analysis and fruits (approximately 500) from all mother trees were collected. From the CSO, leaves and fruits were collected from each clone. The fruits collected in each population were subsequently bulked making sure that each individual mother tree or clone is represented in bulk collections.

This following part of the study was conducted at the nursery of Institute of Wood Science and Technology, Bangalore. From each population, 750 fruits were sampled from the bulk collections, out of which 250 were used in estimating fruit emptiness, whereas the rest were used for germination studies. In each population, five replicates of 50 fruits each were sampled for estimating fruit emptiness. Every fruit was cracked and the number of empty locules was counted and expressed as proportion emptiness in relation to total number of locules (subsequently referred to as fruit emptiness). The mean of each replication was computed thereafter. Fruits used for germination were subjected to pre-germination treatment with cow dung slurry prior to sowing¹⁷. Five replications of 100 pre-treated fruits from each population were sown in randomized block design at the nursery. The number of seeds germinated was monitored everyday for 40 days after 10 days of sowing. At the end of the observation period the percentage of seeds that germinated was calculated and transformed for further analysis. Fruit emptiness and germination data were then subjected to ANOVA.

The following part of the study was carried out at the Conservation Genetics Laboratory of the School of Ecology and Conservation (SEC), GKVK Campus, University of Agricultural Sciences, Bangalore. The leaf samples collected from each population were subjected to DNA extraction following a CTAB (cetyl trimethylammonium bromide) extraction method¹⁸. DNA was quantified based

Table 1. Details of sites for collection of leaves and fruits

Region	Place	Population	Range	Division	Latitude (°N)	Longitude (°E)	Altitude (m)
Northern	Verrampali	UIS	Virnoli	Haliyal	15°27'	74°79'	599
	Verrampali	SPA	Virnoli	Haliyal	15°27'	74°79'	599
	Karka	CSO	Dandeli	Haliyal	15°28'	74°56'	573
Central	Kunehusur	UIS	Chordi	Sagar	14°17'	75°39'	711
	Kunehusur	SPA	Chordi	Sagar	14°17'	75°39'	711
Southern	Devamachi	UIS	Titimathi	Hunsur	12°30'	76°03'	933
	Devamachi	SPA	Titimathi	Hunsur	12°30'	76°03'	933

UIS, Unimproved population; SPA, Seed production areas; CSO, Clonal seed orchard.

Table 2. Mean fruit emptiness percentage, germination percentage and Jaccard's dissimilarity index for seven populations of teak in Karnataka

	Proportion emptiness	Germination (%)	Dissimilarity index
UIS (North)	0.73 ± 0.04	14.43 ± 1.47 (6.25)	0.235 ± 0.051
SPA (North)	0.78 ± 0.04	21.69 ± 2.83 (13.75)	0.253 ± 0.061
CSO (North)	0.82 ± 0.02	17.07 ± 4.59 (9.00)	0.245 ± 0.055
UIS (Central)	0.78 ± 0.13	34.61 ± 3.14 (31.75)	0.238 ± 0.060
SPA (Central)	0.70 ± 0.03	32.17 ± 1.86 (28.00)	0.241 ± 0.052
UIS (South)	0.69 ± 0.06	50.65 ± 2.31 (56.25)	0.210 ± 0.048
SPA (South)	0.67 ± 0.02	60.31 ± 7.24 (67.75)	0.206 ± 0.051
<i>F-value</i>	5.285	81.521	50.30
<i>P-value</i>	0.0001	0.0001	0.0001
CD	0.156	11.35	**

**All pair-wise *t*-test comparisons between populations are significant at $P = 0.05$, except between UIS (South) and SPA (South); UIS (Central) and SPA (Central); SPA (Central) and CSO (North); SPA (Central) and UIS (North) and UIS (Central) and UIS (North).

on the spectrophotometric measurement of UV absorbance at optical density (OD) 260 nm and was diluted to working concentration (20 ng). Genetic analysis was carried out employing DNA-based ISSR molecular markers. Polymerase chain reaction (PCR) amplification was carried out in a 15 µl reaction mixture containing template DNA (20 ng), primer (0.3 µM), *Taq* polymerase (0.5 units), 10× assay buffer and dNTPs (1 mM). A total of 20 UBC-ISSR primers were screened, of which 10 primers that gave consistent results and a higher number of polymorphic bands were selected. Amplified PCR products were separated on a 1.5% agarose gel stained with ethidium bromide (0.5 µg/ml). The gel was visualized under a UV light and captured using Herolab Gel Documentation Unit.

Binary coding was used to score the gels¹⁹. Presence of a PCR-amplified product was scored as 1 and its absence as 0. Based on the absence or presence of amplified products, Jaccard's dissimilarity index was computed by comparing all the possible pairs of individuals (nC_2 pairs) for a given population using the presence or absence data of ISSR markers. The mean dissimilarity index across the populations was compared using Student's *t*-test and one-way ANOVA. Shannon index of diversity for pooled populations was computed using POPGENE ver. 2 software.

ANOVA showed significant variations among the populations for fruit emptiness (*F*-test, $P < 0.001$; Table 2) as well as for percentage of seed germination (*F*-test, $P < 0.001$; Table 2). Fruit quality in terms of fruit emptiness and seed germination was found to be superior in populations (UIS and SPA) from the southern region of the state by having lowest fruit emptiness (0.69 ± 0.06 and 0.67 ± 0.02 for UIS and SPA respectively) and highest germination percentage (50.65 ± 2.31 and 60.31 ± 7.24 for UIS and SPA respectively). Provenance variation for fruit quality of teak is well documented and evidence indicates that fruits from moist regions have better quality in terms of fruit emptiness^{12,20} and seed germination⁹. According to Champion and Seth's²¹ classification of for-

est types in India, the southern teak population of Karnataka falls under subtype 3B/C1a (very moist teak-bearing forest), whereas the northern population under 3B/C1b (moist teak-bearing forest).

Several studies on teak have revealed a close association between seed germination and fruit characteristics. For example, fruit characters like fruit weight²², and size and percentage of two-seeded drupes²³ were positively associated with germination parameters, whereas characters like percentage emptiness, kernel number per fruit^{9,10} and mesocarp and drupe/shell had a negative effect on seed germination⁷. In the present study, it has been observed that higher levels of fruit emptiness resulted in lower levels of germination although the association was not statistically significant ($r = -0.733$; NS at $P = 0.05$; $df = 5$). Such negative association of low germination percentage with fruit emptiness in teak has also been reported⁹.

The 10 ISSR primers adopted to screen the teak genotypes generated 75 loci ranging between 5 and 9 loci. The Shannon gene diversity index after pooling three unimproved stands was 0.375 ± 0.275 and pooled value for three SPA population was 0.359 ± 0.289. These values are considered to be higher than those reported in outcrossing woody perennials studied through isozyme and RAPD markers²⁴. The above diversity estimates were also found to be higher than values reported for teak populations screened in India and elsewhere^{25,26}. One reason for obtaining higher values in this study may be due to the larger sample size used. Nicodemus *et al.*²⁶ while estimating the genetic variation of 10 teak populations in peninsular India, sampled only nine individuals per populations and reported an average gene diversity index of 0.300. Another reason for the higher diversity could be the location of the source of samples. For this study, samples were collected from the central region of the Western Ghats, and according to an earlier report teak populations growing in the Western Ghats range are genetically more diverse than those from Central India²⁶.

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Significant variation for Jaccard's dissimilarity was observed among the populations (F -test, $P < 0.001$; Table 2). Genetic diversity based on Jaccard's dissimilarity was highest for SPA from the northern region (0.253 ± 0.061) and lowest for SPA from the southern region (0.206 ± 0.051). This is also evident from the frequency distribution of Jaccard's dissimilarity indices for the seven populations (Figure 1). In SPA from the northern region, only 20.83% of pairs of individuals had dissimilarity indices less than 0.20, whereas it was 52.12% in case of SPA from the southern region. These results are in contrary to an earlier study on teak which showed that genetic diversity decreased with increasing latitude²⁶.

Association of low genetic diversity with the southern region is also not expected as the area is characterized by moist climatic and suitable soil conditions considered ideal for teak growth compared to the northern region of the state, where climatic conditions are harsh. Eviatar *et al.*²⁷ have reported that climatic stress appears to be a major factor for determining patterns of genetic diversity.

The CSO population registered a high diversity value (0.245 ± 0.055). The percentage of bands shared by this population was also higher (97.34; Figure 2) than all populations, except for SPA from the northern region (98.67). This is expected since clones from various

regions of the state have been assembled in the orchard, thereby generating high genetic diversity within the orchard. However, a loci amplified by primer UBC 807 present in all populations was not detected in the orchard population. Maintaining a high level of genetic diversity at the early stages of breeding is essential if opportunities for selection in the future are to be maximized and inbreeding minimized²⁸. However, it remains to be seen whether this variation is adequately transferred to the ensuing progenies.

From the present study, high genetic diversity of the populations was seen to be positively associated with fruit emptiness ($r = 0.782$ at $P < 0.05$; $df = 5$; Figure 3) and negatively associated with seed germination ($r = -0.867$ at $P < 0.05$; $df = 5$; Figure 4), indicating that high genetic diversity within a population may be detrimental to quality seed production. This is in contrary to a wealth of evidence which points out that higher level of genetic diversity is positively associated with reproductive fitness²⁹. In teak, it is suspected that high genetic diversity of a population may be associated with the high flowering asynchrony within it, which impedes random mating. Flowering asynchrony within teak CSO has been extensively documented^{30,31}. Gunaga and Vasudeva³² have reported that clones from a teak CSO in the southern

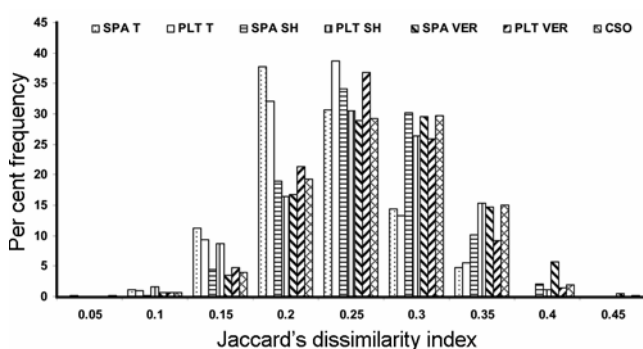


Figure 1. Percentage of frequency distribution of Jaccard's dissimilarity index for seven populations of teak in Karnataka.

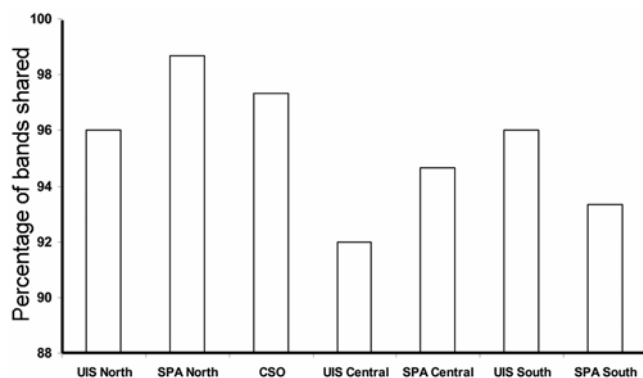


Figure 2. Percentage of bands shared among seven populations of teak in Karnataka.

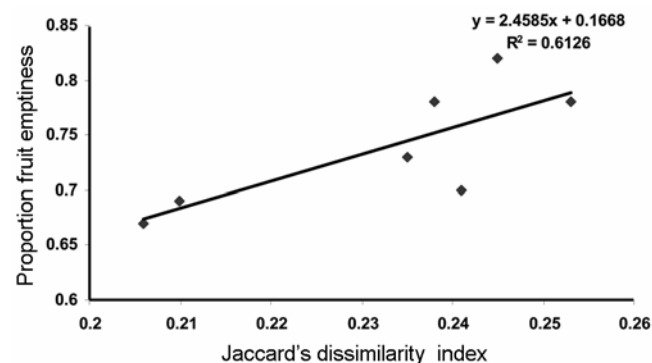


Figure 3. Correlation between Jaccard's index of parent population with proportion emptiness (significant at $P = 0.05$; $df = 5$).

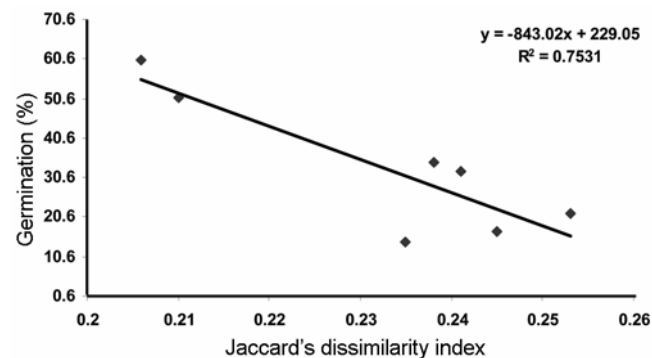


Figure 4. Correlation between Jaccard's index of parent population with seed germination (significant at $P = 0.05$; $df = 5$).

region of Karnataka had lower variation in flowering period compared to those from the northern region. It can be therefore assumed that the low genetic diversity in our southern populations may have facilitated uniform flowering of the individuals in the population. As a result, higher out-crossing rates may have been promoted by encouraging more pollinators and also reducing the chance of close-related mating. Studies have shown that low seed quality in teak is due to the high abortion rate of self-pollinated flowers^{13,33}.

It is seen that maintaining high levels of genetic diversity in teak populations may not be warranted. High genetic dissimilarity among individuals in a population could potentially lead to high flowering asynchrony, which could ultimately affect the reproductive success of the population. However, maintaining a broad genetic base is equally essential to ensure evolutionary flexibility of the population³⁴. Therefore, a compromise between these two components may be a prerequisite for ensuring high reproductive success of populations and maintaining an ideal genetic base. This becomes essentially important in CSOs wherein clones from different geographic regions are assembled. Vasudeva *et al.*³⁵ reported that assembling clones from diverse origin in a CSO may be disadvantageous to seed production, since flowering is severely affected as clones tend to retain the reproductive phenology of the parent location, resulting in asynchrony in flowering. Perhaps the practice of assembling teak clones from diverse origin in a CSO could well be one of the factors responsible for low quality of seed obtained from orchard populations.

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Comparative morphometric, physiological and chemical studies of wild and cultivated plant types of *Withania somnifera* (Solanaceae)

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Ashwagandha (*Withania somnifera* Dunal.) is an important medicinal plant cultivated in about 4000 ha in India. It is mainly distributed in the northwestern region of Madhya Pradesh and also in limited areas of different states. However, wild collection of the species continues to be a source of raw drug in Ayurvedic preparations. In the present communication, results of a comparative study of the wild type distributed locally in Anand, Gujarat and the superior variety, Jawahar Ashwagandha (JA-20), are presented. Results revealed conspicuous differences between the cultivated and wild-type plants in most of the characters studied. One of the major differences between cultivated and wild-type plants is that the former are annual, whereas the latter are perennial. Photosynthetic rate was higher in the wild type, which was reflected in its higher biomass production. Another distinguishing character was the floral structure which favours self-pollination in the cultivated plants because of short stigma covered with anther lobes, which is in contrast to the wild type having long, projected stigma inviting cross-pollination. The cultivated plants were in full bloom during December–February; however, in the wild type flowering was a continuous process throughout its lifespan. Flow cytometer study revealed the same ploidy level for both the plant types. However, chemical profile showed variation between the two plant types, even though targeted chemical constituents tested in the study were common to both. However, HPLC quantification of these constituents showed superiority of the wild type compared to JA-20.

Keywords: Chemical profile, floral structure, flow cytometer, *Withania somnifera*.

ASHWAGANDHA (*Withania somnifera* Dunal.) is an important medicinal plant cultivated in the northwestern region of Madhya Pradesh and also in different parts of India in about 4000 ha (ref. 1). It is also found growing wild throughout India. Even now, wild collection of the species continues to be a source of raw drug in Ayurvedic preparations. Ashwagandha roots and occasionally its leaf and seeds are used in Ayurveda and Unani medicines. Roots

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