Genetic variation for floral traits among teak (*Tectona grandis* Linn. f.) clones: Implications to seed orchard fertility

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Variations for floral traits among constituent clones can potentially influence the pollination success in a clonal seed orchard. A study was undertaken in a nineteen-year-old Clonal Seed Orchard of teak (*Tectona grandis* Linn. f.) to assess the nature/extent of variation for floral traits among clones derived from diverse provenances. Inter-clonal variation was significant for all the eight floral traits, while within the clone variation was negligible. Pollen grains per stigma varied to a large extent (CV = 42.24%) and the remaining traits exhibited very low levels of variation. The association of length and diameter of stigma with number of pollen grains captured per stigma was positive and significant (r = 0.64 and 0.76 respectively), however, the association of anther filament length with length of stigma was negative (r = – 0.46), suggesting a possibility of conflict between male and female function. Teak clones with larger corolla tube diameter showed a higher fruit set as shown by their positive correlation (r = 0.55). The broad sense heritabilities estimated based on clonal means were higher, suggesting a strong genetic control, hence selection could yield beneficial results. The implications of the results to genetic improvement of teak are discussed in the light of poor fertility among teak clones in seed orchards.

TEAK (*Tectona grandis*. Linn. f.) is an important plantation species in the Indian subcontinent and South East Asia. Presently, about 1.5 million hectares (m ha) of teak plantations exist in India and around 50,000 ha are raised annually[1]. It is one of the most favoured timber-yielding species all over the world because of its versatile range of uses[2]. The species is known to have a great genetic variability in India, with its natural distribution spreading over 8.9 m ha of forests ranging from very dry to very moist conditions[3]. In fact, the Indian subcontinent is considered as the centre of diversity for teak because of the huge genetic variation for economically important traits such as bole form, timber quality, biochemical traits, etc.[4]. Genetic improvement of teak in India started in the year 1954. It has focused on identifying phenotypically superior trees from diverse regions and deploying them in seed orchards[5]. Seed orchards form an important link between on-going tree improvement programme and commercial planting, since seed orchards are intended to supply genetically improved seeds in sufficient quantity[6]. However, fruit production among teak seed orchards has been low[7].

It is well known that the levels of variation in reproductive traits of its constituent genotypes influence fruit production in a seed orchard. Hence understanding variation in floral features is fundamental to the successful operation of any seed orchard. Despite extensive planting programmes and the importance of increasing fruit production in seed orchards, little is known about the clonal variation for reproductive biology of teak[8]. To use floral traits as a criterion while selecting the clones, it is essential to understand their genetic control. Hence, in order to achieve better genetic gain, it is imperative to assess variation and genetic control of the floral and fecundity traits in an orchard[9]. However, in general, reproductive traits have been consistently ignored while selecting plus trees of teak as well as while upgrading the existing seed orchards[10]. Virtually, there are no reports on the extent of genetic control of floral features in teak.

Further, use of morphological markers is an essential step in the early period of tree breeding, when molecular markers are not readily available. Floral traits are believed to be the most conserved traits and affected least by the environment. Hence, any detectable variation in these traits may be attributable to genetic causes and can be effectively used as markers to characterize different clones. This study reports on the variation of floral traits and the extent of genetic control of floral traits among eight teak clones of Karnataka. The implications of these variations for genetic improvement are discussed.

The present study was carried out in a 19-year-old clonal seed orchard (CSO) established at Manchikere, Yellapura Forest Division, South India (74°50′N lat.; 14°52′E long. and 600 m asl). This orchard consists of 24 teak clones, which were derived from phenotypically superior trees located at various provenances of Karnataka[11]. To study the inter and intra clonal variations of various floral traits, three ramets each from eight clones derived from different sources, i.e. Barchi, Gundvamoli, Virnoli, Arasake, Thithimatti, Kulagi and Kakanakote forest ranges were considered (Table 1). About 20 randomly collected, completely bloomed, fresh flowers from each ramet were sampled. Flower diameter, corolla tube diameter, style length, anther filament length and flower stalk length were recorded using a projection microscope (under 4× magnification). In the syngamous flowers of teak, petals join at the base to form a cup. The diameter of the corolla base was measured by gently pulling out the cup-shaped petals and considered as 'corolla tube diameter'. Total stigma length, stigmatic diameter, and number of pollen grains deposited per stigma were assessed

under a compound microscope. The number of pollen grains per stigma was counted by considering one-day-old flowers collected after a bright, sunny day. Teak possesses bifid stigma. The length of the two arms of the bifid stigma and diameter of the stigmatic surface were measured and the average computed for each flower. Two randomly picked anther filaments per flower were considered for measuring the length. Clonal mean values of various floral traits were based on observations on at least 60 flowers. Number of fruits developing per inflorescence was counted from five randomly selected inflorescences from each ramet to compute per cent fruit set during fruit maturation period.

In order to estimate the genetic parameters, data were subjected to ANOVA to decompose the variability into genetic and environmental components. While establishing a clonal seed orchard, each ramet was prepared through grafting of vegetative buds derived from plus trees (or ortet) onto a rootstock. The ramets of a clone are genetically similar. Hence variation between ramets within a clone was interpreted as 'environmental variation'; mean sum of squares due to 'between clone source' was used to compute 'genetic variation'. A simple model (Table 2) was adopted[12].

Broad sense heritability was calculated based on clonal means as (Table 2):

$$H^2 = \sigma_c^2 / (\sigma_c^2 + \sigma^2 / r).$$

Genotypic coefficient of variation, phenotypic coefficient of variation and genetic advance were computed following Burton and Devane[13]. While selecting the plus trees of teak, the comparison tree method has been adopted wherein one plus tree out of six comparison trees is selected. Here selection intensity has been approximated to be at 20%, for which the selection differential[14] was 1.40. Genetic gain was computed following Johnson et al.[15].

ANOVA suggested that all the floral traits studied showed significant inter-clonal differences, while the intra-clonal variations were negligible indicating a strong genetic basis for these variations (Table 3). Corolla tube diameter, flower diameter, stigma length and stigmatic diameter exhibited low variation among teak clones (CV = 2.05, 2.89, 4.19 and 5.44% respectively). However, pollen grains deposited per stigma varied to a large extent (CV = 42.24%), as it is also influenced by several external factors such as pollinator behaviour, weather conditions of the day, etc. The number of pollen grains per stigma was very low, indicating a poor pollination success. Similar results among teak clones have also been reported from Indonesia, where the number of pollen grains per stigma varied from one to three[16]. The flowers borne by clone 19 were larger (10.03 mm petal diameter)

Table 1. Pass-port data of teak clones considered in the study

Clone number	Clone ID	Forest range	Latitude N	Longitude E	Altitude m asl
2	My Ha D2	Barchi	15°17′	74°38′	573
7	My Ha V3	Gundvamoli	15°06′	74°36′	570
9	My Ha V5	Virnoli	15°06′	74°36′	570
13	My S A1	Arasake	13°53′	74°28′	571
19	My Hu T3	Thithimatti	12°13′	76°00′	850
24	My Hu T8	Thithimatti	12°13′	76°00′	850
32	My Ha K1	Kulagi	15°11′	74°41′	500
37	My M K3	Kakanakote	11°55′	76°11′	690

Table 2. Model adapted for the estimation of environmental and genetic variation

Source	d.f.	Expected mean square
Within clones (between ramets)	$(r - 1)$	
Between clones	$(c - 1)$	$\sigma^2 + r(\sigma_c^2)$
Error	$(r - 1)(c - 1)$	σ^2

where r is the number of ramets, c the number of clones, σ^2 mean sum of squares due to error (environmental variance) and σ_c^2 mean sum of squares due to clones (interpreted as genetic variance).

Table 3. Comparison of floral traits among teak clones (mean ± SD)

Clone ID	Flower stalk length (mm)	Flower diameter (mm)	Corolla tube diameter (mm)	Style length (mm)	Total stigma length (mm)	Stigmatic diameter (mm)	Anther filament length (mm)	Pollen grains/stigma	Fruit set (%)
My Ha D2	1.87 ± 0.10	7.00 ± 0.06	1.36 ± 0.02	2.80 ± 0.27	0.635 ± 0.03	0.293 ± 0.02	2.63 ± 0.20	0.200 ± 0.05	1.199 ± 0.08
My Ha V3	4.81 ± 0.20	8.23 ± 0.23	1.53 ± 0.02	3.62 ± 0.32	0.670 ± 0.01	0.274 ± 0.01	3.61 ± 0.23	0.067 ± 0.03	0.946 ± 0.02
My Ha V5	1.15 ± 0.10	8.87 ± 0.15	1.35 ± 0.01	4.33 ± 0.25	0.586 ± 0.02	0.276 ± 0.01	3.23 ± 0.23	0.067 ± 0.03	1.145 ± 0.08
My S A1	2.20 ± 0.18	8.40 ± 0.15	1.56 ± 0.03	4.30 ± 0.27	0.240 ± 0.03	0.237 ± 0.02	3.84 ± 0.17	0.016 ± 0.03	1.187 ± 0.07
My Hu T3	1.66 ± 0.17	10.03 ± 0.27	1.37 ± 0.04	5.33 ± 0.24	0.649 ± 0.02	0.416 ± 0.02	2.99 ± 0.24	0.230 ± 0.08	0.934 ± 0.07
My Hu T8	3.64 ± 0.21	8.37 ± 0.38	1.47 ± 0.03	3.46 ± 0.19	0.613 ± 0.02	0.278 ± 0.03	3.13 ± 0.28	0.133 ± 0.03	1.245 ± 0.05
My Ha K1	3.01 ± 0.23	7.23 ± 0.23	1.36 ± 0.01	3.08 ± 0.17	0.594 ± 0.02	0.267 ± 0.01	2.65 ± 0.19	0.083 ± 0.03	1.529 ± 0.37
My M K3	1.37 ± 0.11	7.67 ± 0.15	1.05 ± 0.04	2.70 ± 0.22	0.436 ± 0.02	0.234 ± 0.01	2.99 ± 0.30	0.050 ± 0.05	0.429 ± 0.13
Grand mean	2.47	8.23	1.38	3.71	0.55	0.28	3.14	0.104	1.078
CV (%)	7.06	2.89	2.05	7.37	4.19	5.44	7.71	42.24	14.92
CD at 0.05 level	0.077	0.418	0.055	0.479	0.055	0.055	0.424	0.077	0.0928

CV, Coefficient of variation; CD, Critical difference; SD, Standard deviation.

and possessed longer style length (5.33 mm) and stigma length (0.649 mm). Several workers have documented variations of floral features in teak. For instance, Palupi and Owens[17] have shown that style length ranges from 4.0 to 6.0 mm among the teak clones of Java. Nagarajan et al.[8] have shown that petal diameter among teak clones of Tamil Nadu and Maharashtra varies from 7.44 to 8.06 mm. As evident from the results, teak clones of Karnataka have larger flower diameter than those from Java, Tamil Nadu and Maharashtra. These variations may be mainly due to genotypic origin.

Among species where reproductive success is limited by the pollen, any floral trait that contributes to pollination success should be selected[18]. Since teak is a self-incompatible, out-crossed and insect-pollinated species, larger flowers are important for effective pollination[19]. A larger corolla diameter is likely to increase the floral display, improving the chances of such flowers being pollinated. In fact, corolla tube diameter and fruit set per cent among teak clones were strongly positively associated ($r = 0.55$, $P < 0.05$ at 22 d.f.). Since style and stigma are the pollen-capturing parts, these floral traits are also important for effective pollination. The association of stigma length and stigma diameter with the number of pollen grains captured was positive and significant ($r = 0.64$ and 0.76 respectively, $P < 0.05$; Figure 1). However, the association between style length and number of pollen grains captured per stigma was weak but positive ($r = 0.19$, non-significant). Clone number 19, which had a larger stigma, recorded the highest average number of pollen grains per stigma (Table 3). In contrast, clone 37 that was characterized by smaller floral features had the lowest average pollen grains captured per stigma. This suggests that floral traits are important in determining the level of pollination success and they need to be included as a criterion while selecting a plus tree in teak, since insufficient insect pollinators and their effectiveness appear to be major causes for limited fruit set in teak[7].

Flower features are developmentally most related traits and exhibit allometric relations. Generally, relative sizes of androecium and gynoecium in bisexual species are positively related. Although the anther filament length and style length were weakly positively related ($r = 0.35$, $P = 0.06$) in teak, the association of length of anther filament with stigma length was negative ($r = -0.46$, $P < 0.05$; Figure 2). Consequently, clones with longer anther filaments may be efficient in pollen donation (male function) but tend to constrain the reception of pollen grains (female function). This was corroborated by the significant negative association between anther filament length and number of pollen grains captured per stigma ($r = -0.536$, $P < 0.05$). Such conflicts between male and female functions in a bisexual flower may lead to male and female specialist individuals. These issues are important in calculating fertility variations of constituent clones in a seed orchard.

The final fruit set in teak is influenced by several factors such as pollinator availability, weather conditions, etc. Recently, Gunaga and Vasudeva[9] have reported that pollination success of teak clones was strongly influenced by the coincidence of peak flowering period with rainy season. They have also shown that pollination success could be severely affected by asynchronous flowering among clones and by insistent rainfall resulting in poor fruit set among these clones. Hence it is essential to match the flowering phenology of clones and local rainfall pattern before deploying clones in the seed orchards. Hanumantha and Vasudeva[20] have shown that teak clones differ significantly for pollen viability and vigour. Clones with higher pollen viability and vigour can potentially sire disproportionately higher number of ovules than those with lower levels of pollen viability and vigour under open pollination. This may result in skewing of the parental balance among progeny of seed orchard[21]. In order to encourage exchange of gametes among clones, beehives could be used in the seed orchard[8].

Genetic parameters are important to understand the strength of genetic control of specific traits. Heritability estimates along with expected genetic gains are more useful for selecting best genotypes[6]. Heritability estimates
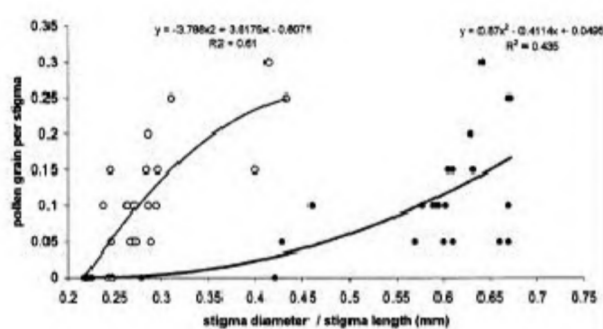

Figure 1. Association of stigma diameter (open circles) and stigma length (closed circles) with number of pollen grains captured per stigma in a teak clonal seed orchard.
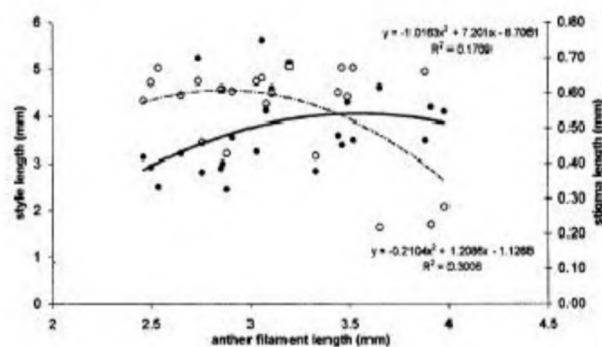

Figure 2. Association of anther filament length with style length (closed circles) and stigma length (open circles) in a teak clonal seed orchard.

Table 4. Estimation of genetic parameters for floral traits among teak clones

Parameter	Flower stalk length (mm)	Flower diameter (mm)	Corolla tube diameter (mm)	Style length (mm)	Total stigma length (mm)	Stigmatic diameter (mm)	Anther filament length (mm)
σ^2_p	1.595	0.91	0.0253	0.842	0.021	0.0093	0.182
σ^2_c	1.585	0.891	0.025	0.817	0.02	0.009	0.162
H^2	0.99	0.97	0.98	0.97	0.95	0.96	0.89
PCV	51.23	11.59	11.53	24.72	26.21	33.96	13.59
GCV	51.07	11.47	11.46	24.35	25.57	33.40	12.82
GA	1.75	1.29	0.22	1.25	0.19	0.13	0.53
G gain	70.99	15.67	15.94	33.67	34.35	45.77	16.88

σ^2_p, Phenotypic variance; σ^2_c, Variance between clones, which is interpreted as the total genetic variance; H^2, Heritability–broad sense; GCV, Genotypic coefficient of variation; PCV, Phenotypic coefficient of variation; GA%, Genetic advance; G gain, Genetic gain (expressed as percentage of the general mean).

are useful in making selection of superior phenotypes (plus trees) on the basis of phenotypic performance of quantitative characters. In the present study the variance between clones has been interpreted as the total genetic variation, which has arisen due to the covariance of ramets of the same clone, ignoring the cloning effects. The variance between ramets within clones has been interpreted as a measure of environmental variance. However, if rootstocks used are different, then they can potentially influence the magnitude of variation. Since the rootstocks of these clones are rather uniform and randomly assigned, this error may be minimal.

The variance components and broad-sense heritability estimates for the different traits are presented in Table 4. In all the parameters, phenotypic variance was greater than genotypic variance, suggesting a role of the environment. Heritability estimates on clonal mean basis were higher for flower stalk length (0.99), followed by corolla tube diameter (0.98), and it was least (0.89) in anther filament length. These heritability estimates are on the higher side, since they are based on clonal mean and rootstock effects have not been removed[22]. However, narrow-sense heritability values would be smaller than these. In a progeny test data from a four-year-old trial in Kerala, Nagarajan et al.[23] have reported a heritability of 0.45 to 0.75 on family mean basis for basal area and height. The genetic gain observed among different floral traits suggests that maximum gain could be obtained for floral stalk length (70.99), followed by stigmatic diameter (45.77). These parameters are important in increasing the pollen capture and hence reproductive success. The least gain is expected for corolla tube diameter and flower diameter, suggesting that they are highly conserved (Table 4).

There are no comparable datasets on teak floral traits in the available literature. However, Bagachi[14] estimated the genetic gain for stem form, vigour traits such as DBH, and clear bole under different selection differentials in teak. The range of genetic gain obtained in that study was low and varied from 0.88 to 16.95%. It was concluded that plus trees of Shimoga provenance were best for further selective breeding. The high levels of heritability estimated in this study suggest that observations made in the seed orchard may provide a useful tool for selecting better clones for establishing second-generation seed orchards. Hence it appears that genetic parameters estimated in this study can be extrapolated with some confidence. Recently, Chawhaan et al.[24] have also reported moderately higher heritability values in teak for traits such as fruit size, fruit weight and seed weight, and have opined that reproductive traits may be under additive gene action.

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Control of collar rot in mint (*Mentha* spp.) caused by *Sclerotium rolfsii* using biological means

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Eight isolates of different species of *Trichoderma* and two isolates of *Gliocladium virens* were tested *in vitro* for their antagonistic activity against *Sclerotium rolfsii*, the cause of collar rot in mint. The most effective isolates of *T. harzianum* and *T. virens* were selected for disease-control studies in pots under greenhouse conditions. Inoculation of *Mentha arvensis*, *M. citrata*, *M. piperita* and *M. spicata* with the selected isolates of *T. harzianum* resulted in disease control ranging from 66.67 to 100%. Reduction in disease was accompanied with significant increase in herb and oil yield.

MENTHA (family Labiatae) is an important essential-oil-yielding herb grown throughout the world. Commercial cultivation is done to obtain its oil, which has different chemical constituents of economic importance, viz. menthol, menthone, methyl acetate, terpenes, etc. These constituents are used in medicinal preparation, toothpaste, mouthwash, perfumery, cosmetics and as flavouring agents. The menthol mint crop is extensively cultivated in India and about 70% of the international annual requirement is met from crops raised in the central region of the Indo-Gangetic plains[1]. A survey conducted by Central Institute of Medicinal and Aromatic Plants, Field Station (CIMAP, FS), Pantnagar, India revealed that the crop is severely affected by collar rot and wilt disease. The disease is caused by *Sclerotium rolfsii* Sacc which causes considerable damage to the crop. Disease intensity in the field ranged from 5 to 20%. Though collar rot and wilt disease of *Mentha* were reported way back in 1933 by Goto[2] from Japan, no further studies were undertaken on this disease. The first attempt to control the disease was by Pandotra and Ganguly[3] using chemical means. *S. rolfsii* is a pathogen of several crops and is not easy to control by conventional means[4,5].

In the past few years, management of diseases using biological antagonists has been increasing continuously. This is influenced by the idea that they may be potential alternatives to the use of chemicals for managing the plant diseases caused by soil-borne pathogens[6–8]. The present investigation is an attempt to control the collar rot disease of mints caused by *S. rolfsii* using fungal antagonists.

The collar rot pathogen, *S. rolfsii* has a wide host range. The collar rot of menthol mint *(Mentha arvensis)* was

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