Clonal variation for seed germination in teak (*Tectona grandis* Linn. f.)

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Large variations for per cent seed germination and dormancy release pattern were observed among half-sib families of eight teak clones derived from a 20-year-old clonal seed orchard. Overall germination percentage was on the lower side. Although initial germination was influenced by traits such as fruit diameter and 100 fruit weight, germination at 140 days after sowing was independent of these traits. Interestingly, there was a perfect negative association between age of the ortet (mother tree) from which the clonal material was originally derived and the per cent germination of its progeny supporting 'genetic load accumulation with-age' hypothesis. Hence care must be taken not to include clones of older ortets while establishing future clonal seed orchards.

CLONAL seed orchards are established through vegetative propagation of phenotypically superior trees and managed as isolated plantations to get genetically superior seedlot through inter-mating of these superior types. They form a crucial link between commercial plantations and tree improvement programmes, as they are intended to supply quality seeds in sufficient numbers. However, in India, fruit set and germination of seeds derived from teak seed orchards are found to be low[1–3]. Even in the international scenario, the problem of poor fruit set and low germination of seed orchard-derived teak seeds has been observed[1,4]. Hence it is imperative to understand the variation for seed germination among clones in order to have a holistic breeding strategy for future. An idea of the available variation in seed germination behaviour and its relation with other seed characteristics of a species will be helpful to select the best available source of seeds[5,6]. Further, such data are also needed to genetically upgrade the orchards by selective culling of inferior types. With this background the present investigation was carried out with seeds (botanically fruits) of eight teak clones collected from a 20-year-old Clonal Seed Orchard (CSO) established at Manchikere, Yellapura forest division of Karnataka, South India, with an objective to assess the clonal variation for germination and dormancy release pattern.

The present teak clonal seed orchard was established during 1980 and is well-isolated from natural/artificial stands of teak trees by over five kilometers. It is being managed by the Karnataka Forest Department adopting standard orchard techniques to encourage higher flowering and fruiting. Germination study was carried out on eight clones by selecting three ramets each per clone (treated as 'replications'). The details of the clones considered and their origin are shown in Table 1. In this study, seeds from different ramets were subjected to pre-germination treatment with cow dung slurry[7] and due care was taken to keep the identity of each ramet intact during the pre-treatment period. At the end of the treatment, mesocarp (outer cottony layer) was removed by thrashing. The seeds were then soaked in water overnight before sowing in a nursery bed at the College of Forestry, Sirsi. Such pre-treated seeds were sown separately on standard raised beds, at depths equivalent to the smallest diameter of the seeds. A minimum of 500 seeds were used per ramet.

Daily germination counts were recorded for a period of 30 days from the start of germination trial and then onwards observations were taken once in a month up to six months after sowing. The germination data were expressed as the percentage of seeds that had germinated at the end of 21 days after sowing (DAS) as well as at 140 DAS; further, peak value (PV) and germination rate were also computed and analysed[8].

Analysis of variance (ANOVA) showed that teak clones differed significantly with respect to percentage germination at 21 DAS as well as at 140 DAS. The clonal superiority with respect to germination at 21 DAS in the order of merit is clone 37, clone 9, clone 24, clone 7, clone 19, clone 13, clone 32 and clone 2 while, the superiority at 140 DAS is clone 13, clone 9, clone 7, clone 19, clone 37, clone 24, clone 32 and clone 2. Germination parameters estimated at 21 DAS such as germination rate and peak value were not significantly influenced by clonal identity (Table 2).

Overall germination percentage was on the lower side. Interestingly, several authors have also reported poor germination of seeds from clonal seed orchards compared to that from natural stands. Prasad and Jalil[9] observed that germination of seeds from orchards varied from 4.2 to 37.8% and seeds of natural stands showed higher germination, which varied from 13.93 to 54.52%. Indira and Basha[2] have also reported that per cent germination of

Table 1. Passport data of teak clones considered for the investigation

Clone number	Clone ID	Origin of clone	
		Forest range	Forest division
24	MyHuT8	Thithimathi	Virajpet
19	MyHuT3	Thithimathi	Virajpet
37	MyMK3	Kakanakote	Mysore
13	MySA1	Arasake	Shimoga
32	MyHaK1	Kulagi	Haliyala
7	MyHaV3	Gundvamoli	Haliyala
9	MyHaV5	Virnoli	Haliyala
2	MyHaD2	Barchi	Haliyala

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Table 2. Germination behaviour (mean ± SD) of different teak clones in nursery

Clone ID	Germination percentage at 21 DAS[$]	Peak value	Germination rate	Germination percentage at 140 DAS
MySA1 (13)	0.90 ± 0.2 (5.41)	0.045 ± 0.040	0.38 ± 0.07	11.09 ± 4.0 (19.27)
MyHaV5 (9)	3.11 ± 1.6 (9.53)	0.182 ± 0.180	1.56 ± 1.20	8.65 ± 4.3 (16.62)
MyHaV3 (7)	1.63 ± 0.8 (7.15)	0.092 ± 0.090	1.00 ± 0.59	8.46 ± 0.5 (16.81)
MyHuT3 (19)	1.49 ± 1.7 (6.30)	0.096 ± 0.090	0.63 ± 1.20	6.81 ± 1.6 (15.01)
MyMK3 (37)	3.43 ± 2.5 (10.04)	0.172 ± 0.170	2.18 ± 2.30	5.13 ± 2.3 (12.76)
MyHuT8 (24)	2.17 ± 1.8 (8.11)	0.129 ± 0.120	1.29 ± 1.30	4.85 ± 2.9 (12.35)
MyHaK1 (32)	0.14 ± 0.2 (1.57)	0.007 ± 0.007	0.05 ± 0.07	0.81 ± 0.02 (4.96)
MyHaD2 (2)	0.06 ± 0.1 (0.85)	0.003 ± 0.003	0.01 ± 0.03	0.39 ± 0.4 (2.76)
F test	*	NS	NS	**
CD	5.27	–	–	5.98
SEM (±)	0.94	0.02	0.27	1.38
CV (%)	47.54	93.62	124.56	24.93

[$]DAS, Days after sowing; numbers in the parentheses indicate the arc sine transformed values.
NS, Non-significant; *, Significant at 0.05 *P* level; **, Significant at 0.01 *P* level.

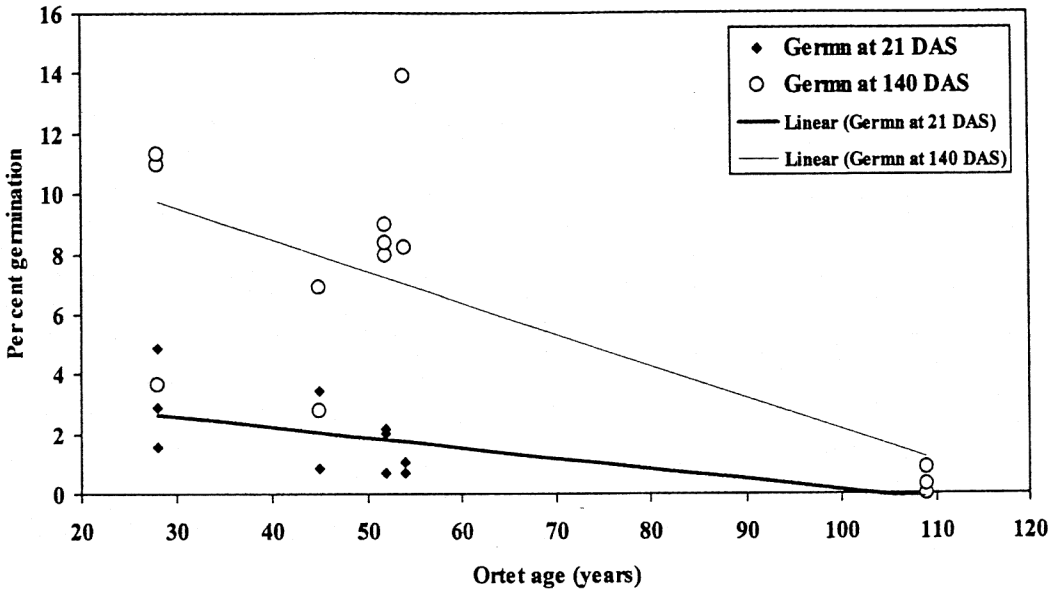

Figure 1. Effect of ortet age on initial and final seed germination of teak clones.

seeds from seed orchards was very poor (0.97 to 16.39%) when sown under nursery conditions. In the present study, only the fully matured and 'about-to-drop' seeds were collected and stored for one year before sowing. Hence low germination may not be due to the prolonged 'after ripening period'. Further, the mesocarp of fruits were physically removed before sowing, hence the influence of a putative water-soluble germination inhibitor present in the mesocarp is precluded[10].

Poor germinability among seeds of CSOs may be related to the higher 'physiological age' of the mother plants from which these clones are derived[11]. Generally 'plus trees' selected for establishing seed orchards are of older age, and hence it is hypothesized that they may inherit higher genetic load[1]. It is argued that among woody plants, there will be accumulation of deleterious mutations with the age of the tree in growing meristems due to copying errors of the DNA. Since trees do not have 'germ-line', these accumulated errors may enter gametes[12,13]. In other words, the genetic load would increase with the age of the 'plus tree', which would pass on to the clonal orchard through vegetative propagation. In order to test whether the age of the ortets (mother tree from which the ramets were derived) has any influence on the seed germination of the respective families, age of the ortet (obtained from the records of the Forest Department) and per cent germination at 21 DAS and at 140 DAS were plotted as shown in the Figure 1. It is interesting that there was a perfect negative association between the two (correlation coefficient $r = -0.902$ and -0.756,

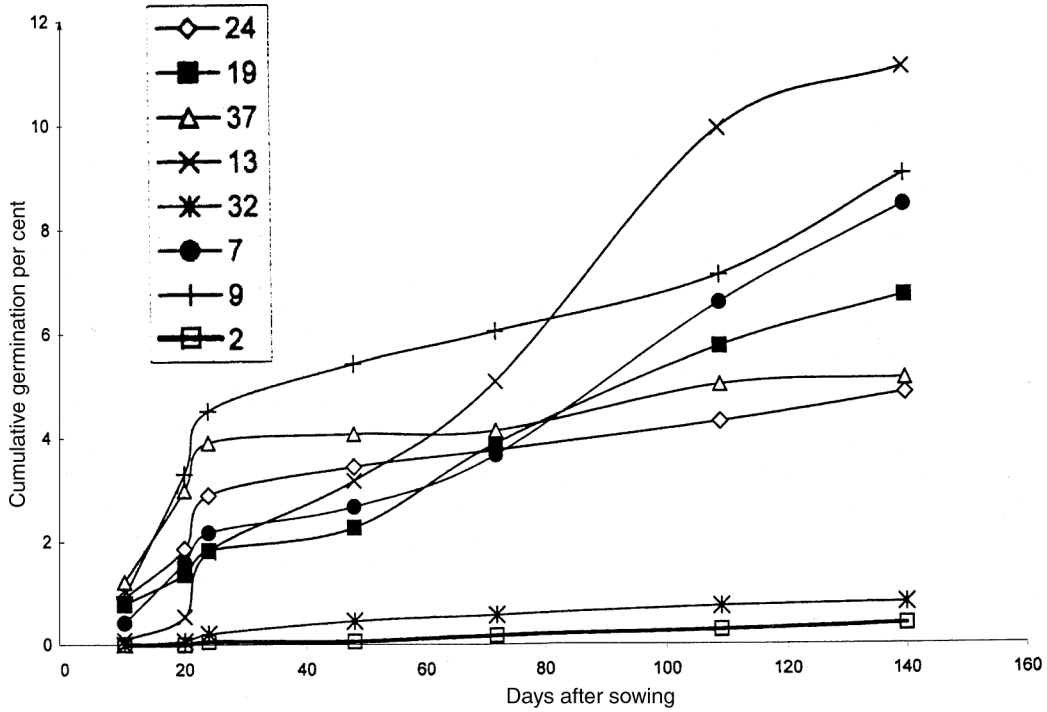

Figure 2. Seed dormancy release pattern of different teak clones. Numbers in the legend refer to clone number in Table 1.

Table 3. Association of germination percentage with a few seed traits[$] among teak clones. Values are Pearson's correlation and Spearman's rank correlation coefficients (shown in parentheses)

Germination per cent at	Fruit diameter	100 Fruit weight	Mesocarp thickness	Fruit density
21 days after sowing	0.847** (0.833**)	0.747* (0.643[ns])	0.691* (0.691*)	–0.564[ns] (–0.559[ns])
140 days after sowing	0.432[ns] (0.286[ns])	0.637[ns] (0.310[ns])	0.388[ns] (0.523[ns])	–0.514[ns] (–0.417[ns])

[$]Data obtained from ref. 21.
*Significant at 0.05 *P* level at 6 d.f.
**Significant at 0.01 *P* level at 6 d.f.
[ns]Non-significant.

respectively, significant at 0.05 *P* level), suggesting that progenies derived from older ortets tend to show lower seed germination. Further, in the present study, since open-pollinated seeds from the CSO are used, it is logical that genetic load may also have been contributed via male gametes. However, this effect would be random in all the clones.

Some of the strongest evidence in support of this hypothesis comes from the comparisons of per generation mutation rates occurring in short and long-lived species[13,14], which have shown that the mutational load could be substantially high in woody trees than in herbs. Aizen and Rovere[15] have shown in a dioecious conifer (*Austrocedrus chilensis*) that the proportion of aborted pollen increased with age and/or the size of the tree supporting the 'genetic load with-age accumulation model'. Recently, Hanumantha and Vasudeva[16] have also hypothesized that pollen germination among different teak clones may be influenced by the ortet age.

Seed germination in teak may be influenced by seed traits such as diameter, thickness of mesocarp, 100 fruit weight and fruit density[17,18], however, a few reports do not agree with this[19,20]. We also tested this association through correlation analysis (Table 3). Per cent germination at 21 DAS was significantly influenced by fruit diameter and 100 fruit weight; however, this initial association did not hold at 140 DAS, suggesting that variation in seed traits alone cannot explain the clonal differences in germination.

The dormancy release pattern of different progenies is graphically represented in Figure 2. There were two distinct peaks of germination, one between 20 to 25 DAS and the other after 72 DAS. Generally, the clones exhibiting lower germination did not conform to any pattern, which showed a monotonic increase in cumulative germination with time. The clones, which showed higher germination (clone 13, clone 9 and clone 7), clearly exhibited an increased activity towards this end. Clone 2 (My Ha D2) and clone 32 (My Ha K1), which showed a monotonic increase in cumulative germination, failed to germinate even one per cent of total seeds sown. Lower seed germination of these clones suggests that these clones could be discarded from further breeding pro-

grammes involving specific crosses. Further care must be taken not to include clones of older ortets while establishing future clonal seed orchards.

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Detection and phylogenetic affiliation of *Wolbachia* sp. from Indian mosquitoes *Culex quinquefasciatus* and *Aedes albopictus*

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***Wolbachia* sp. specific, 900 bp, 16S rRNA and, 650 bp, *wsp* genes were PCR amplified from two mosquito species *Culex quinquefasciatus* (strain NCCS and NIV) and *Aedes albopictus*. On the 16S rRNA based sequence analysis, *Wolbachia* sp. from *Cx. quinquefasiatus* resembled the earlier reported *Wolbachia* sp. from *Cx. pipiens* and related species from B super group whereas that of *Wolbachia* sp. from *Ae. albopictus* showed homology with members of A super group. The *wsp* gene sequence phylogeny correlated with the 16S rRNA data with *Wolbachia* sp. from *Cx. quinquefasciatus* resembling the B group with highest homology to *Cx. pipiens* and related species whereas that of *Wolbachia* sp. from *Ae. albopictus* was highly homologous to the *w*Alb A strain. The nucleotide differences between the earlier reported *w*AlbA and *Wolbachia* sp. (*w*Alb A*) studied from *Ae. albopictus* in this work were so significant that it formed a separate lineage in the A group of phylogenetic tree. These results indicated that *Wolbachia* sp. from *Cx. quinquefasciatus* were similar to previously reported species while *Wolbachia* sp. from *Ae. albopictus* would represent a novel strain of *Wolbachia* sp.**

MANY species of invertebrates are host to bacterial endosymbionts which are important in the nutritional ecology of the hosts[1] while those which are parasites can be important as agents driving evolutionary changes such as evolution of sex determination system of host[2,3]. Bacteria belonging to genus *Wolbachia* have recently been recognized to infect a high proportion of insects, mites, isopods and filarial nematodes and are maternally transmitted from parent to offspring[4]. These intracellular α-proteobacteria were reported for the first time in the ovaries of the mosquito *Culex pipiens*[5] and named as *Wolbachia pipientis*. It causes crossing incompatibility between infected males and uninfected females[6].

These bacteria have attracted scientific interest due to their ability to manipulate host reproduction, leading to distinct phenotypic effects in the host such as parthenogenesis, feminization, male killings and cytoplasmic incompatibility[7–11]. They are also known to enhance the

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