Pak. J. Bot., 48(2): 511-518, 2016.

FRUIT SIZE AND SAMPLING SITES AFFECT ON DORMANCY, VIABILITY AND GERMINATION OF TEAK (*TECTONA GRANDIS* L.) SEEDS

MUHAMMAD AKRAM* AND FAHEEM AFTAB

Department of Botany, University of the Punjab, Lahore-54590, Pakistan
**Corresponding author's email: m.akramks@yahoo.com; Tel: +924299231152,*

Abstract

In the present study, fruits (drupes) were collected from Changa Manga Forest Plus Trees (CMF-PT), Changa Manga Forest Teak Stand (CMF-TS) and Punjab University Botanical Gardens (PUBG) and categorized into very large (≥ 17 mm *dia.*), large (12-16 mm *dia.*), medium (9-11 mm *dia.*) or small (6-8 mm *dia.*) fruit size grades. Fresh water as well as mechanical scarification and stratification were tested for breaking seed dormancy. Viability status of seeds was estimated by cutting test, X-rays and *In vitro* seed germination. Out of 2595 fruits from CMF-PT, 500 fruits were of very large grade. This fruit category also had highest individual fruit weight (0.58 g) with more number of 4-seeded fruits (5.29%) and fair germination potential (35.32%). Generally, most of the fruits were 1-seeded irrespective of size grades and sampling sites. Fresh water scarification had strong effect on germination (44.30%) as compared to mechanical scarification and cold stratification after 40 days of sowing. Similarly, sampling sites and fruit size grades also had significant influence on germination. Highest germination (82.33%) was obtained on MS (Murashige and Skoog) agar-solidified medium as compared to Woody Plant Medium (WPM) (69.22%). Seedlings from all the media were transferred to *ex vitro* conditions in the greenhouse and achieved highest survival (28.6%) from seedlings previously raised on MS agar-solidified medium after 40 days. There was an association between the studied parameters of teak seeds and the sampling sites and fruit size.

Key words: Fresh water scarification, Seed germination, Luxury wood, Seed size and dormancy, Seed viability, Teak.

Introduction

Teak (*Tectona grandis* L.) is known world over for its significance in the timber industry. Generally distributed in tropical or subtropical regions of the world, teak is indigenous to India and the Southeast Asian region (Myanmar, Thailand, Laos) where it is a major source of wood (Tiwari *et al.,* 2004). In Pakistan, teak-wood is appreciated for its quality and durability. Its wooden products (mainly furniture) are considered to be of finest quality and thus fetch huge value. Teak is usually imported as the plant is not indigenous to Pakistan but its aesthetic appeal and durability has made it stand amongst the popular timbers with an ever-increasing demand. Being a tropical tree species, teak offers potential for its growth and multiplication assessment in Pakistan. Such efforts though are limited in scale so far despite a reasonable scope. With more and more teak trees growing in Pakistan it may potentially even reduce the burden on other fine timber-producing trees such as our currently threatened species of *Dalbergia sissoo*.

Teak, on a larger scale is generally propagated through seeds though other techniques such as rooting of coppice (Palanisamy & Subramanian, 2001) and forced softwood shoots (Akram & Aftab, 2009) have also shown promise for exploitation at the nursery level. The germination response of teak seeds is generally poor and perhaps strongly influenced by several factors including after-ripening, "a phenomenon sometimes classified as a kind of dormancy" (Tiwari *et al.,* 2004). Dormancy is retained by hard endocarp that may not be easy to break until seeds could be pretreated. The presence of large number of chemical inhibitors in the mesocarp may also be a contributory factor. The complex nature of dormancy and the means of overcoming it are major problems in the nursery. Extensive work has therefore been carried out on many different methods of pretreatment to improve the germination of harvested fresh drupes of teak (Manonmani & Vanangamudi, 2003). None of these is applicable for all types of teak drupes; in fact different seed sources require different pretreatments. Although pre-treatments may vary in length and intensity, they all, in general, aim at softening the hard endocarp, removing or reducing the effect of the soft mesocarp and thus shortening the dormancy period. Seed number and size varies within the population collected from different localities. Such ecological variations occur in seeds due to different climatic factors. Cross pollination, humidity and light prevailing in different provenances contribute in variable seed size production. Seed size is a remarkable constant character (Hendrix, 1984) that may affect growth and fitness of plants and subsequently transferring the elite characters for population regeneration (Chacon *et al.,* 1998). Sivakumar *et al*. (2002) reported that large drupes of teak have relatively high fresh weight with high germination potential. Similarly, numerous studies on the other plant species demonstrated that seed number and size of an individual plant or even within the species may vary greatly (Thompson, 1984; Peco *et al.,* 2003).

Viability and dormancy of teak drupes can be tested by various methods. Amongst these methods, use of X-rays is considered to be a non-destructive test for the evaluation of seed viability and quality (Chen & Sun 1991) and internal seed structure of teak (Kamra, 1973). There are contradictory views regarding type of seed dormancy of teak drupes. Physical dormancy was reported by Sivakumar *et al*. (2002). One recent report demonstrates that teak drupes generally have mechanical and not physical dormancy (Slator *et al.,* 2013). However, to overcome such obstructions, scratching outer covering to remove mechanical dormancy and soaking drupes in freshwater (to remove physical dormancy) and cold stratification in general have been suggested (Schmidt, 2007).

In vitro seed germination holds promise for enhancing germination potential of teak on agar medium (Yashodha *et al.,* 2005) for early growth and good quality seedling production. It saves time and labor cost against germination under nursery conditions which is useful for tree species having high seed dormancy and low germination potential. However, this necessitates further work as this problem has never been worked out in sufficient detail in teak in the past.

In keeping with the above information and especially limitations associated with various aspects of teak seed biology, the aim of the present investigation was to determine relationship of teak fruit size grades with fruit weight and number of filled locular seeds. Another major objective was to find suitable means for assessment of seed viability and breaking seed dormancy in teak. Subsequent germination potential of such seeds collected from different sampling sites both under *In vivo* and *In vitro* conditions was also determined. An overall main aim of this study was thus to understand several aspects of teak seed biology and overcoming limitations in order to work out a strategy to improve seed propagation of teak.

Materials and Methods

Seed collection: Teak fruits, generally known as seeds (Indira *et al.,* 2000), were collected from 40-70 year-old trees from three sampling sites, i.e., Changa Manga Forest Teak Stand (CMF-TS), Changa Manga Forest Plus Trees (CMF-PT) and Punjab University Botanical Garden (PUBG), Lahore, Pakistan. These areas are located at 31 °05′ and 31 °33′ North latitude and 73 °58′ and 74 °20′ East longitude, respectively. Mature dried fruits were collected from each sampling site during February-March and pooled to a composite sample. The dried calyxes of fruits were removed and further sun-dried for a week and used in our experiments.

Seed viability status

Size grading on the basis of 'fruit number' and 'fruit weight': A sample of one kg fruits from each sampling site (CMF-PT, CMF-TS, PUBG) was randomly collected. Fruits were divided into four categories on the basis of their size, i.e., very large (≥ 17 mm *dia.*), large (12-16 mm *dia.*), medium (9-11 mm *dia.*) or small (6-8 mm *dia.*) fruits. Less than 6 mm *dia.* fruits (~ 8%) were not included in this study because of their poor development. The data were collected for the number of fruits per fruit size grade per sampling site. Similarly, 30 fruits from each sampling site as well as from each fruit size category were randomly separated and used for weight data of individual fruits.

X-rays radiography: To check fleshy seed in each locule, a sample of 100 fruits from each sampling site and fruit size grade was placed vertically on wooden board and exposed to X-rays (15-20 kilovolt potential, Philips). The images of fruits were taken on X-rays film. The number of fruits with either dark or white cavities (representing empty or filled locules, respectively) was recorded on X-rays film and in this way they were categorized into empty, 1, 2, 3 or 4-seeded fruits. The data were recorded for percent number of fruits containing seeds in their locules per fruit type and size grade from different sampling sites.

Cutting test: A sample of 50 fruits from each sampling site and group of fruits were transversely placed on marble plate and cut with sharp knife. Cut fruits were counted for 1, 2, 3 or 4-seeded or empty fruits.

Seed dormancy

Fresh water scarification: One hundred fruits from each sampling site and size grade were soaked in fresh water for 24 h, and then dried under direct sunlight (32-40°C) for one week. This method for these fruits was repeated 3-5 times during April to August.

Mechanical scarification: Fifty fruits from each sampling site and size grade were mechanically scarified. Scarification was carried out manually by holding fruits firmly and removing exocarp (outer seed layer) with sharp knife carefully. After completely removing exocarp, endocarp was then scratched by rubbing seeds on sand paper until only thin layer of endocarp was evident.

Stratification: A composite sample of 100 fruits from each sampling site and for each of the three chilling treatments was first hydrated in fresh water at 20-25ºC for 24 h, decanted and placed in plastic bags. These hydrated seeds were then placed at 4ºC for 4, 6 or 8 weeks. Seeds from each chilling treatment were then sown to test germination response.

Seed germination

Germination in soil: Randomized block design was used with three replications. Nursery beds (6 × 2 × 0.3 m; l × w × h) were prepared at Changa Manga forest nurseries. The beds were prepared by adding canal silt (2 inch thick layer) on the soil surface. Each bed represented a block and each block was further divided into 1.2 m^2 plots for replication and simplification of data collection. Seeds after fresh water scarification, mechanical scarification or stratification, were sown individually 6 cm apart in beds during May-June and data regarding percent germination were collected after 40 days.

***In vitro* seed germination on agar and sterilized sand medium:** Fleshy seeds were surface sterilized with 0.1% $HgCl_2$ for 15 minutes followed by 6% solution (v/v) of NaOCl with one drop of Tween-20 for another 15 minutes. Seeds were then washed 3-5 times with sterile distilled water and inoculated in culture vessels (25 × 150 mm) containing MS (Murashige & Skoog, 1962) or WPM (McCown & Lloyd, 1981) agar-solidified media (5-8 ml) devoid of sucrose but supplemented with activated charcoal (1%). The pH of the medium was adjusted to 5.8 with 1N HCl or NaOH and autoclaved at 121°C at 15 lb $inch^2$ for 15 min. Similarly, sterilized seeds were also germinated in culture vessels (30 × 200 mm) filled with fine sand (25 g in each vessel) soaked in

MS or WPM basal salts (10-15 ml) without sucrose (pH 5.8). One seed was inoculated per culture vessel with a total of 50 seeds each for agar or sand-medium. In sand-medium, seeds were buried 5 mm deep while in case of agar-solidified medium seeds were embedded on the surface of medium. All cultures were placed under 16 h photoperiod (35 µmol $m^{-1}s^{-2}$). The data for seed germination (%) were recorded on the protrusion of radical from the seeds and the experiment was repeated thrice. At day 25, *In vitro*-germinating seedlings were transplanted in polythene bags filled with canal silt and covered with polythene sheet to minimize dehydration under natural conditions in glasshouse and observed their percent survival and further growth after 5, 20, 30 or 40 days of their transplantation.

Data analyses: Before data analyses, Leven's test was performed to determine the homogeneity of variance. Due to equality of variance shown by Leven's test, analysis of variance (ANOVA) was then performed. Mean values were compared and significance of dependent variables was determined by Tukey's honestly significant difference (HSD) test ($p < 0.05$ using SPSS v 12.0.

Results and Discussion

Size grade effect on number and weight of teak fruits: Less than 6 mm *dia.* fruits were found to be in least number (~8%; data not shown). On an average, 2595, 2870, and 3381 total number of fruits per kg fruit mass were recorded from each sampling site, i.e., CMF-PT, CMF-TS and PUBG, respectively. Generally, fruits collected from CMF-PT were heavier as compared to other provenances. Large fruit grades per unit weight comprised of smaller number as compared to other grades amongst the three sampling sites (Fig. 1). There were 1115 small, 690 medium, 565 large and 500 very large fruits collected from CMF-PT followed by CMF-TS and PUBG (Fig. 1). In accordance with our study, seed size effect on the production of total number of seeds has earlier been reported in teak (Indira *et al.,* 2000) as well as in other plant species (Thompson, 1984; Mtambalika *et al.,* 2014). Within the natural population, seed size is strongly correlated with the production of high seed mass in family umbelliferae. Within individual tree as well as amongst the family demonstrate distribution of variance not comparable due to different seed mass production (Thompson, 1984). The distribution of trees in different regions influences the production of different seed mass. Plant population in its native regions meets all natural resources necessary for seed production and maturation ensure high survivability. In the present study area, teak plants lack few natural assets as compared to its native regions of south East Asia. It may be teak population growing in CMF-PT supplied with more natural resources as compared to other provenances confirmed high seed mass production. However, the natural resources that support high seed mass production become available until teak plantation is established.

Figure 2 shows weight of an individual fruit per fruit size grade per sampling site. Very large fruits were heavier than smaller fruits, i.e., the individual weight of large fruits was highest (0.58 gm) from CMF-PT as compared to CMF-TS (0.51 gm) and PUBG (0.41 gm). This variation in fruit weight might be due to microclimatic conditions and dwindling resources acquired during fruit development of teak over the season prevailing in Changa Manga and Lahore sampling sites and may further be related to the variability in pollination as also suggested by many workers (Thompson, 1984; Murali, 1997; Loha *et al.,* 2006; Hanley *et al.,* 2007).

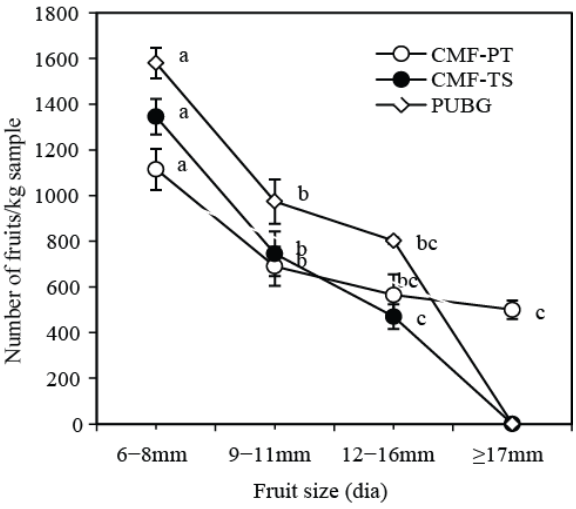

Fig. 1. Fruit size grades and number of fruits per kg fruits sample of teak collected from different sampling sites. The values indicated by different small letters are significantly different according to Tukey's HSD test ($p<0.05$). Vertical bars are ± SE of the means.

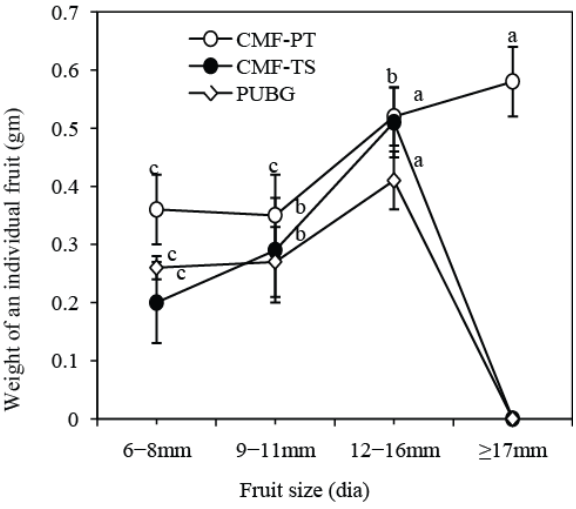

Fig. 2. Fruit size grades and the weight of an individual teak fruit collected from different sampling sites. Data were recorded of 30 fruits for each fruit size grade and sampling sites. The values indicated by different small letters are significantly different according to Tukey's HSD test ($p<0.05$). Vertical bars are ±SE of the means.

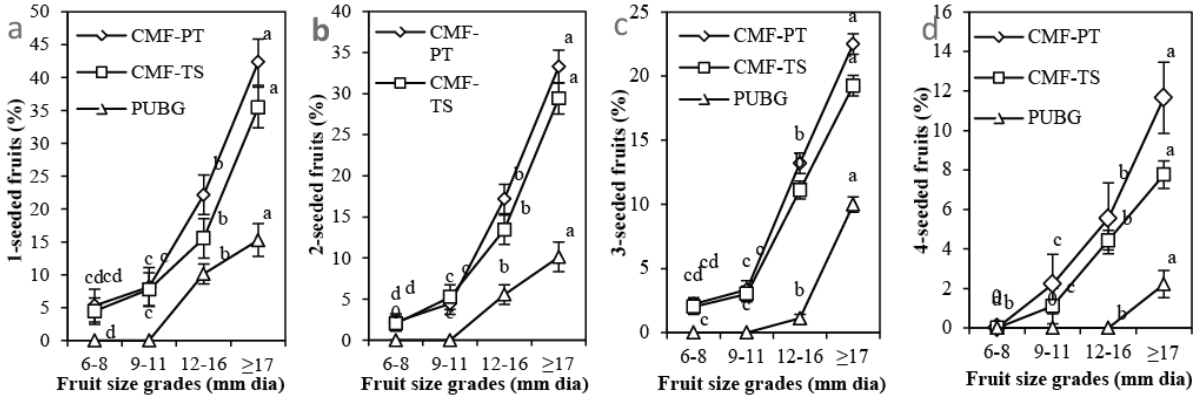

Fig. 3. Relationship between fruit size grades (a-d) and corresponding number of seeded fruits of teak. X-Rays radiography was done from one hundred fruits collected from each sampling site. The values indicated by different small letters are significantly different according to Tukey's HSD test ($p<0.05$). Vertical bars are ± SE of the means.

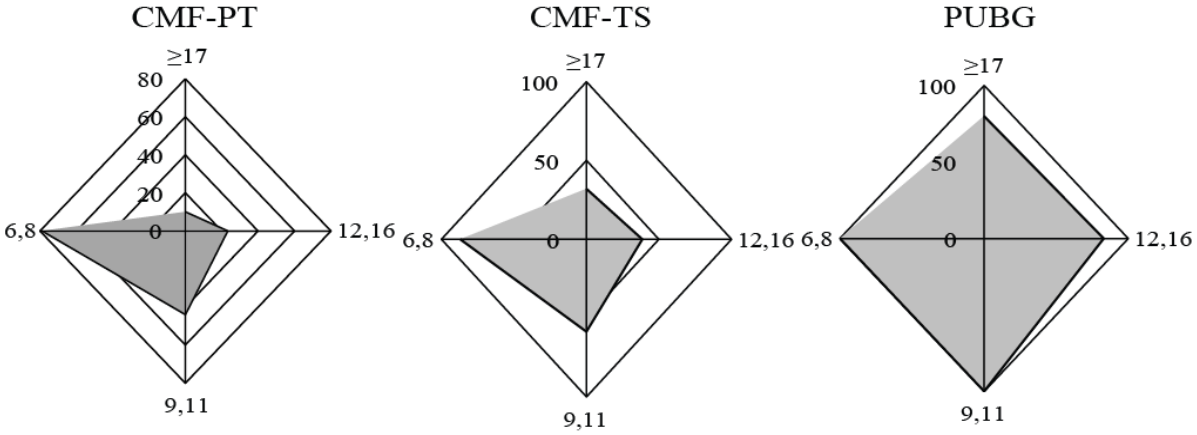

Fig. 4. Radar indicates the occurrence of empty fruits relative to its center point within a specific sampling site. Dark area indicates the proportion of empty fruits towards the specific fruit size grade. Fruit size grades are marked at the corresponding sites on each Fig.

Seed viability determination by X-rays radiography and transverse fruit cut: Figure 3 reveals the presence or absence of fleshy seeds within fruits as visualized by X-rays radiography. Similarly, X-rays radiography has also been tested previously in teak (Kamra, 1973) as well as in other plant species (Martin *et al.,* 1998; Skrzyszewska & Chlanda, 2009). Skrzyszewska & Chlanda (2009) demonstrated that X-rays radiography is very useful technique for the estimation of morphological variations and seed quality of silver fir. X-rays radiography is superior over the use of indigo-carmine staining to determine viability of Scot pine and Norway spruce seeds (Kamra, 1972). He also confirmed this technique useful for estimation of seed soundness of different tropical species (Kamra, 1976). In our study, radiographic images indicate that some fruits were completely filled thus appearing as white-denser areas. Empty locular fruits appeared as dark cavities as earlier been demonstrated by Liu *et al*. (1993) in tomato seeds. A significant linear relationship between fruit types and seed filling percentage was observed from different sampling sites (Fig. 3a-d). Most of the fruits were 1-seeded (Fig. 3a) followed by 2-seeded (Fig. 3b), 3-seeded (Fig. 3c) or 4-seeded fruits (Fig. 3d). Seed filling percentage was invariably higher in fruits collected from CMF-PT followed by CMF-TS and PUBG. The type of fruits was positively correlated with fruit size grades, i.e., percent number of filled fruits (3 and 4-seeded) was higher within very large and large seed grades and lower within medium and small ones. Most of the fruits from CMF-PT were one-seeded (42.33%) followed by 2-seeded (33.3%) or contained 3 seeds (22.34%) whereas a small number of fruits (10.19%) were 4-seeded. Fruits from other localities were comparatively low in seed filling percentage. Similarly, Fedorkov (2001) demonstrated the use of X-rays for estimation of seed quality of Scots pine collected from different provenances and climatic zones.

Figure 4 depicted the extent of occurrence of empty fruits in reference to various fruit size grades in each sampling site. Grey area within each box reveals the tendency towards fruit-emptiness in a specific fruit size grade from each sampling site. As evident from Fig. 4, the incidence of fruit-emptiness increased sharply from CMF-PT to PUBG through CMF-TS.

Half tone photographs clearly depict the internal status of fruits (Fig. 5). Most of the fruits collected from PUBG were empty regardless of fruit size grades (Fig. 5a). Similarly, fruits collected from CMF-TS were also poor in fleshy seeds with only 20 1-seeded fruits out of 50 (Fig. 5b, c, d). On the other hand, most of the fruits collected from CMF-PT contained fleshy-seeds; only 10% fruits from different fruit size grades were usually found empty.

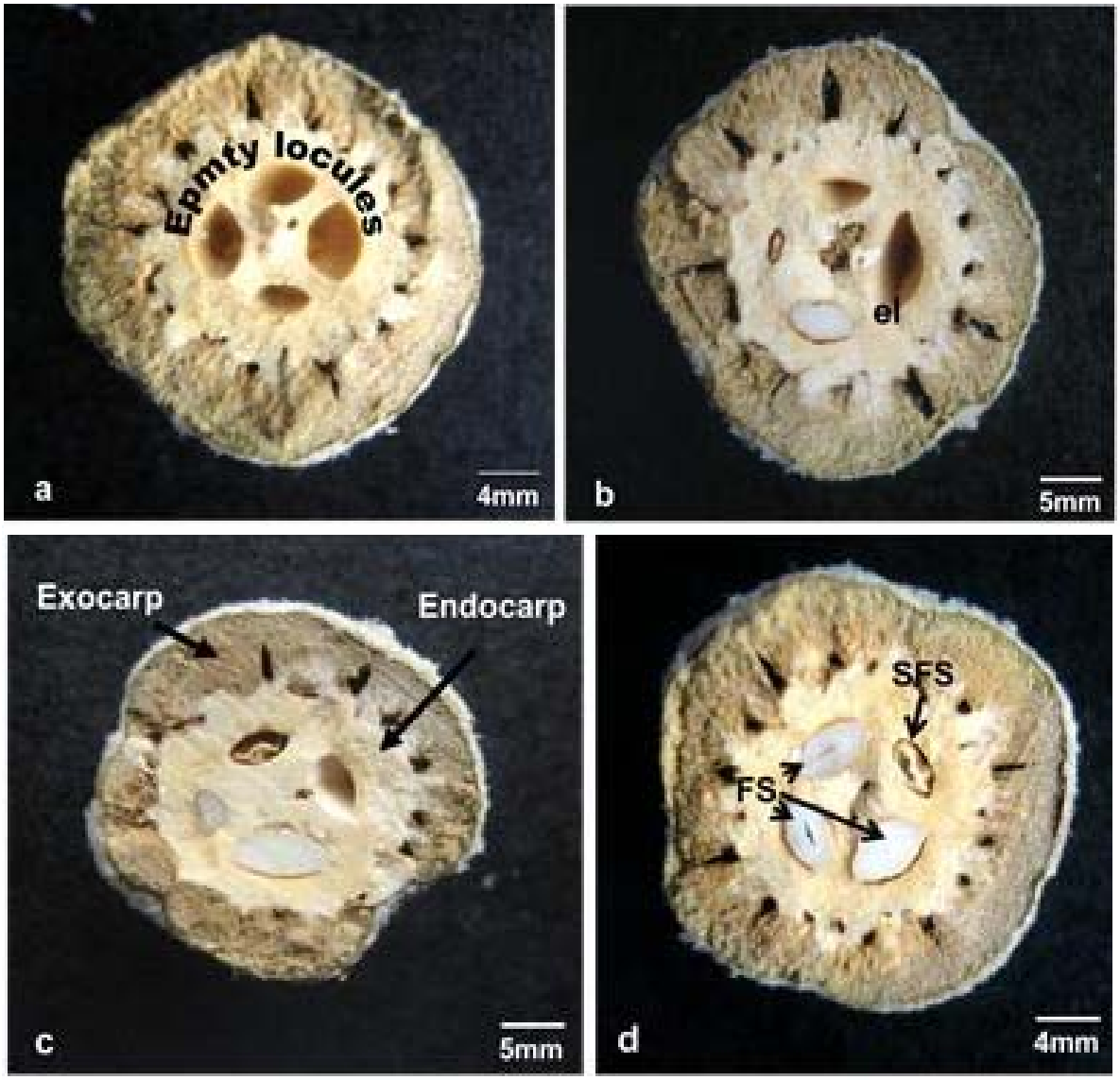

Fig. 5. Transverse cutting clearly depicted filled or empty fruits of teak. a) Empty fruit came from PUBG. b) Fruit with one seed came from CMF-TS. c) Two seeded fruit from CMF-PT. d) Three seeded fruit from CMF-PT. el: *empty locule*, FS: *fleshy seed*, SFS: *shriveled fleshy seed*.

Seed dormancy and germination

Fresh water scarification: Fresh water scarification of fruits for 24 h had a significant effect on subsequent germination. In general, there seemed a strong correlation between the rate of germination and fresh water scarification. Similar observations have also been made by Yadav (1992). He soaked teak fruits in water for 6 h to break physical dormancy with increased germination. Within the specified fruit size grades (very large, large, medium and small), a positive correlation between the size and growth was observed, i.e., greater the *dia*., better was the growth rate. Small fruits from PUBG, however, could not germinate, except for very large (11%) and large size grades (7%) after 40 days of sowing. Nonetheless, amongst all fruit size grades and sampling sites, the CMF-TS had highest rate of germination (44.32%) from very large fruits (Fig. 6). Similar results have also been reported in previous studies on teak (Indira & Bhasha, 1999; Indira *et al.,* 2000) as well as in other plant species (Cicek & Tilki, 2007; Suresha *et al.,* 2007). Very large seeds therefore are considered to be more competent for *In vivo* establishment and survival of seedlings in the field (Cicek & Tilki, 2007), whereas smaller ones have correspondingly lower survival abilities (Armstrong & Westoby, 1993). In the present study, 13 and 12 days were required for very large seeds to germinate from CMF-TS and CMF-PT, respectively after pre-soaking treatment. Similarly, germination was also observed from all fruit grades of CMF-PT with maximum rate of 41.52% (Fig. 6). Sampling site had also significant relationship with seed germination similar to the studies of Hathurusingha *et al*. (2011).

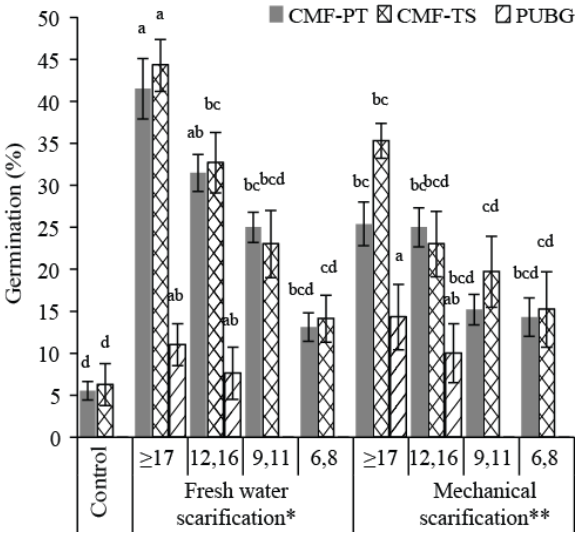

Fig. 6. Effect of fresh water and mechanical scarification on subsequent germination and emergence of teak seeds from each fruit size grade and sampling site. The values over bars indicated by different letters are significantly different according to Tukey's HSD test ($p<0.05$). *100 seeds, **50 seeds per fruit size grade per sampling site. The data were recorded after 40 days.

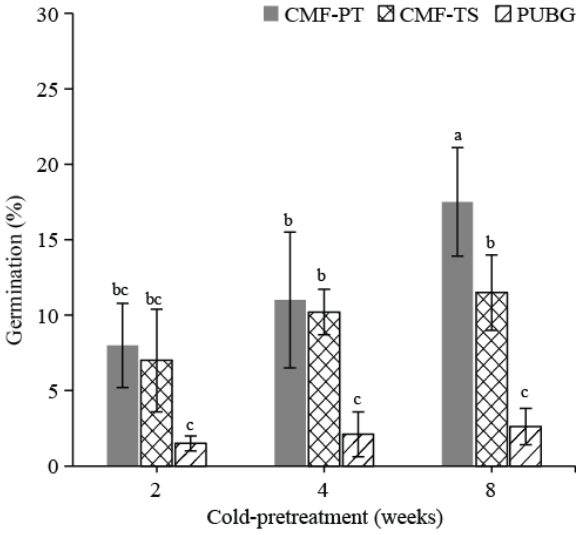

Fig. 7. Effect of cold-pretreatment (4°C) on subsequent germination and emergence of teak seeds collected from different provenances. Different small letters over bars indicate significantly different results according to Tukey's HSD test ($p<0.05$). Data were recorded after 40 days.

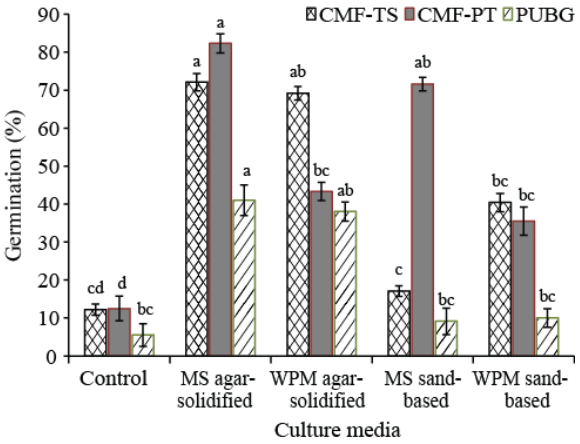

Fig. 8. Effect of *In vitro* culture media on fleshy seed germination and emergence of teak collected from different sampling sites after 25 days of culture. The values over bars indicated by different letters are significantly different according to Tukey's HSD test ($p<0.05$). Fifty fleshy seeds per culture medium and sampling site were inoculated on the culture medium.

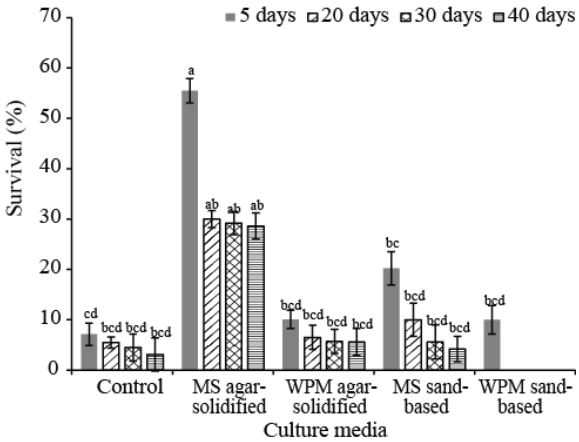

Fig. 9. Survival rate of *In vitro*-germinated seedlings after four different time periods under glasshouse conditions (25 ± 5°C, natural day/night period at relative humidity of 75%) in polythene bags. The values over bars indicated by different letters (from three experimental runs) are significantly different from each other according to Tukey's HSD test ($p<0.05$).

Mechanical scarification: Mechanical scarification was less effective as compared to fresh water scarification (Fig. 7). Similar to fresh water scarification, very large seeds germinated earlier (after 15 days) with 25.42% or 35.32% germination from CMF-PT or CMF-TS, respectively. However, very large fruits collected from PUBG had 14.33% germination response after 30 days whereas medium and small fruits did not germinate (data not shown).

Cold stratification: Cold-pretreatment affected significantly the subsequent seed germination at 4°C for 2, 4 or 8 weeks. Generally, the time period varied as earlier germination was observed from CMF-PT followed by CMF-TS and PUBG. Eight-week cold pretreatment enhanced germination (17.5%) in seeds of CMF-PT followed by CMF-TS (11.5%) and PUBG (2.6%) after 40 days of sowing (Fig. 7). On the other hand, seeds stratified for 2 or 4 weeks germinated with relatively lower rate after the same sowing period.

***In vitro* seed germination on agar and sterilized sand media:** Seed germination on plain agar (control) was very poor, i.e., 12.22, 12.56 and 5.5% collected from CMF-TS, CMF-PT or PUBG, respectively. Significant difference was observed for seed germination on

different *In vitro* media. The rate of germination of fleshy seeds from CMF-PT was highest (82.33%) on MS agar-solidified medium as compared to control (12.56%) (Fig. 8). The rate of *In vitro* seed germination on woody plant medium (WPM) was low on both agar-solidified (43.33%) and sand media (35.55%) collected from CMF-PT. Improved germination on MS agar-solidified medium is not well understood. It is assumed that the differences in media components in general and probably high concentration of nitrogen contents in MS medium in particular might have contributed to better germination of teak seeds as reported for other plant species (Samuel *et al.,* 2009; Sambe *et al.,* 2010).

Growth of *In vitro* germinating seedlings: Figure 9 reveals seedlings on MS agar-solidified medium with vigorous growth and highest survival rate (55.5%) after five days of transplantation as compared to WPM (10.11%). There was strong interaction between survival rate and *In vitro* medium on subsequent seedling's growth. Sand medium was least effective with 20.22% and 10% plant survival on MS and WPM, respectively. However, survival rate was reduced with the passage of time (5-40 days) on all media types. Plants got necrotic on WPM sand-based medium after five days of transplantation in soil.

Forty-day after shifting to glasshouse, seedlings survival percentage on MS agar-solidified medium was 28.55% while 5.55% on WPM. These surviving plants were acclimatized and shifted to soil in growth tunnel. So far no mortality has been seen in these plants.

In conclusion, seed filling was found to be better in fruits collected from CMF-PT and the type of fruits was positively correlated with fruit size grades. Very large fruit size category (≥ 17 mm *dia.*) from all sampling sites for instance though in lesser frequency was with most filled fleshy locular seeds. We therefore also conclude that viability and internal seed status can effectively be determined with X-rays radiography. Dormancy in teak can most effectively be broken down by fresh water scarification. Finally, highest germination potential of fleshy seeds was achieved by *In vitro* seed culture on MS agar-solidified medium with subsequent maximum survival rate in greenhouse conditions.

Acknowledgements

Higher Education Commission (HEC), Pakistan is gratefully acknowledged for the provision of funds for this study in the form of research project No. 1155 awarded to FA. We are thankful to Dr. M. Fazal, SDFO and Changa Manga Forest staff especially Mr. Malik Altaf Hussain for help in nursery experiments.

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(Received for publication 28 February 2015)