REPRODUCTIVE BIOLOGY OF TEAK (*TECTONA GRANDIS* LINN.f.) IN EAST JAVA, INDONESIA

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ABSTRACT

Reproductive biology of teak (*Tectona grandis* Linn. f.; Verbenaceae) in a CSO in East Java, Indonesia was investigated. The causes of low fruit and seed set were identified. Fruit maturation in relation to the low fruit germinability was studied. Three clones, representing low, intermediate and high fruit production capacity, were selected for the study. Clone 5 (low fruit-production capacity) was early flowering. Its peak flowering period started before the CSO peak-flowering period and coincided with the peak rainy season. Clone 12 (intermediate fruit-production capacity) was a late flowering clone. The peak flowering period was reached toward the end of the CSO peak flowering period and after the peak rainy season had passed. Clone 17 (high fruit-production capacity) had peak flowering that coincided with the CSO peak-flowering, toward the end of the peak rainy season. The reproductive success of clone 5 (0.1%) was lower than clone 12 (0.4%) and clone 17 (0.5%), although clones 5 and 17 had a higher mean numbers of flowers per inflorescence (1948.1 and 2007.2 Fl/Infl, respectively) than clone 12 (1331.6 Fl/Infl). In general, low fruit and seed set were the major constraints in fruit production. However, since one seed per fruit is more desirable, the low seed set is not considered to be a problem. That left low fruit set as the major problem.

Fertilization occurred within 24 h after flower opening (AFO). Thus, flowers that aborted after 24 h AFO were considered as fertilized. Percentage of pistils remaining 48 h AFO for clones 5, 12 and 17 were 30, 42.5 and 40%, respectively. The mean numbers of fruits per inflorescence at maturity for clone 5 was 10.375 (0.5%), clone 12 was 17.875

(1.3%) and clone 17 was 35.563 (1.8%). The abortion of fertilized pistils occurred during seed and fruit development and maturation. A major cause of seed abortion was abnormal development of endosperm. During early stages of development this caused the zygote to abort. In later stages it restricted embryo development, causing seed abortion. Failure in endosperm development may result from a high incidence of self-pollination. Clonal variation did not affect fruit quality, but maturation stage did. Fruit collected at 24±1 w AFO (M-3), when fruit water content was about 11%, gave the highest germination capacity (57.67%). The germination value of fruit at M-3 was also higher than M-1 (16±1 w AFO) and M-2 (20±1 w AFO). Although these fruits germinated slower than fruits collected at 28±1 w AFO (M-4), the higher germination capacity is more advantegous.

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ABA	abscisic acid
ACFTSC	ASEAN - Canada Forest Tree Seed Centre
AFO	after flower opening
ASEAN	Association for South East Asia Nations
C	clone
CIDA	Canadian International Development Agency
Coll	collection
CSO	clonal seed orchard
d	day
FAA	formalin-acetic acid-alcohol
Fl	flower
FM	epifluoresence microscopy
Fr	fruit
GC	germination capacity
GV	germination value
h	hour
ha	hectare
Infl	infloresence
ISTA	International Seed Testing Association
KPH	Kesatuan Pemangkuan Hutan

LM	light microscopy
m	meter
min	minnute
mo	month
O	ovule
P	progeny
PERS	preemergent reproductive success
PNFI	Petawawa National Forestry Institute
PP	Perum Perhutani (Forest State Enterprise)
PV	peak value
S	seed
SDW	seedling dry weight
SEM	scanning electron microscopy
SI	self-incompatibility
SPA	seed production area
SPSS	Statistical Package for Social Scientists
T	tree
UV	ultra violet
w	week
yr	year
Z	zygote

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CHAPTER 1

INTRODUCTION

1.1. Background

Teak (*Tectona grandis* Linn. f.) is a tropical deciduous tree species . It is a member of the family Verbenaceae, order Laminales (Troup, 1921) with a chromosome number of 2n=36 (Gill *et al.*, 1983). Economically, teak is the most important timber tree species of Indonesia and is well accepted in local and international markets. The properties of teak wood, such as strength, stability, durability and resistance to termites, makes the demand for teak wood remains high. It is extensively used for furniture, carving, ship building and other purposes.

Teak in Indonesia is thought to have been introduced from India about 700-1500 years ago (Altona, 1922). However, an isozyme study reveals that teak in Indonesia is genetically more similar to the provenances in Thailand and Tanzania than those in India (Kertadikara and Prat, 1995). The species has been distributed naturally throughout the country, but the main growing area is Central and East Java. Clear-cutting with artificial regeneration has been employed in the management of teak forests in Java since the last century. As a consequence, most of the teak forests in Java now are human-made forests (plantations). The natural teak forest in Indonesia is about 31 531 ha (Kartasubrata, 1993).

Large-scale teak planting programs have been set up in several tropical countries and considerable effort has been dedicated to improving the genetic and physical quality of the species. The total area of teak plantations in Indonesia is approximately 617,000 ha (Kartasubrata, 1993) with the annual planting goal of 8,000-12,000 ha (Perum Perhutani, 1985). The annual planting program requires a constant supply of seeds with good physical and genetic qualities.

At the moment the main source of teak fruits in Indonesia is from seed production areas (SPAs), which are upgraded stands. With the annual planting goal of about 8 000 - 12 000 ha, the need for fruits is about 160 000 - 240 000 kg of fruit annually (approx. 20 kg of fruits per ha) (Darmono, 1994). Fruit production from SPAs provides most but not all of the required amount. Fruit production from clonal seed orchards (CSOs) has been used to supplement the demand since 1991, but an adequate fruit supply is still lacking. The high fruit requirement of 20 kg per ha has been adopted because of the anticipated low survival rate and low germination capacity. Two approaches can be used to meet the demand.

Firstly, the demand may be met by increasing fruit production from CSO. Up to 1992 Perum Perhutani (PP) had established 917 ha of CSO from a target of 1 200 ha, with populations of 100 trees per hectare (Rochmini, 1994). Although most CSO have started to produce fruits, production is very low, about 0.1-0.5 kg/tree (data from CSO records). The CSO are expected to completely substitute for the role of SPA in 10-15 years. To meet the demand for fruit, production must be increased to about 1.6-2.4 kg/tree. Secondly, the demand may be fulfilled by increasing the germination capacity of fruits and the survival rate of seedlings, thus decreasing fruit requirement. Several fruit pretreatments have been investigated to increase the germination capacity. The highest germination capacity reached in the field so far is about 40-50% with fruits from SPA, and about 1-10% with fruits from CSO (pers. comm.). Germination capacity may also be increased by harvesting fruits at optimal maturity. To accomplish this, an investigation of fruit maturation in order to determine the optimum time for harvesting is needed. In addition, studying the processes of pollination, fertilization, and seed and fruit development and maturation in a CSO will give a broad understanding of when and why flowers, fruits and seeds abort. This, in turn, may lead to possible solutions to increase fruit production. The low survival rate of seedlings (about 60%) may be due to several factors such as adverse climatic conditions and seedlings of low vigour. Optimum transplanting time and a standard selection for vigorous seedlings need to be investigated. The use of stumps as transplanting material increases the survival rate and ease of handling.

1.2. Objectives

In order to understand and manage fruit production, we must determine the time and causes of flower, seed and fruit loss, and understand the development of both embryo and seed which relate to fruit viability.

Specifically, my research is to determine:

1. Reproductive phenology for teak in the CSO at Cepu, East Java;
2. Relative time of fertilization and the timing and causes of flower abortion;
3. Embryo development and the timing and causes of embryo, seed, and fruit abortion;
4. Preemergent reproductive success; and
5. Fruit and seed maturation.

CHAPTER 2

LITERATURE REVIEW

2.1. Reproductive phenology and reproductive success

Teak distribution in Indonesia is mainly in Java, Kangean, Bali, South Sulawesi, and Nusa Tenggara Islands, at a latitude of approximately 7°S and a longitude range from 106-123°E. The optimum environmental conditions for regeneration, growth and timber quality of the species includes annual rainfall between 1200 and 3000 mm with a 3 to 5 mos dry period, deep and well drained alluvial soil with pH between 6.5 and 8.0, and the optimum light between 75 and 100% of the full daylight (Kaosa-ard, 1981).

Environmental conditions that produce rapid early growth in teak also seem to promote the initiation of flowering. Thus, the height or size of the tree generally has greater influence than age on initiation of flowering under plantation conditions. Flowering may start as early as the first year when trees are still at the seedling stage. Generally, however, in Thailand and India flowering is initiated after 5 to 6 yrs in seedling based stands (Kaosa-ard, 1986a), whereas in Papua New Guinea it is between 2 and 4 yrs (Suangtho and Lauridsen, 1990). Good quality fruits were produced after the trees 15 to 20 yrs old (Haig *et al.*, 1958) and the first large crop of about 15 to 150 kg/ha was obtained from 20 to 25 yrs old SPA (Hedegart, 1976).

A study in Northern India showed the period of flowering to fruit maturation to be 8.5 mos, and this was related to leaf flushing and leaf shedding (Seth and Kaul, 1978). Leaf-flushing started in June and continued through July followed by formation of flower buds. These flowers opened during September and October, followed by fruit development through November, when fruit ripening began. The fruits matured in March and fell during April and May which coincided with leaf shedding. A study carried out in Northern Thailand showed that flowering began about July and fruits developed to their full size about 50 d (7 w) after pollination. The fruits required another 70 to 150 d (10 to 21 w) to mature in December or January (Hedegart, 1976). That is, from flowering to fruit maturation in Thailand took about 120 to 200 d (4-7.5 mos), slightly less than in Northern India.

Reproductive success is defined as the number of eggs which develop into viable offspring (Wiens *et al.*, 1987). This is divided into preemergent and postemergent phases. Preemergent reproductive success (PERS) is defined as the number of eggs that complete development and survive to enter the ambient environment. So, PERS is affected by progeny/zygote (P/Z) ratio. Postemergent reproductive success is the percentage of progeny that survive to reproduce following their release into the ambient environment.

In seed plants, PERS is measured by the number of ovules that mature into viable seeds released from a maternal plant. In angiosperms, flowers (Fl) are the immature module, and fruits (Fr) the mature module, therefore, PERS is product of Fr/Fl and P/Z ratios. In cases where data on zygote formation are not available, seed/ovule (S/O) ratio can be used as an estimate for P/Z ratio. S/O ratio tends to overestimate PERS since not all seeds will germinate and become normal plants; and not all ovules are fertilized and develop into zygotes. However, the formula is a good approach to rank species, clones or individual trees with respect to their reproductive success (Owens, 1995).

$$\mathrm{PERS} = \frac{\text{Fruit}}{\text{Flower}} \times \frac{\text{Seed}}{\text{Ovule}}, \text{ where}$$

Fr/Fl ratio is calculated from the number of fruits per inflorescence (Fr/Infl) divided by the number of flowers per inflorescence (Fl/Infl).

S/O ratio is calculated from the number of seeds per fruit (S/Fr) divided by the number of ovules per fruit (O/Fr).

2.2. Flowering, pollination and fertilization

Siripatanadilox (1974) studied the flower development of teak in Northern Thailand from the observable primordia until fruit formation. It is not clear how long before flower bud emergence primordia can be distinguished. About 11 d after flower bud emergence, the megaspore-mother cells and the pollen-mother cells formed, and within another 17 d egg cells and pollen developed, and matured after a few more days. Blooming occured about 9 d after flower buds appeared.

Flowers are arranged in a panicle, an inflorescence in which the lateral branches arising from the peduncle produce flower-bearing branches instead of single flowers. An individual inflorescence contains about 1200 to 3700 flowers, and the flowering period of an inflorescence lasts from 2 to 6 w, during which 100 to 300 flowers appear per day (Hedegart, 1976).

Teak flowers are small (6 to 8 mm in diameter), whitish, actinomorphic and hermaphroditic. They have six petals joined in a single whorl. The pistil has an ovary with four ovules and a style with a forked stigma. Style and stamens are about 6 mm long, however, the style usually is slightly longer than the stamens (Bryndum and Hedegart, 1969).

Individual flowers have about a one-day cycle. Flowers begin to open about midnight, but the style is not exposed until sunrise, at which time, or shortly after, it is receptive. The optimum pollination period is between 11:30 and 13:00 h (Hedegart, 1973). In the evening of the same day, or the next morning, the corolla together with the stamens or the complete flower falls off.

The production of large numbers of flowers may aid the reproductive success of the plant. Woody perennials producing hermaphrodite flowers which exhibit self-incompatibility are characterized by having some of the lowest fruit to flower ratios (Sutherland, 1986). The delay of several months between floral initiation, anthesis, and subsequent seed maturation in *Banksia* spp. may reduce reproductive success through losses due to insect or bird damage to the inflorescence and developing seeds (Fuss and Sedgley, 1990). Therefore, the more flowers produced the greater the potential of providing seeds for the next generation. There are at least five hypotheses to explain the production of excess flowers (Guitian, 1993). (1) The attraction hypothesis suggests that production of excess flowers increases the plants overall attractiveness to pollinators resulting in more effective pollination and improving overall fertilization rates (Willson and Price, 1977; Stephenson, 1979). (2) The selective abortion hypothesis refers to the condition where excess flowers allow the plant to selectively abort "poor quality" fruits (Stephenson, 1981; Bawa and Webb, 1984; Stephenson and Winsor, 1986). (3) The male function hypothesis implies that excess flowers, although morphologically hermaphroditic, act primarily as male organs and donate pollen as would unisexual flowers (Sutherland and Delph, 1984; Sutherland, 1986). (4) The resource availability hypothesis suggests that excess flowers allow the plant to exploit unexpected favorable conditions in terms of resource availability (Stephenson, 1981). And, (5) the ovary reserve hypothesis proposes that excess flowers may constitute a reserve supply of ovaries in the case of high mortality during the flowering period (Ehrlen, 1991).

Stephenson (1981) synthesized several studies and concluded that the number of female flowers exceeds fruit set even when all of the flowers are pollinated and that pollination does not limit fruit set in many species. Flowers are inhibited from setting fruits if other pollinated flowers and juvenile fruits are developing. This indicates that flowers and young fruits compete for limited maternal resources, thus, fruit set is resource-limited.

An *in vivo* study reports that teak pollen-tube growth reached a maximum of 9.3 mm in 24 h, with an overall mean of 5.53 mm (Egenti, 1978). If the average length of teak pistils is approximately 6 mm (Bryndum and Hedegart, 1969), then not all pollen tubes are able to reach the micropyle within 24 h after pollination.

There are two bee species, *Heriades binghami* (Megachilidae) and *Ceratina hieroglyphica* (Anthophoridae) reported as important teak pollinators in Thailand (Hedegart, 1976). The major insect pollinators in Nigeria are members of the Nymphalidae (*Euphaedra janatta, Acraea bonasia,* and *Belenois calypso*). Insects added into bags enclosing inflorescences increased the fruit set indicating that teak is basically an insect-pollinated species (Bryndum and Hedegart, 1969). The report also indicated that teak is slightly self-compatible. When insects were supplied into isolation bags where only self-pollination was possible, relatively large numbers of fruits were produced. This indicates that self-pollen can fertilize the egg if no xenogamous pollen is available. Although the number of fruits per inflorescence (5.5) was much less than with insect cross-pollination (26.6), it was essentially the same as in hand-cross-pollination (5.2). An investigation carried out to increase fruit set on *Catalpa speciosa* by hand-cross-pollination failed to set more fruit compared to open-pollination (Stephenson, 1979).

Fertilization is the fusion of female and male gametes. In angiosperms the process of double fertilization involves the fusion of one sperm nucleus with the egg nucleus producing the zygote, and the other sperm nucleus with the diploid polar nucleus producing the triploid endosperm which is considered to fulfill a nutritive function. In the majority of tree genera the two haploid polar nuclei fuse prior to fertilization, and the diploid polar fusion nucleus then fuses with a haploid sperm (Sedgley and Griffin, 1989).

Some reports confirm that the pollen tube penetrates into one of the two synergids, which is termed the receptive synergid or degenerated synergid since it usually degenerates either before pollen-tube penetration into the micropyle or at tube arrival. It is not clear whether either of the synergids can act as the receptive synergid. Huang and Russell (1992) reported that membrane-bound Ca levels are higher in the receptive synergid than in the persistent synergid and egg, suggesting that Ca may be involved in the guidance of the pollen tube during its final growth until the receptive synergid is reached. In some species, the receptive synergid degenerates even before pollination or remains intact at least until the time of pollination (Russell, 1993).

Self-incompatibility (SI) is defined as the inability of a fertile hermaphrodite seed plant to produce zygotes after self-pollination, and is one of the mechanisms to encourage out-breeding in flowering plants. It can occur either in the stigma, style, or ovule, so that pollen does not germinate or pollen-tube growth is arrested on the stigma, in the style or in the ovary (Seavey and Bawa, 1986; Sedgley and Griffin, 1989). In the latter case, called ovarian SI, pollen tubes may stop growing in the nucellar tissue near the embryo sac, as in *Acacia retinodes* (Kenrick *et al.* 1986) , or male gametes may enter the embryo sac but fail to fuse with the egg, as in *Theobroma cacao* (Cope, 1962), or abortion of zygote and/or endosperm may occur after normal fertilization, as in *Rhododendron* spp. (Williams *et al.,* 1984). The nucellar arrest has the advantage that ovules entered by an incompatible pollen tube are not sterilized but remain available for subsequent fertilization by compatible pollen.

Fertilization in teak occurred within one day after pollen adhered to the stigma, and the embryo was distinguished about 12 to 17 d after fertilization (Siripatanadilox, 1974). Fruit set in teak is very poor, commonly about 2% (Bawa and Webb, 1984), and lack of or inadequate pollination is a common reason given. Hedegart (1976) indicated that self-incompatibility in teak may be as high as 96 to 100%, resulting in very low rates of fertilization. The lack of fertilization is not always due to shortage of pollen tubes (lack of pollination) or the specific causes mentioned above but in some cases, results from the release of inhibitors that cause stylar plugging. It was found that in *Sophora japonica*, a deciduous tree originating from eastern Asia, early fertilized ovules inhibited the growth of additional pollen tubes by secreting IAA into the style, thus preventing subsequent fertilization and causing ovule abortion (O'Donnell and Bawa, 1993).

Low fruit set also results from variable development of the embryo sac. Not all ovules contain a mature embryo sac at the time of pollination, and the pollination process itself may inhibit immature ovules from maturing, as in *Prunus armeniaca* cultivar Moniqui Fino (Burgos and Egea, 1993). Malformed ovules also affect viability of the embryo sac, and thus seed set.

In open pollination of teak in Thailand the fertilization percentage averaged only 1.3% and was ascribed to an insufficient number of pollinating insects; whereas the success of control, hand-cross-pollination resulted in 6 to 60% filled seed with an average of 20% (Hedegart, 1973). Egenti (1978) reported that flower abortion after fertilization in teak could be as high as 90% in a panicle and flowers which had not fallen off 14 days after pollination developed into fruits. An *in vivo* study in Nigeria showed that teak pollen was viable until the second day after anthesis, whereas the stigma was receptive for only one day. The highest percentage of fruit set (27%) was obtained when the flowers were pollinated on the day of anthesis, between 13:00 and 14:00 h, slightly later than those in Thailand (11:30-13:00 h) and much later than those in India (08:00-10:00 h) (Mathew *et al.*, 1987). Hand pollination of *Gmelina arborea*, also a member of Verbenaceae, showed self-inviability. This condition is where self-pollinated flowers set fruits that develop to different sizes but with a maximum size only half that of mature open- or cross- pollinated flowers, however, all fruits eventually abort. The aborted fruits from the self-pollinated flowers were found to contain collapsed embryos (Bolstad and Bawa, 1982). Examination of stigmas and styles also revealed that there was no difference in pollen germination and pollen-tube growth rates between self- and cross- pollinated flowers.

2.3. Embryogenesis

Embryogenesis in angiosperms starts after double fertilization. The zygote usually remains undivided for some time, although considerable cellular and cytoplasmic changes can take place (Went and Willemse, 1984). The zygote is located at the micropylar end of the embryo sac, which is usually vacuolated. Embryogenesis in higher plants can be divided into three overlapping phases. The first phase is morphogenesis, during which the polar axis of the plant body (shoot and root apices) is defined. The second phase is embryo maturation, which is characterized by accumulation of storage reserves, whereas during the final phase the embryo prepares for desiccation, becomes desiccated, and enters a period of developmental arrest (West and Harada, 1993).

As early as 1896, Koorders studied the morphology, physiology, and embryology of *Tectona grandis*. The ovule is anatropous, where the micropyle faces towards the placenta, and has only one integument (unitegmic) composed of seven to eight layers of cells. Pal (1951) studied the embryology of some Verbenaceae including teak and reported that the size of the mature embryo sac is about 320 μm, and the organization of the nuclei is of the *Polygonum* eight nucleate bipolar type. The micropylar end of the embryo sac is much broader than the chalazal end, which is very narrow and acute. The synergids are elongated and very large so that the egg is almost covered by them. The egg nucleus is slightly bigger than that of the synergids and the latter persist for some time after fertilization. The antipodal cells are small and ephemeral.

In *Lippia nodiflora*, another member of Verbenaceae, the fertilized egg is dormant for some time. The first division occurs after a fair amount of endosperm tissue has formed and the micropylar and chalazal haustoria are organized. Embryo development is of the Solanad type (Pal, 1951), where the first division is by a transverse wall forming basal and apical cells followed by another division that results in a linear tetrad of the proembryo (Johansen, 1950). The basal cell divides and forms a long suspensor (Johri, 1984), which is thought to function in embryogenesis by pushing the embryo into the nutrient-rich endosperm and, possibly, by serving as a conduit for nutrients and growth factors from the maternal tissue to the embryo (Yeung and Meinke, 1993). The apical cell divides and forms the embryo proper.

The triploid endosperm starts to develop after the second sperm nucleus fuses with two polar nuclei. In teak the endosperm is cellular and reported to have a unicellular, 2-nucleate endosperm haustorium that develops directly from the chalazal chamber, the chalazal haustorium (Pal, 1951). Many of the Verbenaceae develop micropylar and chalazal haustoria in the early stage of endosperm development. The term endosperm proper refers to that portion of the endosperm where the embryo develops (Johri *et al.*, 1992) and is used to distinguish the endosperm from the haustoria. In many dicotyledoneous species the endosperm is substantially degraded by the time the embryo matures (Lopes and Larkin, 1993).

2.4. Seed and fruit maturation

Structurally, teak has a hard, irregular, dry stone fruit. It has a thin papery outer layer which develops from the calyx and is normally removed during processing, a thick corky outer layer, and a stony inner layer. Inside the stony layer are four chambers. Each may contain a seed, but normally only 1-2 seeds are formed. The seed is oval, white and measures about 6 mm long and 4 mm wide. The seed can not be easily separated from the fruit, and therefore, the fruit is the dispersal unit.

Changes in abscisic acid (ABA) content during the development and maturation of fruits, seeds and embryos have been investigated. ABA is found in highest concentration in developing seeds and is relatively low or even absent at maturity of many species (King, 1982). In some species appreciable levels of ABA are consigned to the mature seed (Black, 1983). ABA content is correlated with the inhibition of precocious germination and enhancement of storage reserve synthesis, especially protein, during seed development of soybean (Ackerson, 1984a and b), wheat (Triplett and Quatrano, 1982) and pea (Wang *et al.,* 1987; Barratt *et al.*, 1989).

During seed development, ABA content in *Phaseolus vulgaris* increased as the dry weight increased (van Onckelen *et al.,* 1980), whereas in the subsequent maturation phase, when dry weight was constant, the ABA content decreased gradually. Further research indicated that embryos are held in embryogenetic growth by *in situ* ABA synthesis, as in sunflower (*Helianthus annuus*) (Le Page-Degivry and Garello, 1992). When isolated germinative growth is permitted, but if the ABA content is maintained, embryogenetic growth continues (Kermode, 1990; Black, 1991). The higher the ABA content in the young embryos, the longer the duration in culture before germination begins (the lag phase). The long duration of the lag phase results in an increase in the number of days for *Phaseolus vulgaris* embryos to germinate (Prevost and Le Page-Degivry, 1985). As soybean embryos matured the ABA content declined, and embryos required less time to germinate (Ackerson, 1984b).

Embryo ABA content in soybean was reported to correlate with the ability of immature embryos to germinate and also stimulated growth and protein accumulation during early stages of embryogenesis (Ackerson, 1984a). The inhibition of germination by ABA may be initiated only when the ABA is produced by the embryo itself. Initiation of dormancy may also depend on seed sensitivity to ABA. A study using *Arabidopsis* ABA-deficient mutant showed that neither maternally derived ABA nor applied ABA are able to induce dormancy (Koorneef *et al.,* 1989). The mechanism of precocious germination inhibition by ABA is thought to be on the cell wall. It inhibits wall yield and loosening which are requisites for cell expansion. The effect of ABA on developing embryos generally depends on the embryo age, with sensitivity to the regulator decreasing as embryos approach maturity (Bewley and Black, 1994).

2.5. Seed production and quality testing

Teak fruit production is very low compared to the number of flowers produced and the average germination is only about 40 %. High quality fruit procurement is essential for reforestation since good quality fruit plays an important role in the success of reforestation and stand quality.

A study carried out in India estimating potential germinability of teak, showed that none of the 23 Indian origins of teak had fruits with seeds in all the four chambers. In general, four-seeded fruits were very rare, whereas one-seeded fruits were the most common (Gupta and Kumar, 1976). A similar study conducted by Kaosa-ard (1986) in Thailand showed that about 45% of the seed lots from eight sites had only one seed per fruit, about 28% had no seed, and percentages of fruits containing 2, 3, or 4 seeds were

21, 5, and 1%, respectively. Dabral (1976) suggested that empty fruits in teak develop at the stage when the stony layer starts to harden, due to heavy lignification in the chambers, and is not related to chamber size. Ovules at this time are in the 'watery' stage.

Seed viability refers to the ability of seed to germinate under optimum conditions. In mono-embryo seeds (i.e. one dispersal unit contains one embryo), the viability test is expressed as the percentage of seeds that germinate (ISTA, 1985). Although some teak fruits contain more than one seed, Hedegart (1974) defined teak seed viability as the percentage of fruits containing at least one well-developed sound seed as determined by a cutting test. The definition seems to be practical for teak seed testing and nursery practice. ISTA (1993) put forward a definition used in testing as follows:

> "When a unit produces more than one normal seedling, only one is counted for determining the germination percentage. On request the number of units which have produced one, two, or more than two normal seedlings may also be determined."

Viability of teak fruit varies greatly and usually is very low. A study carried out in Thailand using the cutting test indicated that viability varied from 40 to 85% depending on fruit size, source, year, and the climatic conditions during the flowering and fruit-setting periods (Kaosa-ard, 1986).

The germination of teak is sporadic and might occur from 10 to 90 d or even several years after sowing, however, most germinate about 40 d after sowing (Keiding, 1985). Several factors affect the germination. The optimum range of soil moisture for germination is between 11 (or slightly lower) and 18% (with air humidity of about 70 to

80%). Above 18% soil moisture, the fruit tends to deteriorate and eventually die (Suangtho, 1990). The ambient temperature range in tropical countries is between 27 and 33°C, just about the optimum temperature for teak germination (Gupta *et al.,* 1975). Light is an important factor controlling the germination of teak. Murthy (1973b) reported 40% germination in the open field. In the dark at temperatures between 35 and 37°C it was 34%, and in the dark at 25°C it was only 0.4%.

Fruit selection according to size was used in Indonesia as early as 1930 (Eidman, 1934). It was reported that fruits with a diameter >14 mm germinate faster than smaller ones and give higher growth rates in the first year. Kaosa-ard (1981) found that viability of teak fruit varied according to size. For example, the average viabilities of teak fruit in diameter groups of 9-9.9, 10-10.9, 11-11.9, and ≥12 mm were 41, 53, 63, and 57.1%, respectively. These results were similar to that of Darus (1980) who found that large (>18 mm), medium (8-17 mm), and small fruit (<8 mm) had average germinations of 83, 71, and 32% and a germination period of 2-8, 2-8, and 2-5 w, respectively.

Various methods of fruit treatment have been developed to enhance germination. Basically they are to soften the stony layer, eliminate the effect of the corky layer which is thought to contain an inhibitor, and shorten the period of after ripening, thus increasing germination percentage and shortening the germination period. Several fruit pretreatments that have been studied are: soaking in water for 24 to 72 h; alternate soaking and drying for 24 h over a 1 to 2 w or 1 mo period; soaking in boiling water for 15 min; alternate soaking in Sach's solution and drying (24 h each) over a 1 w period; removing the corky layer (Bedell, 1989; Muttiah, 1975); and, soaking in water for 24 h then allowing to dry for 3 d, repeated over 1 mo period (ISTA, 1985). The results vary and depend on fruit source, production year, time of harvest, and fruit age from time of harvest. Removal of the corky layer seems to give better germination, however, the treatment is not applicable for large-scale nursery practice.

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials

A ten-year-old CSO (planted in 1983) managed by Perum Perhutani/KPH Cepu-BKPH Sekaran at Cepu in East Java was selected for the study. It lies between 7-8° S latitude and 111-112° E longitude with an altitude of between 0 and 25 m above sea level. The annual rainfall is 1,700 mm with four distinct dry months (June through September). The average monthly minimum rainfall of 38 mm occurs in August and maximum of 255 mm in February.

Trees are at 10 x 10 m spacing, 10 to 15 m tall and most had produced many inflorescences during the 1 to 4 yrs prior to the study. Records kept at the CSO identified flowering clones and clones that produced fruit during the previous 4 years. The block selected for sampling contained 24 clones and approximately 160 trees per clone in an area of 44.5 ha.

Using the data available in the CSO on kg of fruit per tree for the previous 4 yrs, the clones were grouped into three categories: nine clones with low fruit-production capacity, less than 0.1 kg of fruit per tree ; nine clones with intermediate fruit-production capacity, between 0.1-0.2 kg of fruit per tree; and, six clones with high fruit production capacity, more than 0.2 kg of fruit per tree.

Three clones and four trees from each clone were selected to observe the detailed aspects of reproductive phenology from the time of flower opening and pollen shed to the time of seed and fruit maturity. The three clones were: clone 5, representing low fruit-production capacity; clone 12, with intermediate fruit-production capacity; and clone 17, with high fruit-production capacity.

Collection of flowers was carried out throughout the flowering period from January through June 1993, and fruit collections were made during the maturation period from June through August 1994. Bamboo scaffoldings were erected adjacent to the selected trees so that samples could be collected repeatedly.

3.2. Methods

3.2.1. General observations on reproductive phenology

Observations on general phenology were carried out on: the time of first flowering, peak flowering and last flowering (per inflorescence and tree); duration of fruit and seed development and maturation; time of fruit shed; and any climatic factors that might adversely affect these processes (i.e. rain or wind causing flower or fruit loss). The number of flowering trees was counted every two weeks to determine flowering period for the overall CSO, whereas fruiting trees were counted at the end of the maturation stage. Observations of vegetative buds using a dissecting microscope were made to determine the time of reproductive bud initiation, although more detailed and precise observations are needed. Observation on the time of flower opening, pollen shed, and stigma receptivity were carried out using a dissecting microscope. This information was needed to determine the collection schedule.

Data kept in the CSO on the numbers of flowering trees and fruit-bearing trees, and fruit production for each clone during 1989 to 1995 were used to determine periodicity of fruit production. Data were statistically analysed using Statistical Package for Social Scientists (SPSS) (Norusis, 1993). Data in proportion were transformed using arcsine transformation, but the original data were used to present the result. Analysis of variance was used to test the effect of clones and production period on the number of flowering trees, number of fruit-bearing trees and total fruit production per clone and fruit production per tree per clone. Significant differences were tested using Duncan's multiple range test with 95% confidence level. In cases when the differences among clones were not significant, data from the three clones were pooled.

Flowers were collected at the same time as described in section 3.2.2., dissected and fixed in FAA (formalin-acetic acid-alcohol) for Scanning Electron Microscopy (SEM) to observe detail of the flower structures. Fixed specimens were dehydrated in an ethanol series, critical point dried, and coated with gold, then observed using a JEOL JS M-35 SEM at 15 kv. Fruits having different water contents were collected. The fruit and seed structures were observed using the SEM and dissecting microscope.

3.2.2. Determining relative time of fertilization and the timing and causes of flower abortion

Flowers may abort due to failure of flowers to be pollinated, lack of fertilization or adverse climatic conditions. Lack of fertilization may be caused by: failure of pollen to germinate; pollen tubes being arrested on the stigma, in the style, in the ovary wall, or in the micropyle; or, lack of fusion of the egg and sperm. Another possible cause is self-inviability when there is self-fertilization but the embryo fails to develop.

Pollen-tube growth rate was measured in order to determine the time of fertilization. In this part of the study, the paraffin technique and epifluorescence microscopy (FM) were used. Some samples were also prepared for the resin technique in case the paraffin technique was inadequate.

During the flowering period, four to six inflorescences per tree were tagged at random on each of the four trees from each of the three clones. On each of the four inflorescences 30 flowers were tagged at random after they opened, at 07:00 h every day for 14 d using different colors of thread. Ten flowers per inflorescence were collected at 0, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, and 72 h after flower opening (AFO). These samples were then divided into three groups: four flowers per inflorescence were fixed in FAA for paraffin embedding and SEM as in Section 3.2.1; three flowers were fixed in glutaraldehyde for resin embedding; and, three flowers were fixed in ethanol-acetic acid (ethanol:acetic acid = 3:1 v/v) for the FM.

Sample sizes at each collection were:

10 Flowers (Fl)/Inflorescence (Infl) x 4 Infl/Tree (T) x 4 T/Clone (C) x 3C =

480 Fl/Collection (Coll) were divided into:

4 Fl/Infl x 4 Infl/T x 4 T/C x 3C = 192 Fl/Coll for paraffin embedding and SEM

3 Fl/Infl x 4 Infl/T x 4 T/C x 3C = 144 Fl/Coll for FM

3 Fl/Infl x 4 Infl/T x 4 T/C x 3C = 144 Fl/Coll for resin embedding

Flowers from the four inflorescences of the same tree were put in the same vial.

Specimens fixed in FAA were dehydrated in a tertiary-butyl alcohol series of solutions (Johansen's solution series) (Johansen, 1940) and embedded in Tissueprep 2 (Fisher Scientific paraffin wax CAS 8002-74-2). The embedded specimens were softened in Gifford's solution at 37 C for 3 to 4 w and serially sectioned using a rotary microtome at 6 µm. After being mounted on microscope slides, the sections were stained with 1% safranin in H_2O and 0.05% fast green in 95% ethanol (Berlyn and Miksche, 1976). They were mounted in Entellan (Merck 7961), covered with cover slips and cured at 37°C for 2 w. These specimens were used to observe the structure of the mature embryo sac, the time of fertilization and to describe early embryo and endosperm development using the light microscope (LM).

The fluorescence test was carried out using samples from collections at 4 to 28 h AFO, omitting collections at 0 h and from 32 to 72 h AFO. The reason for omitting these collections was an assumption that fertilization occurred within 24 h AFO as in Thailand and Nigeria (Hedegart, 1973; Siripatanadilox, 1974; Egenti, 1978). Specimens were fixed in ethanol-acetic acid (3:1 v/v). The ovary wall was sliced on two opposite sides so that the pistils would lie flat and rinsed in water four times at 30 min intervals. They were then cleared in 8N NaOH from 2 to 5 d at room temperature or until the pistils became transparent and stained with 0.1% decolorized aniline blue in 0.1% K_3PO_4 for about 30 min (Kenrick and Knox, 1985; Owens *et al.*, 1991). Clearing at 45 to 55°C was also satisfactory and reduced the clearing time to only 4 to 6 h (Motten, 1992). Cleared pistils were then dissected, mounted on microscope slides with cover slips, gently squashed, and observed using a Leitz microscope equiped with UV epifluorescence. The number of pollinated pistils was determined as pistils that had at least one pollen tube in the style. Numbers of pollen tubes in the style were recorded, but were not counted when they were numerous and twisted. The time was recorded for how long it took for the longest pollen tubes to reach the upper style (the upper half of the style length), and the lower style (the lower half of the style length), and the ovary or micropyle (lower part of the inner side of the ovary wall and/or micropyle). Observations of pollen-tube growth in the ovary were not separated from those that reached the micropyle, since the pollen-tube tip could be shifted due to mounting. To observe variation of number of pistils with at least one pollen-tube reaching the upper style, lower style and the ovary or micropyle among collection time, data from the three clones were pooled. The data in proportion were transformed using arcsine transformation and statistically analysed as in Section 3.2.1. Analysis of variance was carried out to observe the effect of clonal variation and collection time on number of pollinated pistils using SPSS (Norusis, 1993). Significant difference among clones and collection times were tested using Duncan's multiple range test with 95% confidence level. The number of pollinated pistils with the longest pollen-tube at each site among collection times was also counted by pooling the data from the three clones.

Specimens for the resin technique were fixed in 2.5% glutaraldehyde in 0.075 M PO_4 buffer (pH 7.2) at ambient temperature, then rinsed in 0.075 M PO_4 buffer and dehydrated in an ethanol series. Infiltration was conducted using Spurr's resin (Spurr, 1969) and cured for 18 h at 60°C.

3.2.3. Determining embryo development and the timing and causes of embryo, seed and fruit abortion

To determine embryo development, observations had to start at fertilization and continue until fruit maturation. Fruit development and maturation were monitored by measuring fruit diameter and water content. Fruit diameter was measured every 2 w from the day the flower opened until the fruit was ready for harvest. Fruit water content was measured every 2 w starting 6 w AFO, when the enclosing calyx started to change color from green to yellowish green, until harvest. Specimens were placed in plastic bags in a cooler and taken to the Seed Science and Technology Laboratory, Bogor Agricultural University for measurement. Fruit water content was determined on a fresh weight basis with five fruits per replicate. The four trees of each clone represented the four replicates. The fruits were weighed, dried at 105°C for 24 h then reweighed (Hor, 1993).

Sample size at each collection: 5 Fr/T x 4 T/C x 3C = 60 Fr/Coll.

The paraffin technique, as in Section 3.2.2, was used to determine embryo and seed development. Specimens for determining early embryo and endosperm development (the first 3 d AFO) were taken from collection in Section 3.2.2. Developing fruits were collected from four trees from each clone. Thirty flowers were tagged when they opened at 07:00 h every day for 17 d on each of 4 to 6 inflorescences per tree. Collections started 3 d AFO and continued until maturation according to the following schedule:

1. Twice weekly from 3 d to 4 w AFO = 8 Coll
2. Weekly from 5 to 8 w AFO = 4 Coll
3. Biweekly from 10 to 20 w AFO = 6 Coll

Total for 20 w = 18 Coll

Sample size at each collection:

4 Fr/Infl x 4 Infl/T x 4 T x 3 C = 96 Fr/Coll divided into:

2 Fr/Infl x 4 Infl/T x 4 T x 3 C = 48 Fr/Coll for measuring fruit diameter and water content

2 Fr/Infl x 4 Infl/T x 4 T x 3 C = 48 Fr/Coll for paraffin embedding.

In cases when the number of developing fruits previously tagged as inadequate due to high abortion rate, fruits from the adjacent branches of the same inflorescence that were the same size were used.

As fruits develop the wall becomes very thick and hard, therefore fruit samples collected 4 w or longer after tagging were cut open and all seeds were sliced down two surfaces to allow better fixation in FAA and embedded in Tissueprep 2 using the same procedure as in Section 3.2.2. Some sections of fruits in the later stages were used for an insoluble carbohydrate test using the periodic acid-Schiff's reaction, and for a protein test using periodic acid-Schiff's-amido black reaction (Jensen, 1962).

3.2.4. Determining preemergent reproductive success

At fruit maturity, four inflorescences were taken from each of the 12 trees. The total number of flowers per inflorescence (Fl/Infl) was counted as the number of flowers, floral stalks (where flowers had been shed) and developing fruits. At maturity the number of fruits per inflorescence (Fr/Infl) was counted and Fr/Fl ratio was calculated.

Two hundred mature fruits (50 Fr from each of the four trees) from each clone were cracked and the number of developed seeds per fruit (S/Fr) was counted. The number of ovules per fruit (O/Fr) in teak was four, and S/O ratio was calculated. This observation also provided information on the proportion of empty fruits. Once the Fr/Fl and S/O ratios were determined, the PERS of each clone was calculated.

The total numbers of inflorescences per tree (Infl/T) as well as the number of fruit-bearing inflorescences per tree (Fr-infl/T) and fruit production per tree (Fr-prod/T) for the 12 trees were determined to find out further clonal variation in 1993.

Analyses of variance were carried out based on variation among clones using SPSS (Norusis, 1993). Significant difference in Fl/Infl, Fr/Infl, Fr/Fl, S/Fr, O/Fr, S/O and PERS among clones was tested using Duncan's multiple range test with 95% confidence level. In these analyses, data from the four trees were pooled. Data on Infl/T, Fr-infl/T and Fr-prod/T were analysed similarly.

3.2.5. Determining fruit maturation

The term 'fruit' in this section is used in the same manner as 'seed' in the seed quality tests (fruit is the dispersal unit). The time required for teak fruits to germinate varies greatly, which indicates considerable variation in after-ripening. Other internal factors which may also affect the germination capacity are presence of completely empty fruits, hard fruit coat, and the condition within the embryo (i.e. presence of inhibitor).

This part of the study was conducted at the Seed Science and Technology Laboratory, Faculty of Agriculture, Bogor Agricultural University, and comprised two experiments. Details of the experiments are as follows:

3.2.5.1. Experiment 1

Experiment 1 was conducted in 1993. Fruits were collected from the selected trees (three clones, four trees per clone) in mid-June 1993 when they matured at 18-20 w AFO. They were sun-dried for three days before treatment. The fruit quality testing was conducted based on clonal variation in relation to fruit size. Fruits were separated into three size categories, i.e. small (S) fruits: diameter <10 mm, medium (M) fruits: diameter between 10-14 mm, and large (L) fruits: diameter > 14 mm. Only fruits from three trees per clone were used as replicates due to the low fruit production of one of the trees in each of clones 5 and 12. The number of experimental units was 3 C x 3 size groups x 3 replications, or 27 units.

Twenty-five fruits were used per replicate (modified from Hor, 1993) instead of 50 Fr because of limited number of fruit in each size per clone. Fruits were pretreated by soaking in water for 12 h and then sun-drying for 12 h. This was repeated for 6 d, then fruits were planted with the pedicel down in germination boxes of a size sufficient to hold 25 fruits. The pretreatment was modified from ISTA (1985), based on personal experiences that the repeated treatment over a 1 m period, as the rule put forward by ISTA, resulted in a lower germination. Washed sand was used as the medium, and the tops of the fruits were placed level with the surface. The boxes were put in full daylight and watered daily.

Observations started 1 w after sowing and continued twice a week for 12 w. The parameters observed in Experiment 1 were:

1. Normal seedling dry weight (SDW) at 4 w after sowing as a vigor test. Four weeks after sowing was adopted as the first count instead of 1 w (Hor, 1993). The delayed first count was based on personal experiences that less than 5% of fruits germinate within 1 w after sowing. SDW was obtained by drying normal seedlings in the oven at 70° C for 24 h after excising the cotyledons (Pinthus and Kimel, 1979).
2. Germination capacity (GC) was calculated as the cumulative germination percentage of fruits that had germinated with at least one normal seedling emerging from a fruit at 12 w after sowing, when the test was terminated. The test period was prolonged to 12

w, much longer than the 4 w suggested by Hor (1993), due to the fact that less than 20% of fruits had germinated within 4 w after sowing.

A normal seedling was determined as a seedling with complete essential structures such as radicle, epicotyl, and cotyledons which are indicative of the ability to produce a normal plant under favorable conditions (Copeland and McDonald, 1985). In this study the teak normal seedlings were determined as seedlings with complete essential structures and at least one pair of leaves.

Analyses of variance were carried out to test the effect of clonal variation and fruit size using SPSS (Norusis, 1993). Significant differences in GC and SDW among fruit sizes were tested using Duncan's multiple range test with 95% confidence level. In these cases data from the three clones were pooled due to insignificant difference among clones.

3.2.5.2. Experiment 2

Experiment 2 was carried out in 1994. Three clones and four trees per clone were selected for fruit collection. The three clones were the same clones used in 1993, but the trees were different. Fruits were collected between June through August when they reached maturity. Based on the result from Experiment 1, only fruits with diameter >10 mm were used in Experiment 2. In this experiment, the fruit quality test considered clonal variation in relation to maturity. Fruit water content determination in 1993 showed that fruits started to dry rapidly between 14-16 w AFO during which time they started to mature. This finding was used to determine maturation stages which were estimated from peak flowering period from the end of February to the beginning of March (information from the CSO records). Maturation stages were defined as M-1 (16±1 w AFO), M-2 (20

±1 w AFO), M-3 (24±1 w AFO), and M-4 (28±1 w AFO). Fruits were collected every 4 w within the range of the maturation stages and sundried as in Section 3.5.1. For the germination test, 25 fruits per replicate were pretreated and planted in the same way as in Experiment 1. The number of experimental units was 3 C x 4 maturation stages x 4 replicates, or 48 units. Observations were conducted as in Experiment 1. The parameters observed in Experiment 2 were:

1. Germination capacity (GC), calculated as in Experiment 1.
2. Germination rate (GR), calculated as cumulative germination percentage of fruits that had germinated with at least one normal seedling per fruit per week during the test.
3. Peak value (PV) was calculated as the maximum quotient derived from all of the cumulative germination percentages on any day divided by the number of days to reach this percentage. PV is a measure of the mean daily germination of the most vigorous component of the seed lot, and is a mathematical expression of the break of a sigmoid curve (T-value) representing a typical course of germination (Fig. 1) (Djavanshir and Pourbeik, 1976).
4. Germination value (GV) as a vigor test was calculated according to the following formula (Czabator, 1962):

    GV = MDG X PV, where

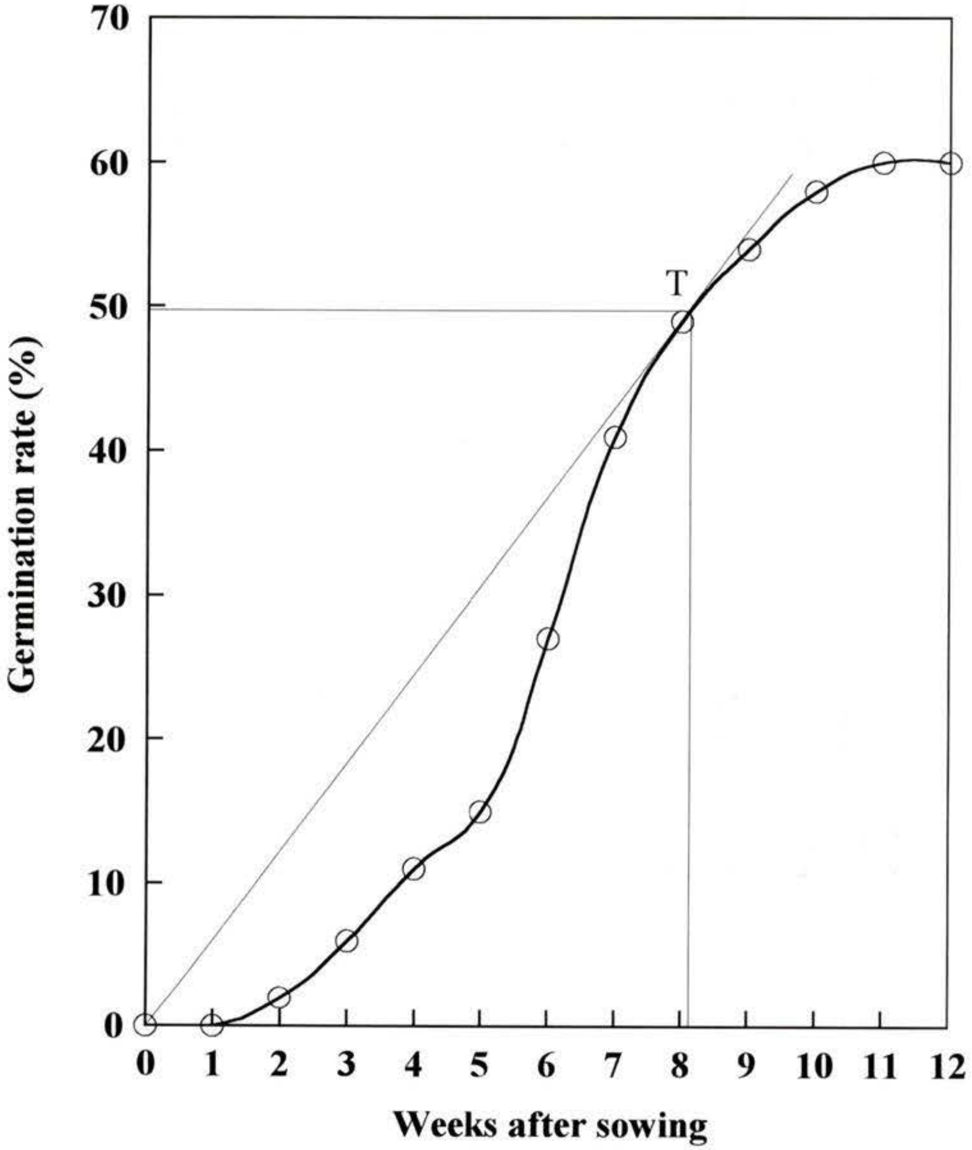
    MDG (mean daily germination) is calculated as the percentage of full-seed germination at the end of the test divided by the number of days in the test.

   The formula is a measurement for seeds of woody perennials having slow germination and combines germination capacity and germination speed.

5. Abscisic acid (ABA) content was analysed in the Biochemistry Laboratory at the Biotechnology Research Institute for Food Crops, Bogor, West Java, Indonesia. The procedure used was according to the methods of Sweetser and Vatvars (1976) with minor modifications. Fruits were collected and seeds were extracted immediately and frozen in liquid N. Seeds (0.2 g) with seed coats still intact were ground in 10 ml 80% aqueous methanol. A known amount of [^{14}C]ABA was added prior to extraction so that final recovery of ABA could be calculated. The sample was extracted and passed through a Sephadex column. ABA was identified and quantified by HPLC using a DuPont absorption column based on retention times of a standard ABA. ABA content was calculated as µg/g fresh weight (fw).
6. Protein content analyses were also conducted, using the Kjeldahl procedure, at the same Laboratory as ABA analysis. This analysis was for total protein content of the seed which consisted of storage and structural proteins. Samples were obtained in the same way as in the ABA analysis. Samples containing 0.2 g seeds with seed coats were ground with concentrated H_2SO_4 to which selenium (as catalyst) had been added, followed by distillation and titration to determine the amount of ammonia nitrogen. Since most proteins contain about 16 % nitrogen, the protein content was calculated as the amount of ammonia nitrogen multiplied by 6.25 (West *et al.*, 1963).
7. Fruit water content (WC) was determined as in 3.2.3 (Hor, 1993).

Statistical analyses were carried out using SPSS (Norusis, 1993). Analyses of variance was used to test the variation among clones and maturation stages. Significant difference in GC, PV, GV, ABA, protein and water content among maturation stages were analyzed using Duncan's multiple range test with 95% confidence level. In these cases data from the three clones were pooled.

Fig. 1. T-value represents the break of the germination curve. Peak value (PV) is calculated as percent germination at T-value divided by the number of days needed to reach T-value.


Germination rate (%)
0
10
20
30
40
50
60
70
T
0
1
2
3
4
5
6
7
8
9
10
11
12
Weeks after sowing

CHAPTER 4

OBSERVATIONS AND RESULTS

4.1. General reproductive phenology

The period of flowering to fruit maturation of teak in Indonesia is about 6 to 8 mos (Table 1). Vegetative buds flush in the beginning of the rainy season in October, followed by the appearance of inflorescence buds in December through January. Flowering starts in mid-January and continues until the beginning of May, with peak flowering from mid-February to the end of March. This is followed by fruit development from mid-February through June. Fruit maturation occurs during the dry period of mid-May through September. Fruit collection is usually conducted between the end of August until the beginning of September, after the peak dry season, when fruits easily fall when the trees are shaken. From bud dissections, there is evidence that flower initiation for the following season occurs within the terminal buds during fruit development. These buds become dormant in the dry season when all leaves are shed.

Observations on the three clones show that clone 5, representing a low fruit-production capacity, started to flower in the beginning of January and continued until the beginning of March. Its peak flowering period lasted for about 3 w during mid-January until the beginning of February. Clone 17, representing a high fruit-production capacity, flowered during the end of January to mid-April with the peak flowering period from mid-February to mid-March. Clone 12, representing an intermediate fruit-production capacity, flowered during mid-February until mid-April with its peak occurring in the beginning of

March and lasting about 3 w. Clone 17, which follows the general flowering period of the overall CSO, had the longest flowering period among the three clones (11 w), followed by clone 5 (9 w) and clone 12 with the shortest flowering period (8 w) (Table 2). The data also show that clone 5 flowered earlier than the overall CSO, and the last 2 w of its peak flowering coincided with the beginning of the peak rainy season. Clone 17 started its peak flowering toward the end of the peak rainy season. Clone 12, started to flower in the beginning of the CSO peak flowering period and reached its peak flowering toward the end of the CSO peak-flowering period.

Data obtained from the CSO show irregular fruit production in teak. The number of flowering and fruit-bearing trees from 1989 to 1995 varied among years, but remained the same among the three clones. Over the 7-year period, the trees at the CSO had two good fruit-bearing years, the first in 1989 and the second in 1993. Not all flowering trees produce fruit. The highest percentage of flowering and fruit-bearing trees occurred in 1993 ($p< 0.01$) when 64.6% flowered (Fig. 2A) and 44.1% bore fruit (Fig. 2B). The lowest was in 1995 when only 2.7% of trees flowered and 2.1% produced fruits.

Total fruit production per clone over the 7-year period varied among clones as well as among years. Clone 17 produced more fruits than clones 12 or 5 ($p<0.05$). The highest total fruit production for clones 5, 12, and 17 was 3.68, 12.38, and 121.80 kg, respectively, and occurred in 1993 and the lowest was in 1995 (0.01, 0.01, and 0.32 kg, respectively) (Fig. 3A). These data also show that clones 5 and 17 had a 4-year interval between two good-crop years, in 1989 (2.85 and 65.91 kg for the respective clones) and 1993 (3.68 and 121.80 kg, respectively). This coincided with the number of fruit-bearing in which 7.80 kg and 12.38 kg of fruit were produced, respectively. However, the 7-year data did not confirm a 2-year interval for clone 12.

Usually, fruit production per tree is used as a measure of fruit-production capacity of a clone. In the CSO, the highest fruit production per tree of clone 5 occurred in 1994 (0.33 kg/tree), clone 12 in 1991 (0.36 kg/tree) and clone 17 in 1993 (1.37 kg/tree). The lowest occurred in 1995 and for clones 5, 12, and 17 was 0.01, 0.01 and 0.08 kg/tree, respectively (Fig. 3B).

Teak has a high flower-abortion rate. Clone 5 had the highest abortion rate, followed in order by clones 12 and 17. The percentage of intact pistils per clone 24 h AFO was 32.5, 42.5, and 40%, respectively, for the three clones but this percentage decreased with time. At 72 h AFO only 5% of clone 5 pistils remained intact, whereas clones 12 and 17 had 15% and 20% intact pistils, respectively (Fig. 4).

4.2. Floral structure and morphology

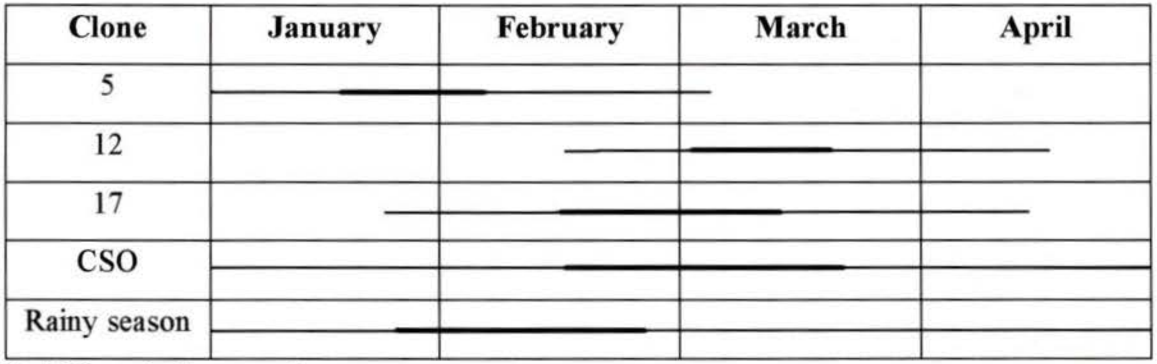
The teak inflorescence is a panicle and has rhythmic branching. The branches are subtended by leaves in a decussate arrangement. The inflorescence appears in the terminal bud and contains flowers ranging from 800 to 2800 (Fig. 5). The size of the inflorescence varies among clones, trees within a clone, and individual inflorescences within a tree. The length of the panicle from the last pair of leaves ranged from 50 to 110 cm. The flowering period for each tree was 5 to 10 w and for each inflorescence was 2 to 6 w, depending on the number of inflorescences on a tree, inflorescence size and climatic conditions. On days with heavy rain, flowers may be knocked off the pedicel before or just after they open.

Table 1. Reproductive phenology of teak (*Tectona grandis* Linn. f.) and season

Stage of development	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**
Reproductive bud initiation	—	—	—	—								
First phase of reproductive bud development			—	—	—	—						
Reproductive buds become dormant						—	—	—	—			
Second phase of reproductive bud development										—	—	—
Flowering	—	━	━	—	—							
Fruit and seed development		—	—	—	—	—						
Fruit and seed maturation					—	—	—	—	—	—		
Rainy season	━	━	—	—	—					—	—	—
Dry season					—	—	━	━	—	—		

Bold lines indicate peak season

Table 2. Flowering period of the three clones and CSO, and rainy season



Bold lines indicate peak season

Fig. 2. Percent flowering (A) and fruit-bearing trees (B) in the three clones from 1989 to 1995, inclusive. A total of 480 trees were used for analyses, 160 trees from each clone. Error bars represent standard error of the means of the three clones.

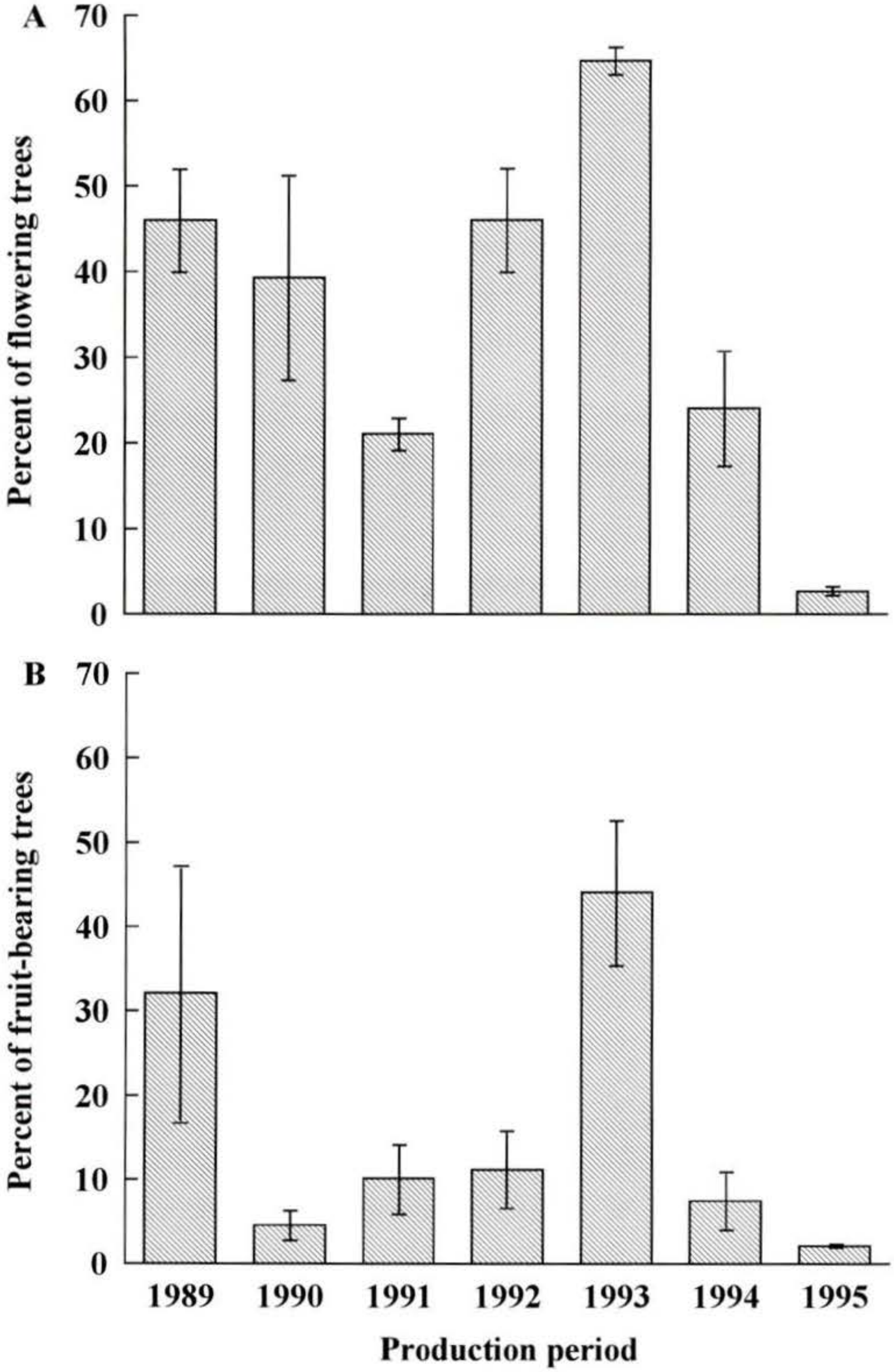

A
B
Percent of flowering trees
Percent of fruit-bearing trees
0
10
20
30
40
50
60
70
1989
1990
1991
1992
1993
1994
1995
Production period

Fig. 3. Total fruit production (A) and fruit production per tree (B) of clone 5 (☐), 12 (▨) and 17 (■) from 1989 to 1995, inclusive.

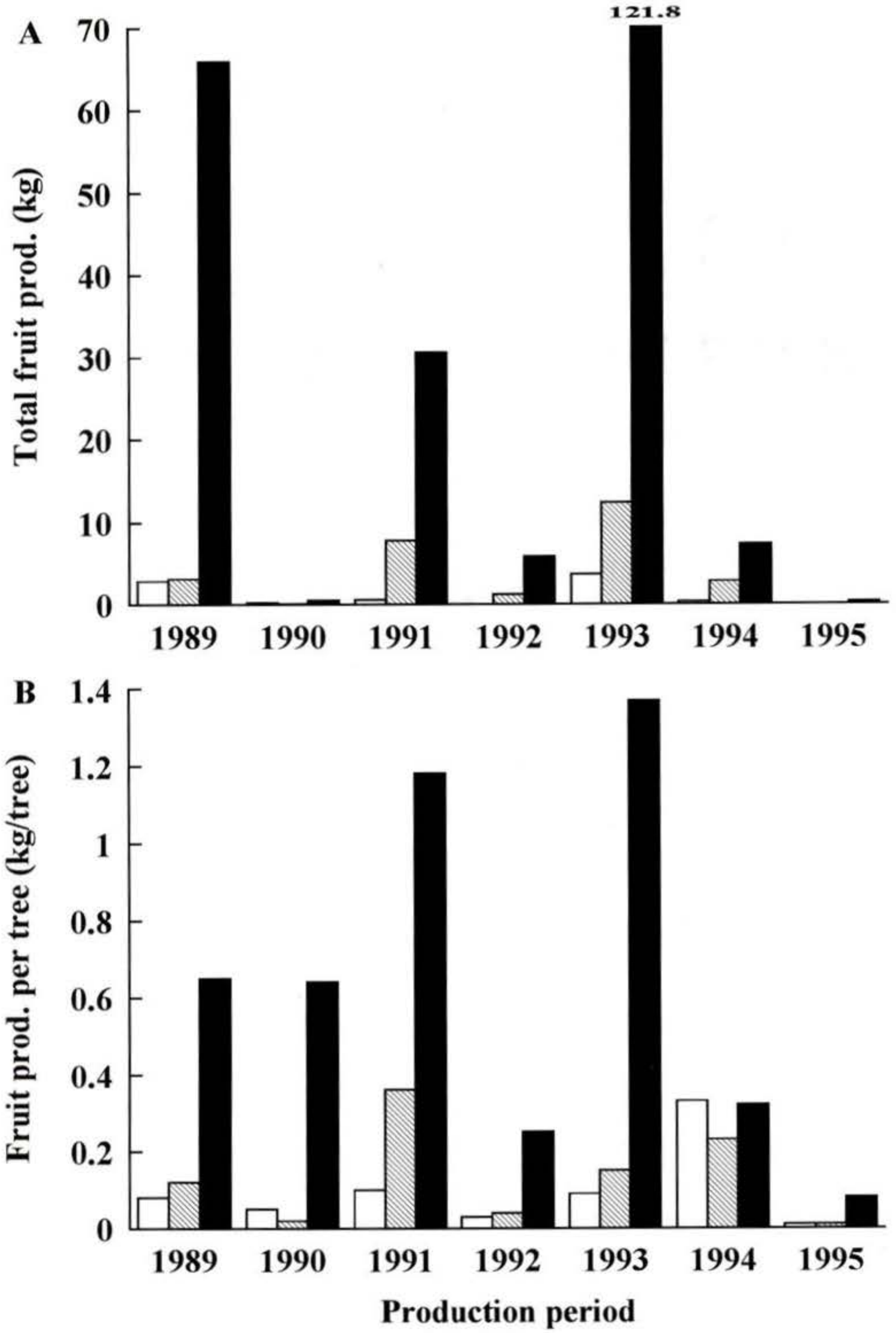
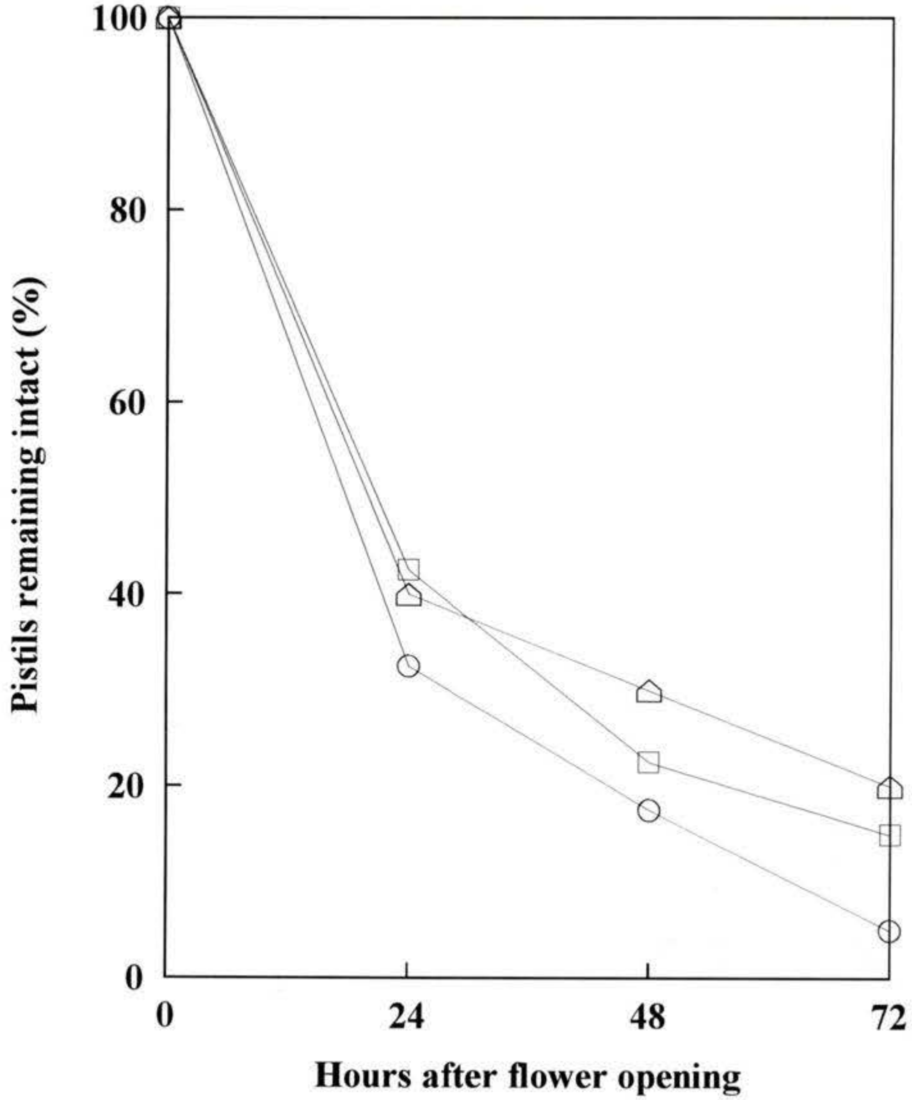

A
121.8
70
60
50
40
30
20
10
0
Total fruit prod. (kg)
1989
1990
1991
1992
1993
1994
1995
B
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Fruit prod. per tree (kg/tree)
1989
1990
1991
1992
1993
1994
1995
Production period

Fig. 4. Percent of pistils remaining intact for 72 h AFO for clone 5 (–⊖–), 12 (–⊟–) and 17 (–⌂–).


Pistils remaining intact (%)
100
80
60
40
20
0
0
24
48
72
Hours after flower opening

The flower is about 6 to 8 mm in diameter, hermaphroditic, whitish, with six petals that join at the base to form a short floral tube (Fig. 6). Six stamens are attached to the petals. Most ovaries contain four locules with one ovule in each locule. In a few cases where five or six ovules were found, one or two locules contained two ovules. The stigma usually is two-forked (Fig. 7), although some with three-forked stigmas were found (Fig. 8). The surface of the stigma is papillate. The style is hollow and is comprised of 10 to 12 cell layers (Fig. 9). It varies in length from 4 to 6 mm and usually protrudes beyond the stamens.

Teak pollen is spherical and has a smooth, reticulate exine with three conspicuous furrow-like apertures where a pollen tube may emerge (Fig. 10). Pollen grains are hydrated and clumped together when shed. Anthers have two chambers and they open by splitting lengthwise between 3 and 5 h AFO.

Flowering in a tree starts on the lower branches and progresses upward. During the flowering period, about 30 to 100 flowers opened in a panicle each day with an average of about 60 flowers opening per day. Clones differed not only in time of flowering during the season but also time of flower opening during the day. Flowers only have a one day cycle. In clone 5, the early-flowering clone, flowers opened between 04:00 and 06:00 h, clone 17 between 05:00 and 07:00 h, and clone 12, the late-flowering clone, between 06:00 and 08:00 h. The style is usually still bent when flowers open, anthers are flat and pollen is not visible. In the three clones the styles straightened and the stigma became splayed and shiny between 09:00 and 11:00 h, at which time anthers began to open and shed pollen. The corollas started to shed as early as 8 h AFO, but in most cases the corollas or entire flowers were shed between 12 and 24 h AFO. If the corolla was not shed, it withered within 3 d AFO. The base of the calyx (calyx tube) elongates and joins at the top enclosing the enlarging globular ovary (Fig. 6). This indicates that fertilization had occurred. At the same time, the stigma dried but remained intact. In later stages the enclosing calyx became much inflated, formed visible calyx lobes and remained until the fruit matured.

4.3. Fruit and seed structure

Teak has a dry stone fruit. The thin papery outer layer which develops from the calyx is usually incorrectly termed the exocarp (Fig. 11). It is removed during processing disclosing the velvety surface of the fruit (Fig. 12). The pericarp has two distinct layers, the corky outer layer and the stony inner layer. The corky layer is thick, the thickness of which causes size differences in fruits among clones. Clone 5 has a thinner corky layer and smaller loci than clones 12 and 17 (Figs. 13, 14, 15). This layer dries as the fruit matures reducing fruit weight. The cork cambium in mature fruits is discontinuous between the corky and stony layers (Fig. 16). The stony layer is very compact and has very thick cell walls (Fig. 17). The surface of the stony layer that is in contact with the seed coat has many pits (Fig. 18). At the micropylar end of the stony layer, a long narrow opening is formed about 3 to 4 w AFO and persists until the seed matures. It may function mechanically for fruit opening during germination.

The dicotyledonous seed is about 5 to 6 mm long and 3 to 4 mm wide (Fig. 19) and enclosed by the stony layer. It is hemianatropous –the hilum is situated between the micropyle and chalaza. The seed coat is 2 to 3 layers of dead cells which originate from the integument. Remnants of endosperm form a layer 2 to 3 cells thick just beneath the seed coat and contain starch grains (Fig. 20). The surface of the seed coat shows ridged cell walls when the seed matures (Fig. 21). A trace of the hypostase and a vascular bundle are present on the dry seed coat. Some fruits contain a well developed seed coat without any developing seed. In these cases the remnant of endosperm was not found.

4.4. The ovule

The teak ovule is hemianatropous, in which the micropyle shifts about 90° toward the future hilum (Fig. 22). It is about 0.5 mm long and 0.2 mm wide when the flower opens and has one integument that is not distinctly separated from the thin nucellus. The integument consists of 6 to 8 layers of cells and the nucellus is 1 to 2 layers of cells. The nucellus only forms around the upper half of the embryo sac where the latter narrows. The nucellus has more dense cytoplasm and stains darker than the integument (Fig. 25). A vascular bundle is well developed when the ovule matures. It ends at the chalazal region where the nucellus and integument merge. The cup-like chalazal region has dense cytoplasm and thick cell walls and is termed the hypostase (van Tieghem, 1901). It is present at the chalazal end of the ovule from the time the embryo sac matures and dries as the seed matures.

The mature embryo sac is elongate and wide at the micropylar end and narrow at the chalazal end where it is embedded in the hypostase. It contains seven cells: the egg cell, two synergids, the central cell with two nuclei, and three antipodals. The egg cell is larger than the two synergids and its nucleus lies at the distal end of the cell. These three cells are anchored at the micropylar end (Fig. 23). The antipodals are small and clumped

Figs. 5-10. Teak floral structures.

Fig. 5. Teak inflorescence is a panicle. Flower (arrow). Bar = 50 cm.

Fig. 6. Teak flower with six anthers and stigma (arrow). The elongated calyx tube encloses the fertilized ovary (arrowhead). Flower bud on the right. Bar = 3 cm.

Fig. 7. Scanning electron micrograph of two-forked stigma with papillate surface (arrow). Bar = 100 μm.

Fig. 8. Scanning electron micrograph of three-forked (*) stigma. Bar = 200 μm.

Fig. 9. Light micrograph of longitudinal section of the hollow style (st) consisting of 10-12 layers of cells. pt, pollen tube. Bar = 100 μm.

Fig. 10. Scanning electron micrograph of pollen at flower opening with three apertures (*) where the pollen tube emerges. Bar = 10 μm.

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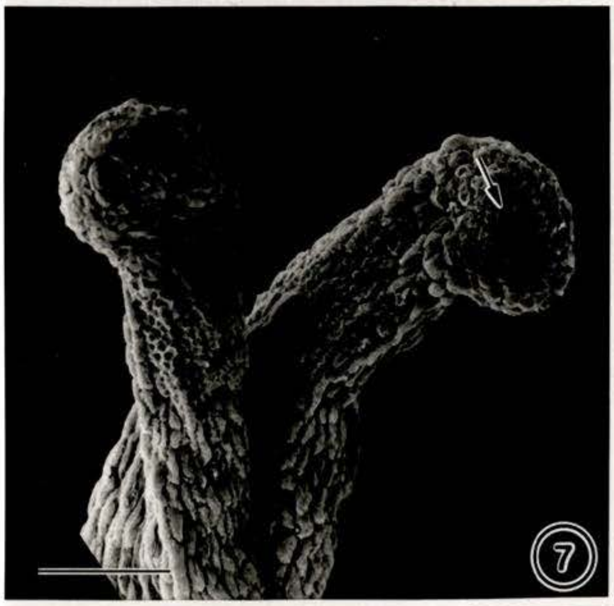

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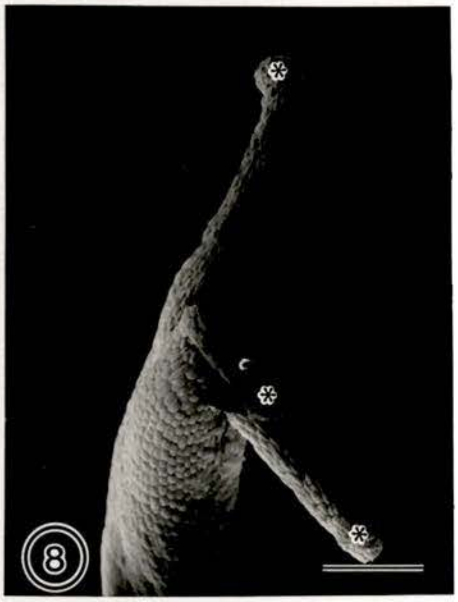

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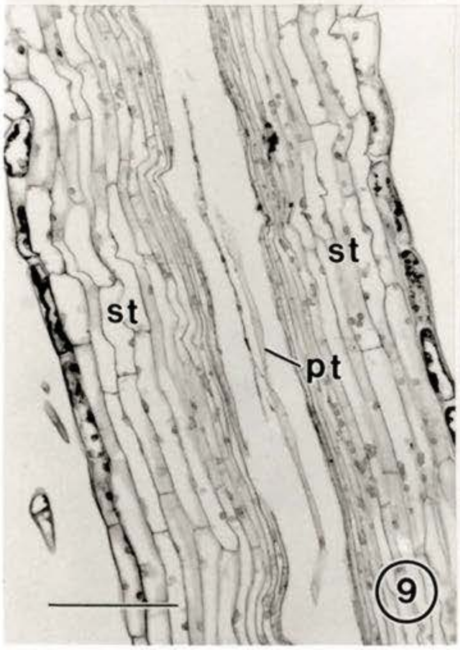

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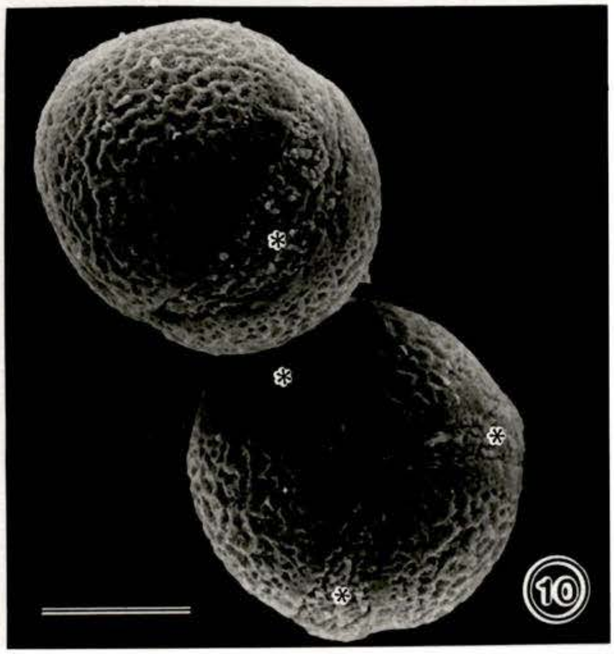

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Figs. 11-21. Teak fruit and seed structures.

Fig. 11. Mature fruits with enclosing calyx (arrows).

Fig. 12. Fruits after calyx removed.

Figs. 13, 14, 15. Cross and longitudinal sections of fruits from clones 5, 12 and 17, respectively, showing fruit coat layers, corky layer (c) and stony layer (s). The fruit diameter of clone 5 is less than in clones 12 and 17, which also have a thicker corky layer and larger loci. Some locules are filled (*),whereas others are empty but contain a well developed seed coat (arrows).

Fig. 16. Scanning electron micrograph of corky and stony layers with cork cambium between (arrowhead). Bar = 100 μm.

Fig. 17. Scanning electron micrograph of stony layer showing the thick cell walls (arrow) and the inner surface that is in contact with the seed coat (arrowhead). Bar = 20 μm.

Fig. 18. Scanning electron micrograph of inner surface of the stony layer showing pits (arrow) inthe cell walls. Bar = 5 μm.

Fig. 19. Mature seed showing a trace of the hypostase and the vascular bundle (arrow). The hilum (arrowhead) is between the micropylar and chalazal ends. Bar = 5 mm.

Fig. 20. Scanning electron micrograph of remnants of endosperm containing starch grains (arrow) beneath the seed coat (arrowhead). Bar = 20 μm.

Fig. 21. Scanning electron micrograph of seed coat surface showing ridged cell walls. Bar = 20 μm.

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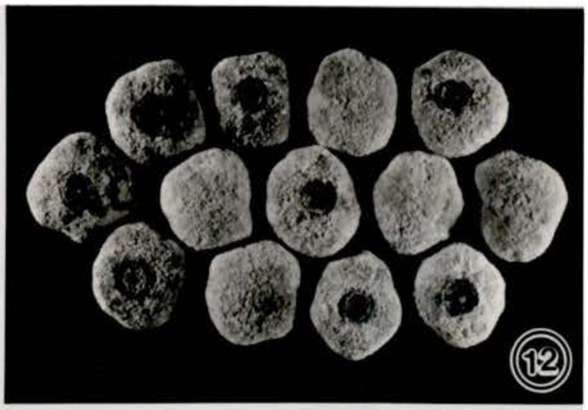
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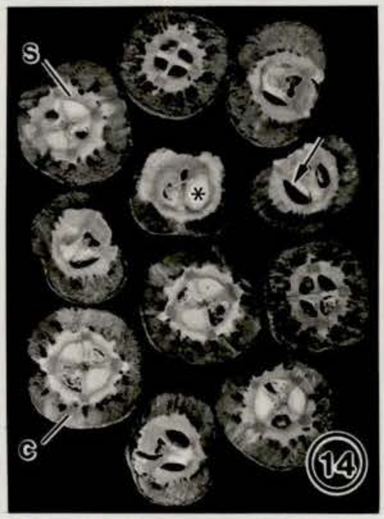
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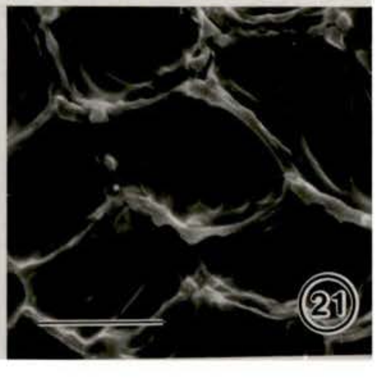
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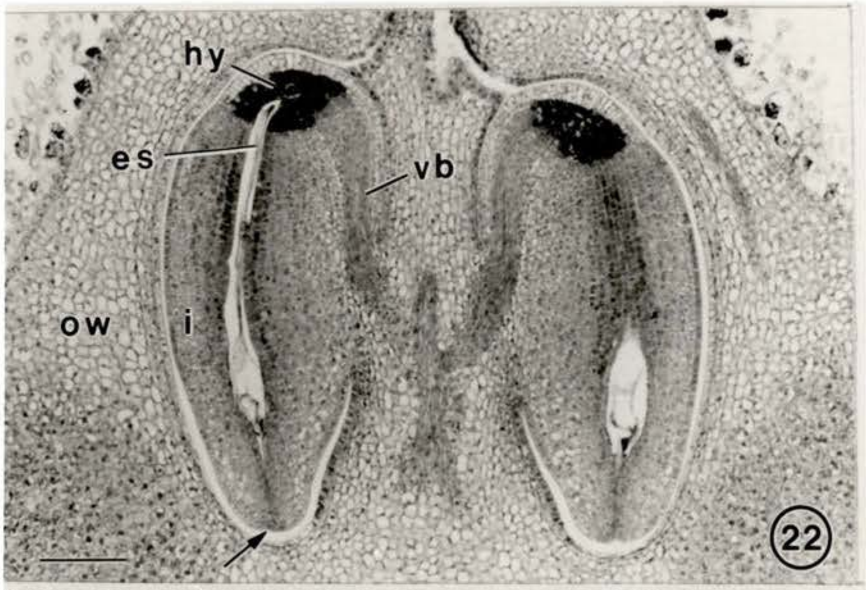
Figs. 22-25. Light micrographs of longitudinal sections of teak ovules.

Fig. 22. Median section of pistil showing two hemianatropous ovules.Pollen tubes grow along the inner side of the ovary wall (ow) to the micropyle (arrow). The hypostase (hy) at the chalazal end is where the embryo sac (es) is embedded and the ovule vascular bundle (vb) ends. The embryo sac is elongate, wide at the micropylar end and narrow at the chalazal end. i, integument. Bar = 10 µm.

Fig. 23. Egg apparatus at the micropylar end consists of the egg (e) and two synergids (sy). Bar = 1 µm.

Fig. 24. Three antipodals (*) within the embryo sac at the chalazal end are embedded in the hypostase. Bar = 0.5 µm.

Fig. 25. Central cell with the two polar nuclei (pn). The nucellus (nu) consists of two cell layers. The integument has 6-8 cell layers. Bar = 1 µm.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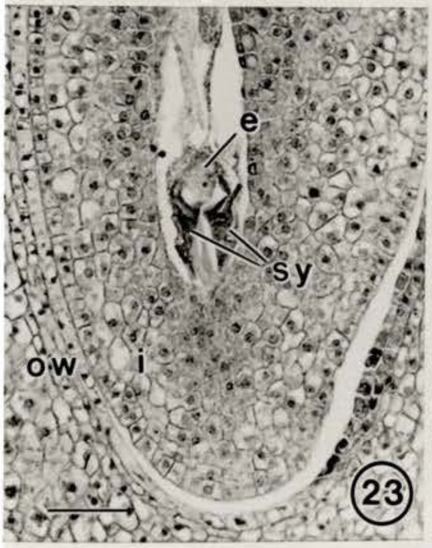
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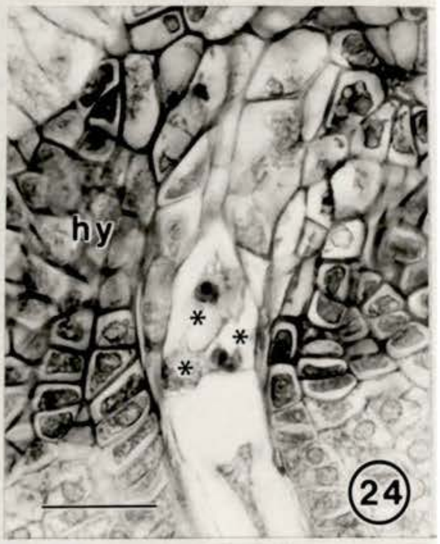
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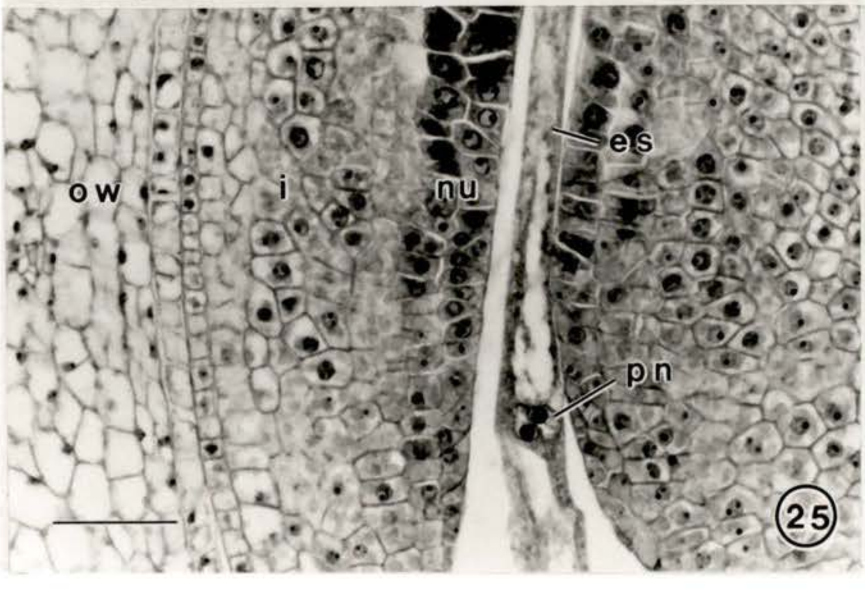
es
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together at the chalazal end of the embryo sac (Fig. 24). They degenerated between 16 and 24 h AFO, just before fertilization. The central cell is vacuolate and has the two largest nuclei in the embryo sac (Fig. 25). They fuse 16 to 24 h AFO to form a secondary nucleus that lies near the egg cell.

4.5. Pollination and fertilization

Pollination occurs when pollen grains are transferred to the stigmatic surface. Most teak pollen grains germinate after they are transferred to the stigma (Fig. 27). Some pollen grains stuck on the stigmatic surface but did not germinate until 28 h AFO. Pollen tubes start to penetrate the hollow style as early as 4 h AFO, although only 1.04% of pistils had at least one pollen tube in the style at that time. After growing between the transmitting tissue cells of the style (Tangmitcharoen and Owens, 1996) the pollen tubes grow along the inner surface of the ovary wall to the micropyle.

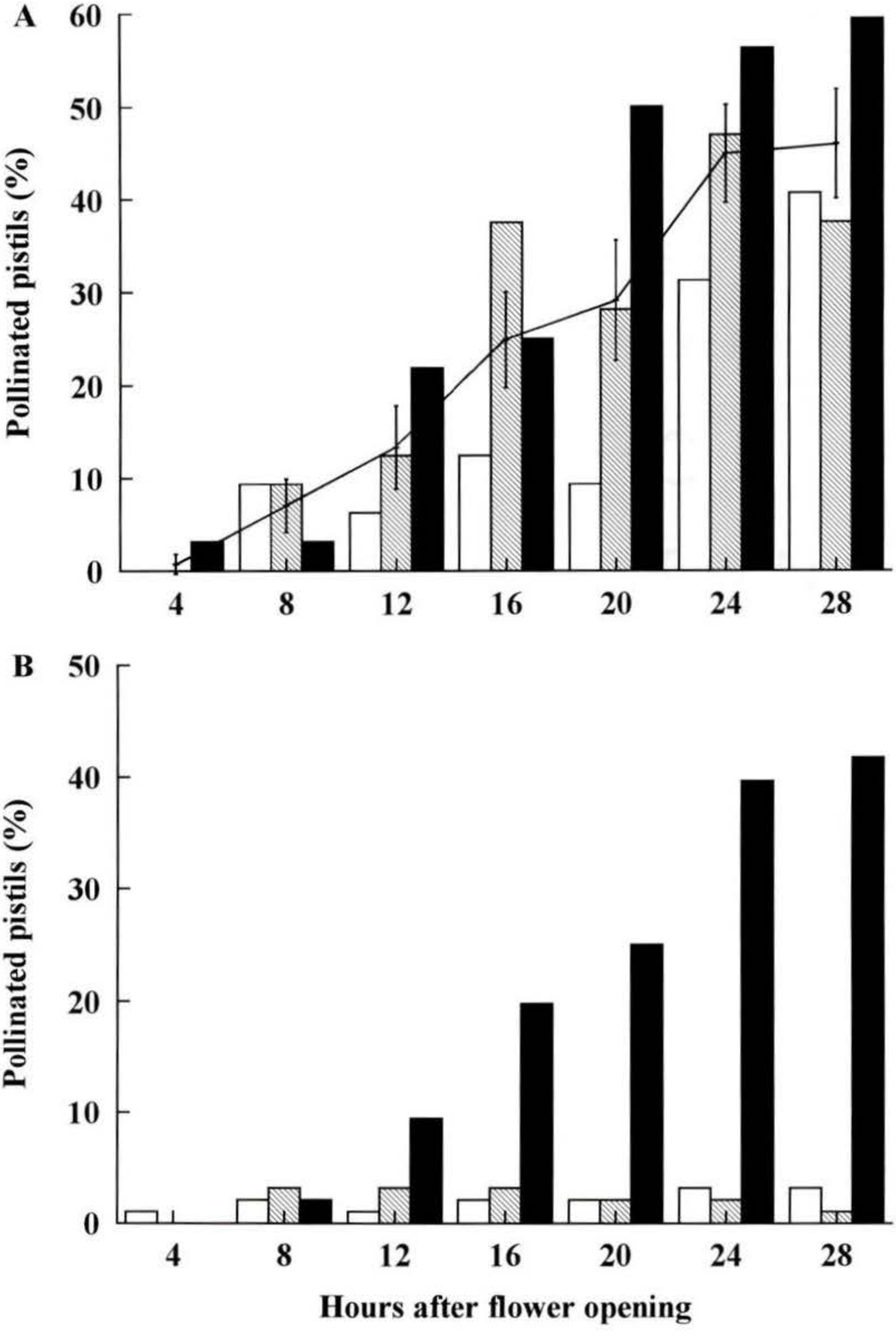
The percentage of pollinated pistils (pistils with at least one pollen tube growing into the style) varied depending on collection time. Results of the fluorescence test showed that the mean percentage of pistils with at least one pollen tube in the style was very low in the first 12 h AFO. It increased sharply at 16 h AFO then continued to increase ($p<0.01$) until it reached its highest percentage at 24 and 28 h AFO (44.79 and 45.83%, respectively). It remained about the same among clones throughout the collections, except for the collection at 20 h AFO, at which time clone 17 had a higher percentage than clone 5 (Fig. 26A).

The number of pollen tubes in the style of pollinated pistils varied among clones and collection times, ranging from 1 to 32. In cases when pollen tubes were numerous and twisted it was difficult to count them accurately. Pollen-tube growth rate varied considerably. The percentage of pistils with at least one pollen tube reaching the ovary or micropyle increased considerably from 8 h AFO (2.08) to 24 h AFO (39.58%)(Fig. 26B). In general, only a few pistils (μ=2.08-2.43%) contained pollen tubes that were arrested in the upper or lower style regardless the collection time.

Several abnormalities of pollen-tube growth were found in open pollinated flowers in the three clones, including pollen-tube arrest in the style, reversal in growth direction, irregular growth by thickening some part of the tube, bending of tube tip in the micropyle forming a hook-shape and swelling of tube tip after entering the micropyle (Figs. 28, 29, 30, 31, 32). Despite the many abnormalities, most pollen tubes were able to reach the ovary or micropyle. There were several instances where pollen tubes formed a plug in the micropyle preventing other pollen tubes from penetrating (Fig. 33).

Fertilization occurs when a pollen tube enters the micropyle and penetrates one of the synergids, usually termed the receptive synergid or degenerated synergid, and deposits two male gametes (Fig. 34). The paraffin sections show that the receptive synergid has more dense cytoplasm than the persistent synergid. The fluorescence test shows the receptive synergid fluoresces brightly. Fertilization is evident when the pollen-tube tip fuses with the fluorescing synergid. This is followed by the appearance of fluorescing debris at the chalazal end of the synergid (Fig. 35) indicating degeneration of the synergid cell wall. Eventually both synergids degenerate as endosperm starts to develop. Paraffin sections reveal that the pollen tube enters a micropyle between 16 and 24 h AFO, supporting the data from the fluorescence test.

Fig 26. Percent pollinated pistils at each collection for clone 5 (□), 12 (▨) and 17 (■) (A) and percent pollinated pistils with the longest pollen tube reaching the upper half of the style (□), the lower half of the style (▨) and the lower part of inner side of ovary wall and/or micropyle (■) (B). The line with error bars represents the means of pollinated pistils of the three clones at each collection. Pollinated pistils were determined as pistils with at least one pollen tube in the style.


A
60
50
40
30
20
10
0
Pollinated pistils (%)
4
8
12
16
20
24
28
B
50
40
30
20
10
0
Pollinated pistils (%)
4
8
12
16
20
24
28
Hours after flower opening

Figs. 27-33 and 35. Epifluorescence micrographs of portions of teak pistils.

Fig. 27.	Style (st) showing pollen germinated on the stigma (arrow) and pollen tubes (arrowhead). Bar = 50 µm.
Fig. 28.	Arrested pollen tubes (arrows) in the style. Bar = 200 µm.
Fig. 29.	Pollen tube reversing direction (arrow). Bar = 100 µm.
Fig. 30.	Pollen tube showing irregular growth and tube thickening (arrow). Bar = 100 µm.
Fig. 31.	Hook-shaped pollen-tube tip (arrow) in the micropyle has failed to reach the synergid (sy). ov, ovule. Bar = 1 µm.
Fig. 35.	Fertilization indicated by entry of the pollen tube (arrow) into the synergid. The fluorescing debris indicates degeneration of the synergid. Bar = 2 µm.

Figs. 32-34. Light micrographs of portions of ovules.

Fig. 32.	Swelling of pollen-tube tip (arrow) in the micropyle. Bar = 0.5 µm.
Fig. 33.	Micropylar end of ovule showing pollen tube filling the micropyle (arrow). Bar = 0.5 µm.
Fig. 34.	Egg apparatus showing two male gametes (arrows) in one synergid. e, the egg. Bar = 0.5 µm.

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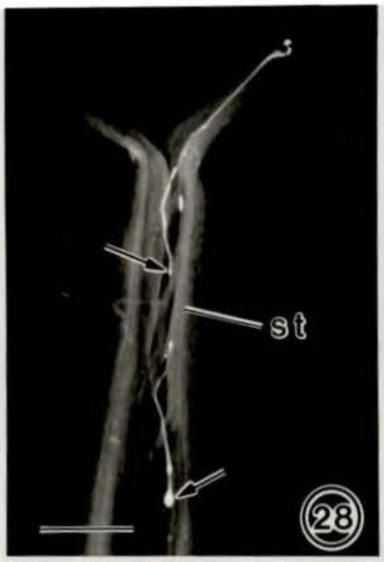

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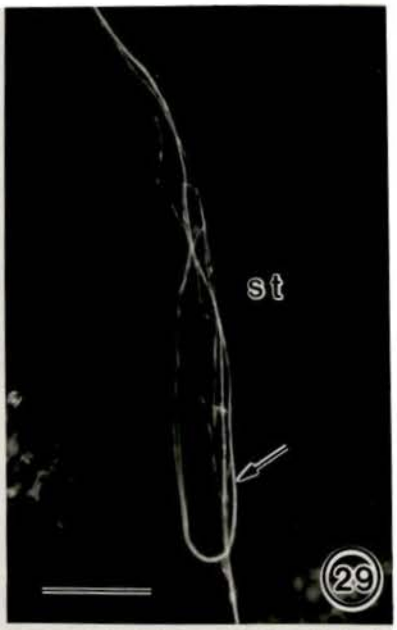

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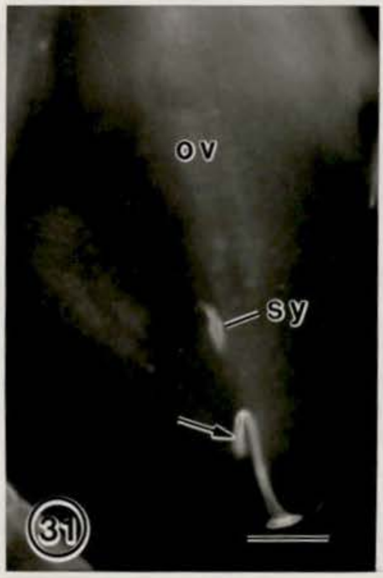

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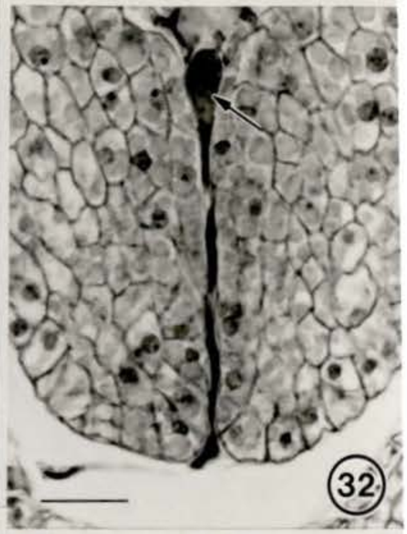

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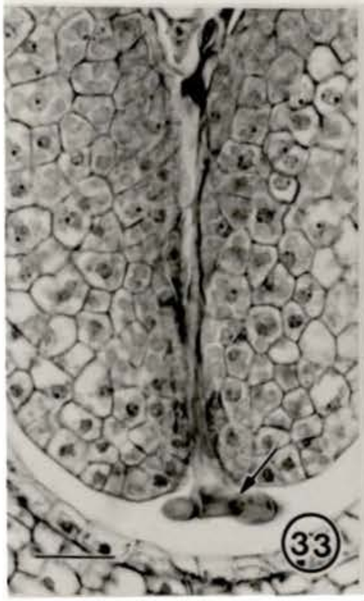

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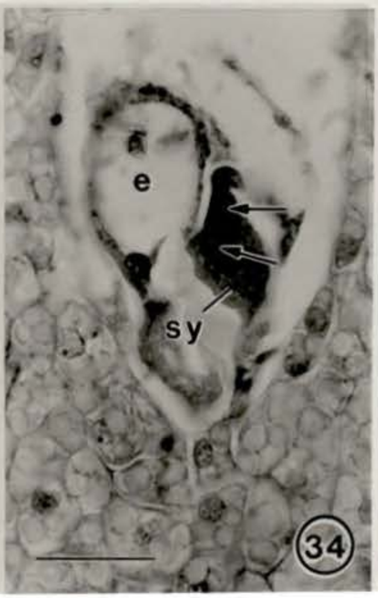

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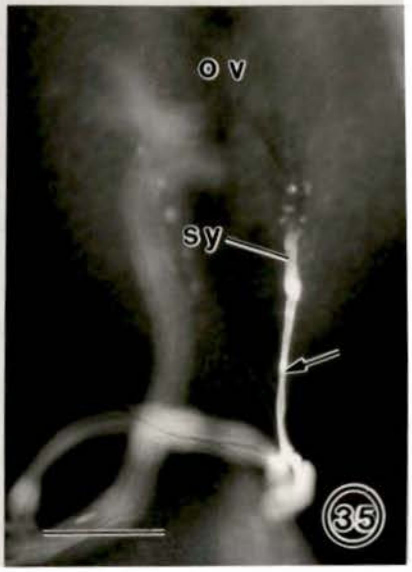

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Insect infestations cause damage in different parts of the pistils at the pollination and fertilization stages. Damage to the stigma prevents pollination; damage to the style causes pollen-tube growth disruption; and damage to the ovary or ovule prevents fertilization or embryo development. Insect infestations also occur during embryo and fruit development. In these cases the insects damage the outer corky layer as the stony layer hardens leaving only epidermal cells of the corky layer. Although the embryo continues to develop in this case, the fruits usually does not germinate.

4.6. Post fertilization

4.6.1. Ovule and endosperm development

At fertilization, one male gamete fuses with the secondary nucleus to form the primary endosperm. This cell divides into two polarized cells forming micropylar and chalazal chambers (Figs. 36, 61), which later become multinucleate. The cell at the micropylar end divides and forms the early endosperm , whereas the cell at chalazal end, the chalazal cell, embeds its tip in the hypostase. The early endosperm starts to develop as early as 32 h AFO. Its cells are either uninucleate or multinucleate and are large and elongate with slightly thickened cell walls loosely connected to each other. They develop very rapidly and occupy almost the whole embryo sac within 1 w AFO (Fig. 37), at which time the ovule has increased in size to about 3 to 4 mm (Fig. 61). Embryo sacs with fully developed early endosperm enlarge (Fig. 38) whereas ones with undeveloped early endosperm remain small (Fig. 39). At this time a more compact group of cells, the endosperm proper, starts to form at the chalazal end of the early endosperm. It develops either from the chalazal cell or from the distal part of the early endosperm. Two groups of cells are very obvious at this stage, the early endosperm at the micropylar end and the endosperm proper at the chalazal end (Figs. 40, 61). The endosperm proper becomes oblong and separates from the surrounding nucellus and integument. Its cells are smaller than the early endosperm cells and are oppressed to each other. The tip of the endosperm proper is embedded in the hypostase where it directly connects to the maternal plant through a projection which functions as the chalazal haustorium (Fig. 41). The endosperm proper continues to develop and occupies about a half of the embryo sac 3 to 4 w AFO. It pushes against the nucellus and the integument which become vacuolate and start to degenerate. The early endosperm cells become compressed and vacuolate. At this time the chalazal end of embryo sac has enlarged and become much wider than the micropylar end.

The endosperm proper continues to enlarge and 4 to 5 w AFO , its cells become vacuolated and empty (Fig. 42). The cells in the vicinity of the embryo start to degenerate, but the cells at the micropylar end of the endosperm proper, where the suspensor is anchored, remain intact and stain darkly. By this time the early endosperm cells have completely degenerated (Figs. 43) and the inner layer of the pericarp, which later becomes the stony layer, becomes lignified. These cells stain darker than the outer layer, which later develops into the corky layer. A long and narrow opening at the micropylar end of the stony layer is formed (Fig. 44) which later functions in mechanical opening during germination.

Between 6 and 7 w AFO the endosperm proper enlarges, filling about half of the ovule, and causing the integument to further degenerate, leaving 3 to 5 layers of vacuolate cells in the outer layer of the integument. The inner and radial cell walls of the outer layer of the integument thicken forming a ‘u’ shape. The cells of the endosperm proper also degenerate as a result of embryo development. At this time, the cytoplasm of the cells at the chalazal end, the hypostase, start to degenerate.

Between 8 and 11 w AFO the ovule reaches its maximum size about 5 mm (Fig. 61). The endosperm proper has enlarged, almost filling the ovule, but most of its cells have degenerated as a result of the developing embryo, leaving only 3-4 outer layers of intact endosperm cells. These cells are densely cytoplasmic and persist until the seed matures. The SEM observations show that these cells are filled with starch grains. The proximal end of the endosperm proper, where the suspensor is embedded, starts to degenerate. The outer integument layer becomes compressed and its cell walls ridged. There is no further ovule enlargement at this stage although the embryo is still developing.

The outer layer of the integument persists as the seed matures 14 to 16 w AFO. The dead cells and the ridged cell walls harden and become the seed coat. The hypostase and vascular bundles are dried but their traces remain very obvious on the seed coat. The 3 to 4 layers of the endosperm remain intact.

Several abnormalities of endosperm development were found. In many cases the early endosperm develops during early embryogenesis but the endosperm proper fails to form. In these cases the embryo may partially develop, but when it does, it aborts as the early endosperm degenerates (Fig. 45). These ovules may abort at an early stage of development or continue to enlarge as the fruits develop but eventually abort. In some cases the endosperm proper did not develop into a globular-shaped as normally occurs, but irregularly-shaped (Figs. 46,47). This abnormal development restricts the development of the embryo, and may cause embryo abortion. In other cases the early endosperm develops toward the funicle or the integument between the micropyle and the chalaza; not toward the chalazal end of the embryo sac (Fig. 48). The cutting test shows that most of the empty locules have empty seeds of various sizes with well developed seed coats. It was also found that in some cases the ovules enlarge although there is no indication that fertilization occurs.

4.6.2. Embryo development

The second male gamete fuses with the egg to form the zygote. Both synergids degenerate after fertilization. The zygote remains at the micropylar end (Fig. 49) and does not start to divide until about 72 h AFO. Some zygotes remain undivided 1 w AFO (Fig. 50). They are much smaller than the early-developing zygotes and it is likely that these zygotes eventually abort.

After remaining at the micropylar end for some time, the zygote divides into a basal cell and an apical cell. The basal cell forms a long suspensor supporting the embryo proper. The base of the basal cell remains attached to the embryo sac wall at the micropylar end. The apical cell develops into an embryo proper 3 to 5 d AFO (Figs. 51, 61), after the early endosperm develops. The suspensor elongates pushing the early globular-shaped embryo between the early endosperm cells into the endosperm proper 1 to 2 w AFO. The embryo then divides to form a late globular-shaped embryo 2 to 3 w AFO (Figs. 52, 61). The early endosperm cells starts to degenerate at that time. In some

Figs. 36-44. Light micrographs of endosperm development in teak.

Fig. 36. Longitudinal section of an ovule 28 h AFO showing micropylar (mc) and chalazal chambers (cc). Bar = 10 μm.

Fig. 37. Early endosperm (ee) 3-5 d AFO filling the embryo sac. nu, nucellus; i, integument. Bar = 10 μm.

Fig. 38. Cross section of fully developed early endosperm. Bar = 10 μm.

Fig. 39. Cross section of undeveloped early endosperm. Bar = 20 μm.

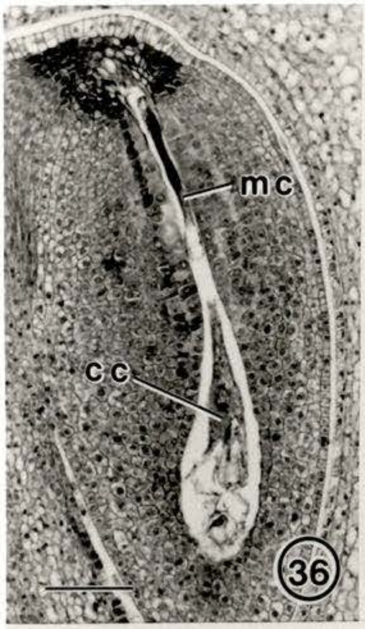
Fig. 40. Endosperm development 1-2 w AFO showing early endosperm comprised of loosely conected large cells and endosperm proper (ep) comprised of more compact smaller cells. Bar = 20 μm.

Fig. 41. Chalazal end of the ovule 1 w AFO showing the chalazal haustorium (arrow) embedded in the hypostase (hy). Bar = 5 μm.

Fig. 42. Layers of integument (i) and endosperm proper when they start to degenerate 4-5 w AFO. The nucellus (nu) has degenerated. Bar = 20 μm.

Fig. 43. Degenerated early endosperm (arrow) 4-5 w AFO. Bar = 20 μm.

Fig. 44. A long narrow opening (arrow) forms in the stony layer (s) which has lignified 4-5 w AFO. Bar = 20 μm.



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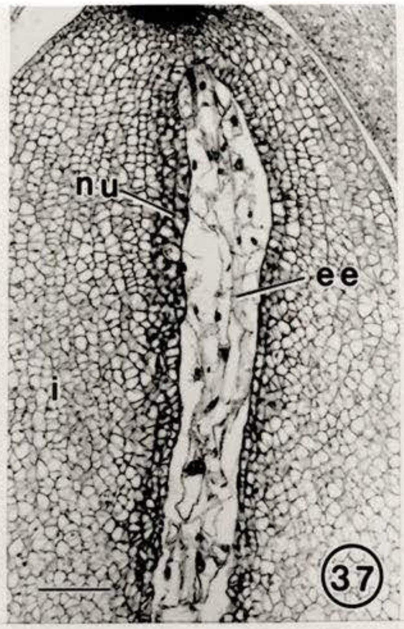

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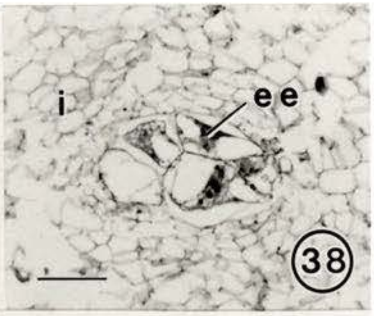

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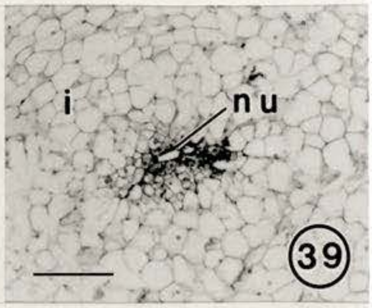

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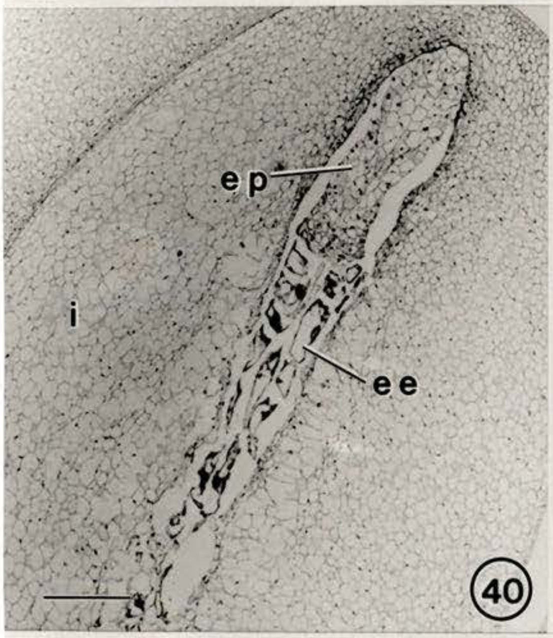

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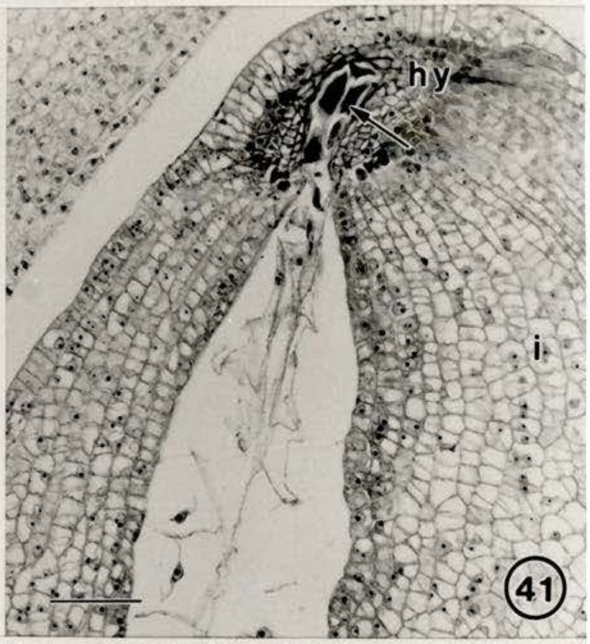

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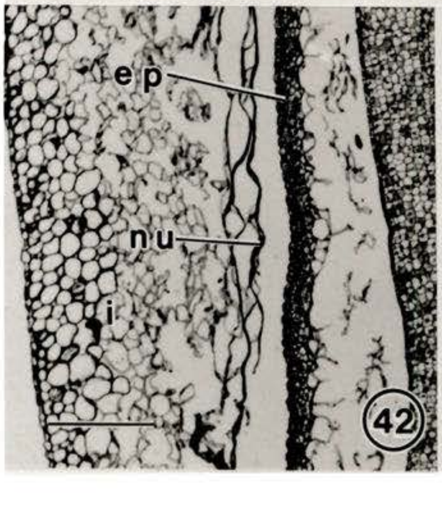

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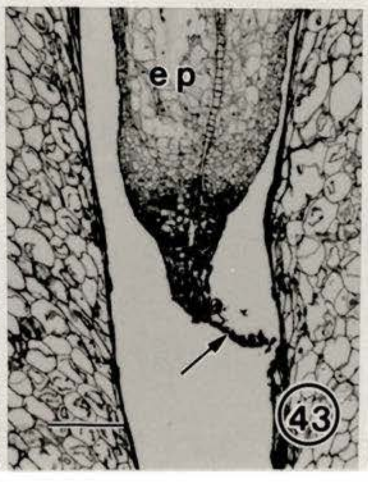

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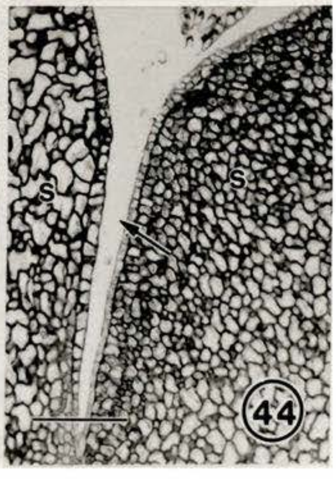


44

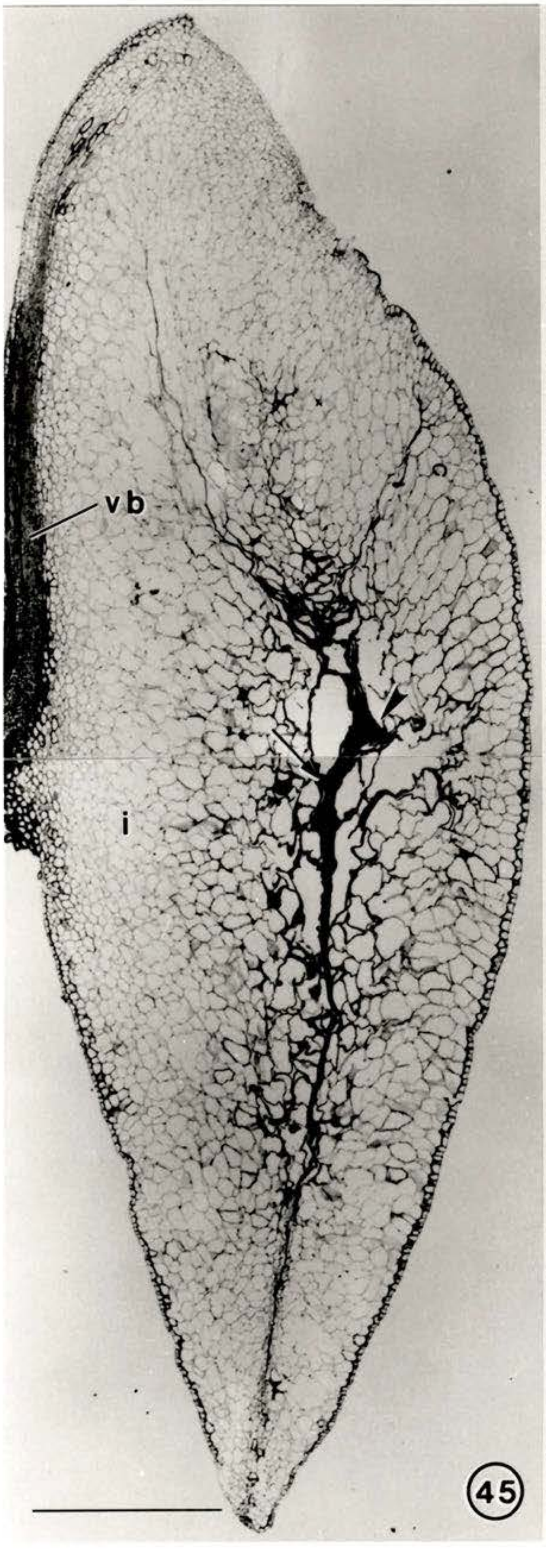
Figs. 45-48. Light micrographs of abnormal endosperm development in teak.

Fig. 45. Longitudinal section of an ovule showing aborted early endosperm (arrow) and embryo (arrowhead) and undeveloped endosperm proper. i, integument, vb, vascular bundles. Bar = 50 µm.

Fig. 46. Cross section of an ovule showing fully developed embryo (arrowhead) and normal development of endosperm proper (ep). Bar = 20 µm.

Fig. 47. Cross section of an ovule showing fully developed embryo (arrowhead) and abnormal development of endosperm proper (arrow). Bar = 20 µm.

Fig. 48. Longitudinal section of an ovule showing abnormal development of early endosperm (arrow) growing toward the side of the ovule. Bar = 10 µm.



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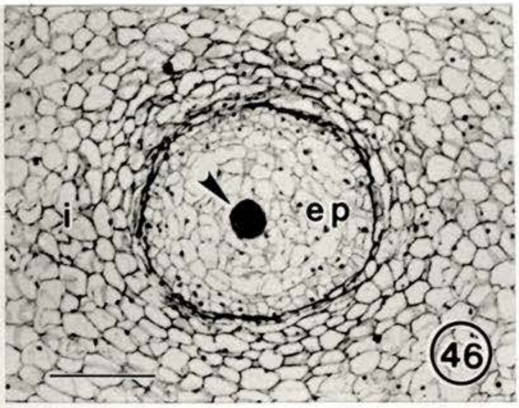

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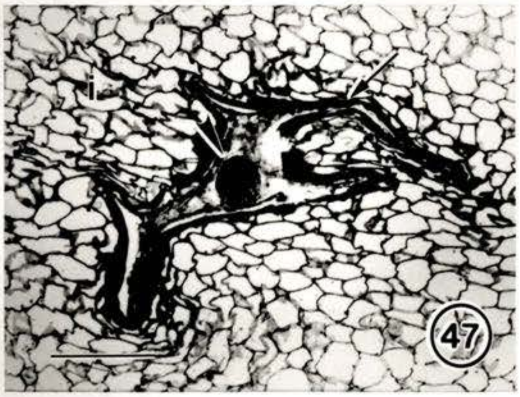

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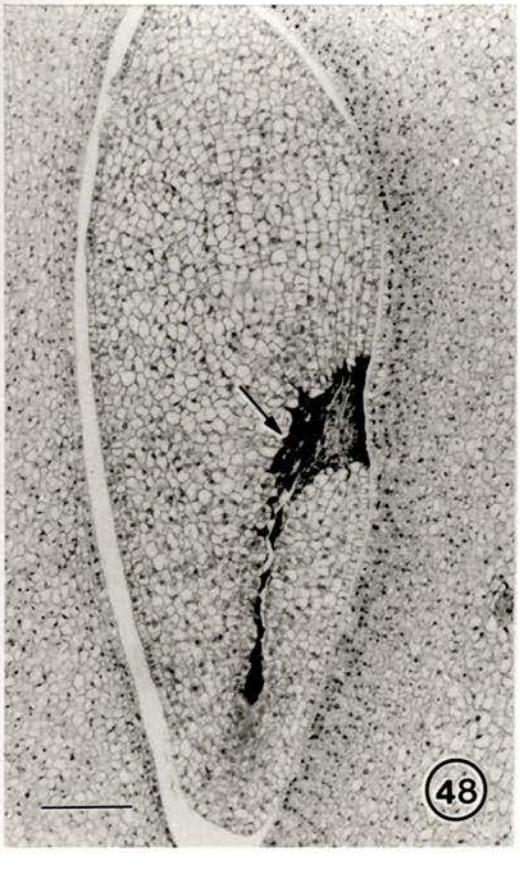


48

Figs. 49-58. Light micrographs showing embryo development in teak.

Fig. 49. Zygote (z) 36 h AFO. The two synergids have degenerated (arrows). Bar = 2 µm.

Fig. 50. Undeveloped zygote 1 w AFO (arrow). Bar = 2 µm.

Fig. 51. Embryo development 3 d AFO showing a basal cell (arrow), suspensor (su) and embryo proper (em). Bar = 2 µm.

Fig. 52. Chalazal portion of the ovule showing club-shape embryo (eb) in the endosperm proper (ep). The early endosperm (ee) has started to degenerate. hy, hypostase. Bar = 5 µm.

Fig. 53. Heart-shaped embryo (eb) with suspensor (su) 4-5 w AFO. The endosperm proper has started to degenerate. Bar = 20 µm.

Fig. 54. The suspensor is anchored at the proximal end of the endosperm proper. Bar = 10 µm.

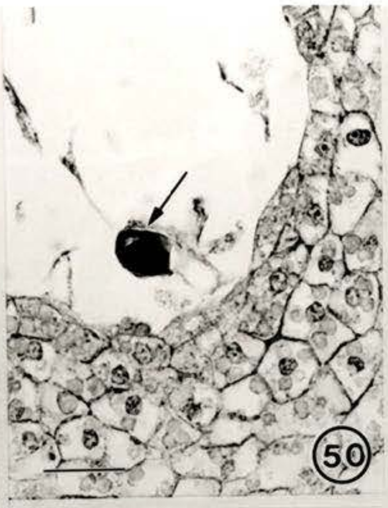
Fig. 55. Two cotyledons (co) are formed 5-6 w AFO. i, integument. Bar = 20 µm.

Fig. 56. The suspensor has degenerated and the provascular bundles (arrowheads) have developed in the embryo axis 8-11 w AFO. The 5-6 layers of endosperm cells remain (arrow) and the integument has degenerated. Bar = 20 µm.

Fig. 57. Cotyledon cells containing few starch grains (arrowhead) 8 w AFO. Only 4-5 layers of endosperm remain (arrow). Bar = 5 µm.

Fig. 58. Cotyledon cells containing many starch grains (arrowhead) 14 w AFO. Bar = 5 µm.


z
49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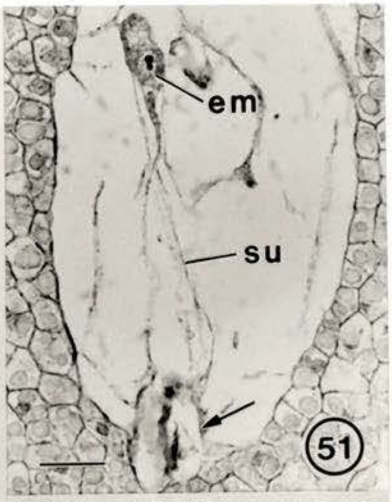
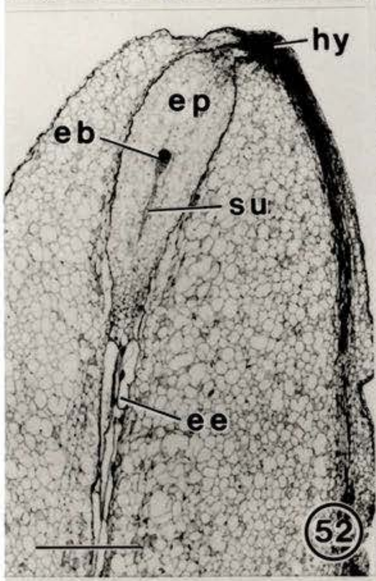
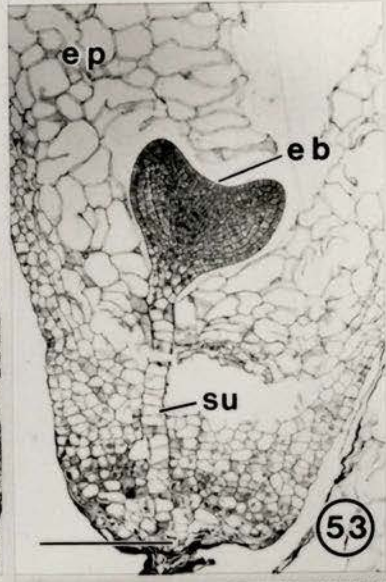
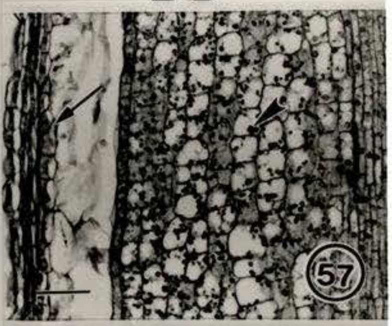

em
su
51


hy
ep
eb
su
ee
52


ep
eb
su
53


ep
su
54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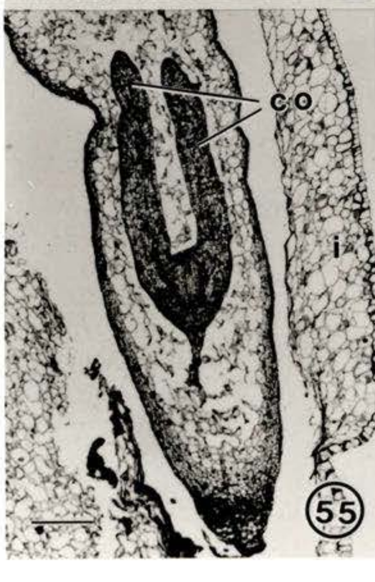
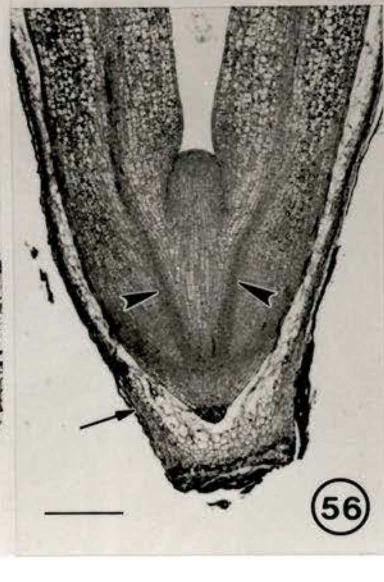

co
i
55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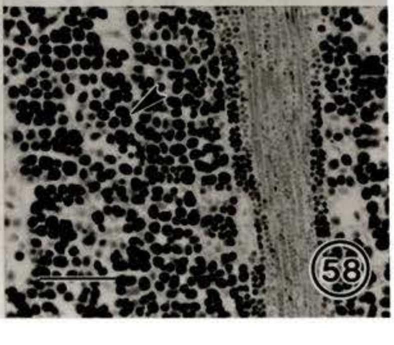
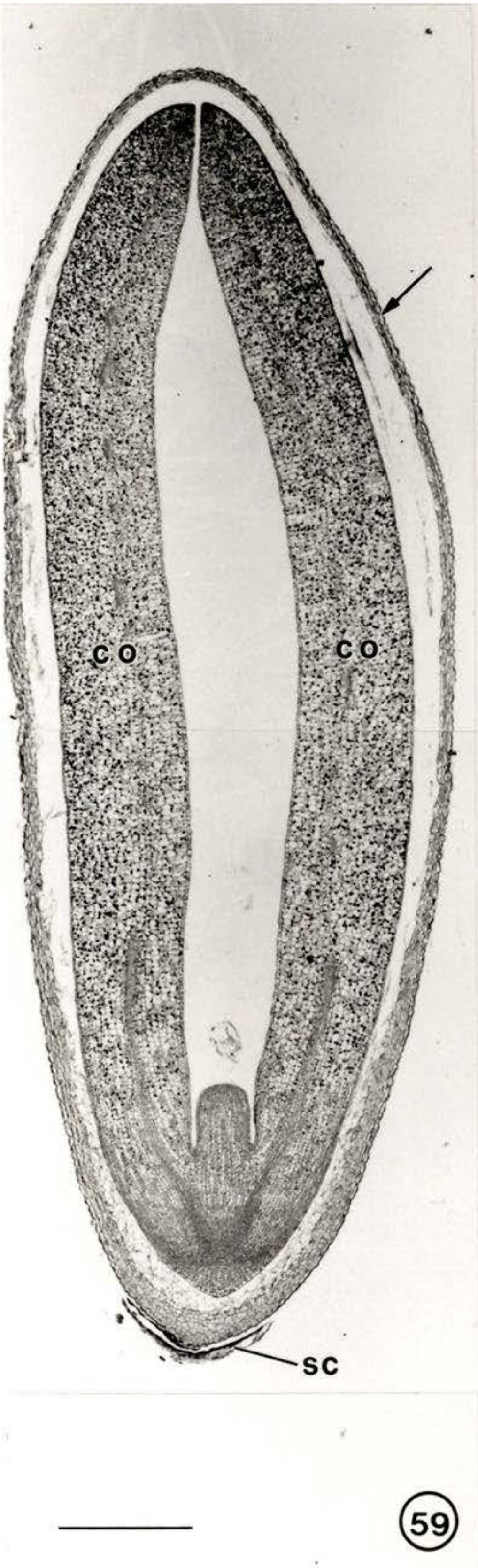

58

Figs. 59-60. Light micrographs of longitudinal sections of teak seeds.

Fig. 59. Immature seed 8-11 w AFO. The cotyledons (co) are long and slender and 4-5 layers of endosperm (arrow) remain. Most of the seed coat (sc) has been removed. Bar = 0.5 mm.

Fig. 60. Near-median section of a mature seed 14 w AFO showing thick cotyledons. Bar = 0.5 mm.



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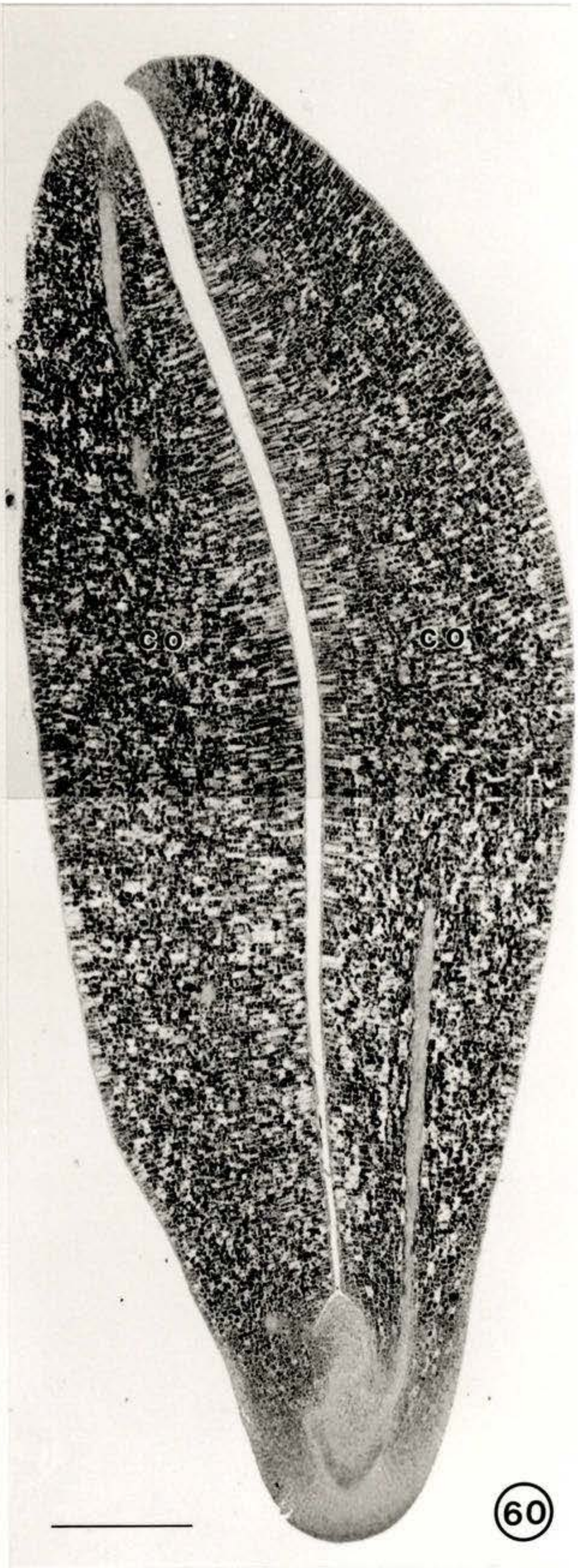
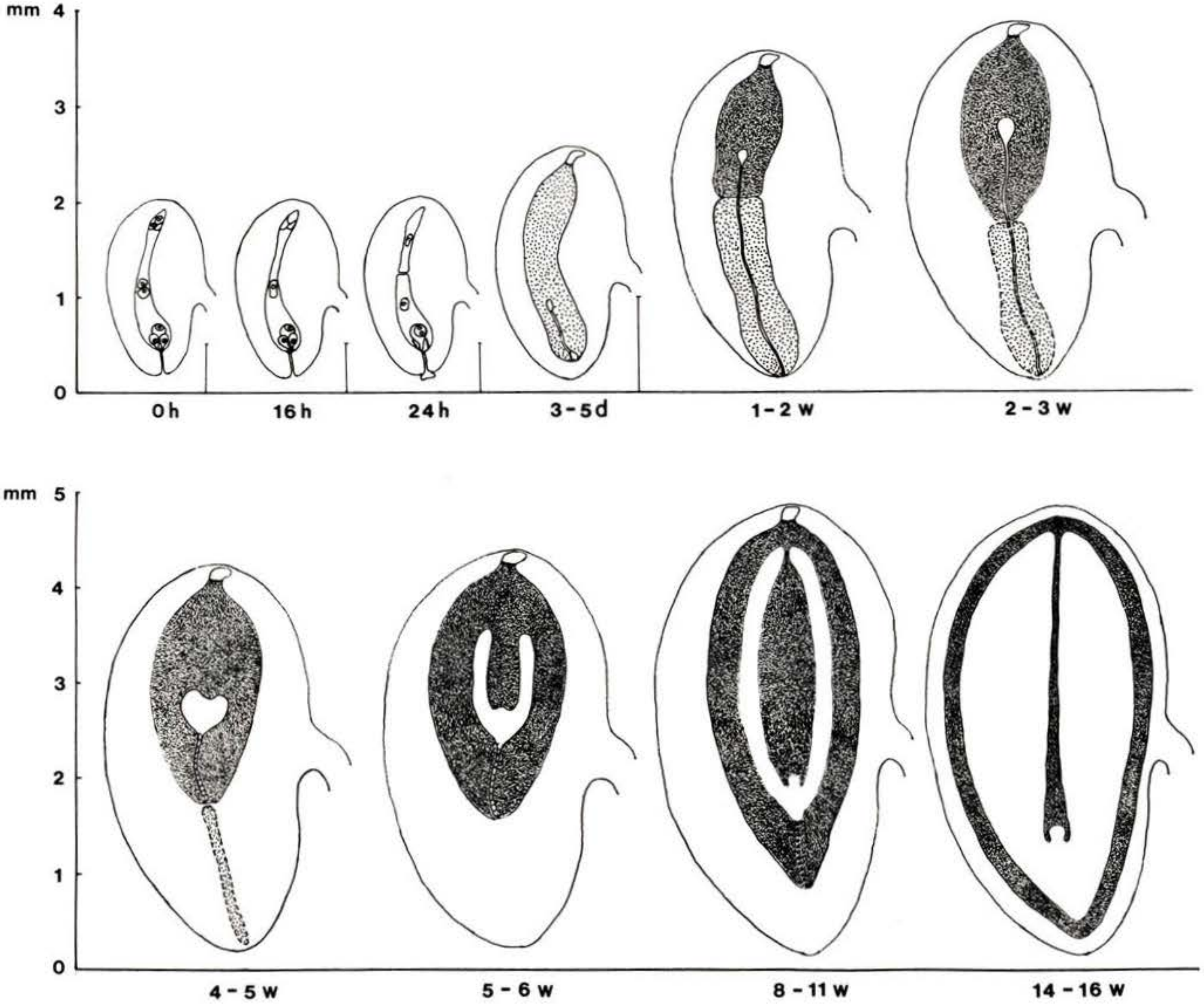


60

Fig. 61. Schematic diagram of teak embryogenesis. The two polar nuclei fuse 16-24 h AFO. Fertilization occurs 24 h AFO followed by formation of micropylar and chalazal chambers. Early endosperm forms 3-5 d AFO when the proembryo starts to develop. Endosperm proper is formed 1-2 w AFO and the embryo is at the early club-shape stage. The early endosperm starts to degenerate 2-3 w AFO when the endosperm proper has enlarged to about half the ovule length. The embryo is in late club-shape stage. The heart-shape embryo is formed 4-5 w AFO, at which time the early endosperm has degenerated. The two cotyledons form 5-6 w AFO and the suspensor starts to degenerate. About 8-11 w AFO the suspensor has degenerated and the embryo is completely developed. Most of the endosperm has degenerated, only 2-4 layers of cells remain and the cotyledons are long and slender. The seed matures 14-16 w AFO when the cotyledons are thick and fill the ovule cavity. Diagrams 0 h, 16 h, 24 h and 3-5 d have been enlarged and the lines on the right side represent the size of the ovule according to the scale. The other diagrams were drawn according to the scale.


mm
4
3
2
1
0
0 h
16 h
24 h
3 - 5 d
1 - 2 w
2 - 3 w
mm
5
4
3
2
1
0
4 - 5 w
5 - 6 w
8 - 11 w
14 - 16 w

cases no indication of embryo development was found, although the early endosperm had formed.

The embryo develops into a heart-shaped stage (Fig. 53) within the endosperm proper 4 to 5 w AFO (Fig. 61) and the embryo provascular bundles start to form. At this stage the early endosperm has degenerated and the suspensor is anchored in the micropylar end of the densely cytoplasmic endosperm proper (Fig. 54). The cotyledonous embryo (Figs. 55, 61) is formed 5 to 6 w AFO, when the suspensor begins to vacuolate and then degenerate. The embryo continues to enlarge causing the endosperm proper to further degenerate. About 8 to 11 w AFO the provascular bundles have completely developed and extend from the embryo axis to the tip of the cotyledons (Fig. 56). At this stage carbohydrate and protein content of the cotyledons is lower (Fig. 57) than at the mature stage when they fill most of the cotyledon cells (Fig. 58).

The ovule develops into its maximum size 8 to 11 w AFO, but the embryo still develops. The cotyledons are slender and long, and the root cap, which originated from hypophysis, starts to develop (Figs. 59, 61). As the seed matures 14 to 16 w AFO, the cotyledons are thick and fill the ovule (Figs. 60, 61).

4.7. Reproductive potential and reproductive success

The number of flowers per inflorescence varied among clones. Clone 12 produced significantly fewer ($p<0.05$) flowers (1331.6 Fl/Infl) compared to clone 17 (2007.2 Fl/Infl) and clone 5 (1948.1 Fl/Infl). Likewise the number of fruits per inflorescence varied among clones. Clone 17 had a higher ($p<0.01$) average number of fruits per inflorescence (35.563 Fr/Infl) than clone 12 (17.875 Fr/Infl) and clone 5 (10.375 Fr/Infl).

The clonal variation was more obvious ($p<0.01$) when comparing fruit to flower ratio in which clone 5 had the lowest ratio (0.0056) followed by clone 12 (0.0134) and clone 17 (0.0178). Clonal variation regarding the S/Fr and S/O ratios were the same since the three clones all had four ovules per fruit. Clone 5 had a S/Fr of 0.9500 and S/O of 0.2388 which was significantly lower ($p<0.05$) than clone 12 (S/Fr = 1.2200, S/O = 0.3050) and clone 17 (S/Fr = 1.2075, S/O = 0.3019). The last two were not significantly different. There was a significant difference ($p<0.01$) in PERS among clones. The overall values of PERS for all three clones was very low. Clone 5 had the lowest (0.1%) followed by clone 12 (0.4%) and clone 17 (0.5%) (Table 3).

There were significant differences among clones ($p<0.05$) in total number of inflorescences per tree (Infl/T), number of fruit-bearing inflorescences per tree (Fr-infl/T) and fruit production per tree (Fr prod/T). Clone 17 had higher Infl/T (144.00) and Fr-infl /T (129.00), than clone 5 (93.25 Infl/T and 70.25 Fr-infl /T) and clone 12 (64.00 Infl/T and 52.00 Fr-infl/T). Fruit production per tree of clone 17 was significantly higher (3358.75 g/T) than in clone 12 (467.75 g/T) and clone 5 (599.25 g/T) (Table 4).

Most mature fruits contained only one seed regardless of clone. A few contained two but rarely did they contain three or four (Table 5). Clone 5 produced the highest percentage of empty fruits (10.5%), followed by clone 12 (4.0%) then clone 17 (1.0 %).

4.8. Fruit maturation and germination

Fruit diameter increased very rapidly (from 1.98 to 13.04 mm) during the first 6 w AFO. The increase then gradually slowed during the next 4 w until the fruits reached a

Table 3. Mean numbers of flowers per inflorescence (Fl/Infl), fruits per inflorescence (Fr/Infl), fruit to flower ratio (Fr/Fl), seeds per fruit (S/Fr), ovules per fruit (O/Fr), seed to ovule ratio (S/O) and preemergent reproductive success (PERS) of the three clones.

Clone	Fl/Infl[1]	Fr/Infl[1]	Fr/Fl	S/Fr[2]	O/Fr[2]	S/O	PERS
5	1948.1^{a}	10.4^{a}	0.0054^{a}	0.9550^{a}	4	0.2388^{a}	0.0013^{a}
12	1331.6^{b}	17.9^{a}	0.0134^{b}	1.2200^{b}	4	0.3050^{b}	0.0041^{b}
17	2007.2^{a}	35.6^{b}	0.0178^{b}	1.2075^{b}	4	0.3019^{b}	0.0053^{b}

[1] Means of four inflorescences per tree and four trees per clone

[2] Means of 50 fruits per tree and four trees per clone

Means followed by the same letter are not significantly different (α=0.05) within the same column (Duncan's multiple range test).

Table 4. Means of total number of inflorescences per tree (Infl/T), fruit-bearing inflorescences per tree (Fr-infl/T) and fruit production (Fr-prod) in grams per tree (g/T) of the three clones in 1993.

Clone	Infl/T[1]	Fr-infl/T[1]	Fr-prod (g/T)[1]
5	64.00^{a}	52.00^{a}	467.75^{a}
12	93.25^{ab}	70.25^{ab}	599.25^{a}
17	144.00^{b}	129.00^{b}	3358.75^{b}

[1] Means of four trees per clone

Means followed by the same letter are not significantly different (α=0.05) within the same column (Duncan's multiple range test).

Table 5. Percentage of mature fruits with 0 to 4 seeds per fruit (S/Fr) in the three clones

No. S/Fr	Clone 5 (%)[1]	Clone 12 (%)[1]	Clone 17 (%)[1]
0	10.5	4.0	1.0
1	85.0	73.5	81.5
2	3.0	20.0	14.5
3	1.5	1.5	1.5
4	0	1.0	1.5

[1] Means of 50 fruits per tree and four trees per clone

maximum diameter ranging from 14.06 to 15.15 mm 8 to 10 w AFO. At 14 w AFO fruit diameter declined slightly as fruits entered maturation stage and started to dry (Fig. 62).

Fruit water content for the three clones averaged 77.34% at 6 w AFO. It then gradually decreased during the following 8 w as fruits started to mature, decreasing to 54.40% 14 w AFO. Rapid water loss then began, decreasing to an average of 26.90% 16 w AFO, 14.69% 18 w AFO, and 11.83% at 20 w AFO (Fig. 63). At 16 w AFO the fruit diameter also became constant (Fig. 62) and fruits were considered to be mature.

4.8.1. Experiment 1

Results from Experiment 1 showed that there were no significant interaction effects between clone and fruit size in either germination capacity (GC) or seedling dry weight (SDW). However, fruit size significantly affects GC and SDW ($p< 0.01$). The small fruits have a much lower GC (10.22%) and SDW (48.50 mg) than medium fruits (36.00% and 124.38 mg) or large fruits (40.00% and 125.21 mg) (Fig. 64A, B).

4.8.2. Experiment 2

Results from Experiment 2 showed that maturation stages M-1(16±1 w AFO), M-2 (20±1 w AFO), M-3 (24±1 w AFO) and M-4 (28±1 w AFO) significantly affected GC, peak value (PV), germination value (GV), abscisic acid (ABA) content and water content. Neither clone nor the interaction between maturation stage and clone affected these parameters. The protein content was not dependent on either clone or maturation stage.

The GC at 12 w after sowing varied among the maturation stages ($p<0.01$) with the highest obtained from fruits collected at M-3 (57.67%), followed by M-2 (45.55%), and M-4 (45%). The lowest GC was obtained when fruits were collected at M-1 (32.91%) (Fig. 65A).

Germination rates varied among the four maturation stages (Fig. 66). The germination curves showed that only 1.3 % of fruits collected at M-1 started to germinate 4 w after sowing. About 2% fruits from M-2 germinated 3 w after sowing, 1.6% fruits from M-3 germinated 2 w after sowing and 1.3% fruits from M-4 germinated 1 w after sowing. Germination rate of fruits from M-4 became constant 8 w after sowing when no more fruits germinated. Fruits from M-1, M-2 and M-3 still continued germinating 12 w after sowing as indicated by an increasing germination rates.

Peak value (PV) indicates the time of the highest germination rates of the test and was very much influenced by maturation stage ($p<0.01$). The time needed to reach the T-values was reduced with later maturation stages (Fig. 66). However, the germination obtained at the respective T-value did not follow accordingly. T-3 was associated with the highest germination followed by T-2, T-4, and T-1. The highest PV (0.87) was obtained from fruits collected at M-4 followed by M-3 (0.70), M-2 (0.53) and M-1 (0.38) (Fig. 65B).

Germination values (GVs) were obtained from PVs of M-1, M-2, M-3 and M-4 multiplied by mean daily germination (MDG) of fruits from the respective maturation stages. The MDGs of the respective maturation stages were 0.4379, 0.5174, 0.6970 and 0.5218. GVs of fruits collected at M-3 and M-4 gave similar GVs of 0.4879 and 0.4540 which were higher than those from M-1 (0.1664) and M-2 (0.2742) (Fig. 65C).

Seed abscisic acid determination showed that fruits collected at M-1 had the highest ABA content (4.77 μg/g fw). The ABA content declined to 3.23 μg/g fw 4 w later (M-2). Fruits collected at M-3 and M-4 had ABA contents between 0.84 to 1.45 μg/g fw (Fig. 67A).

At 16±1 w AFO (M-1) the seed total protein content was 33.24%. It declined slightly 4 w later to 31.05% (M-2) and remained about constant for another 8 w (between 30.07 to 30.49% at M-3 and M-4, respectively) (Fig. 67B).

The water content of fruits collected at M-1 was 14.04% and slightly declined 4 w later to 13.66% at M-2. As the fruits reached M-3 the water content declined to 11.07% and continued to decline 4 w later to 8.72% (Fig. 67C).

Fig 62. Fruit diameter during development and maturation of clones 5 (—○—), 12 (—□—) and 17 (—△—). The darkest line with error bars (—●—) represents the means of fruit diameter of the three clones at each collection.

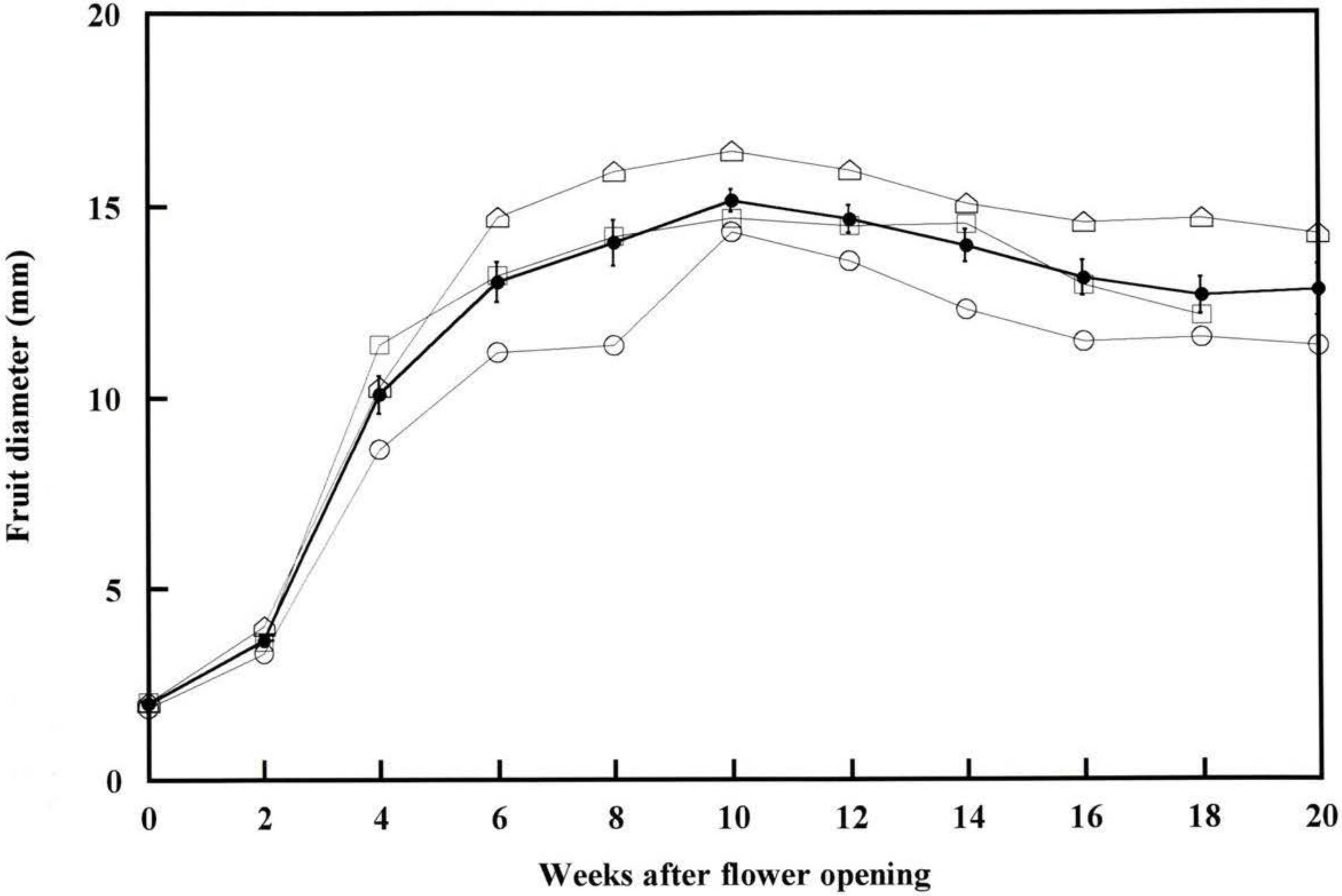

20
15
10
5
0
Fruit diameter (mm)
0
2
4
6
8
10
12
14
16
18
20
Weeks after flower opening

Fig. 63. Fruit water content during development and maturation of clones 5 (⊖), 12 (⊟) and 17 (⌂). The darkest line with error bars (◆) represents the means of the fruit water content of the three clones at each collection.

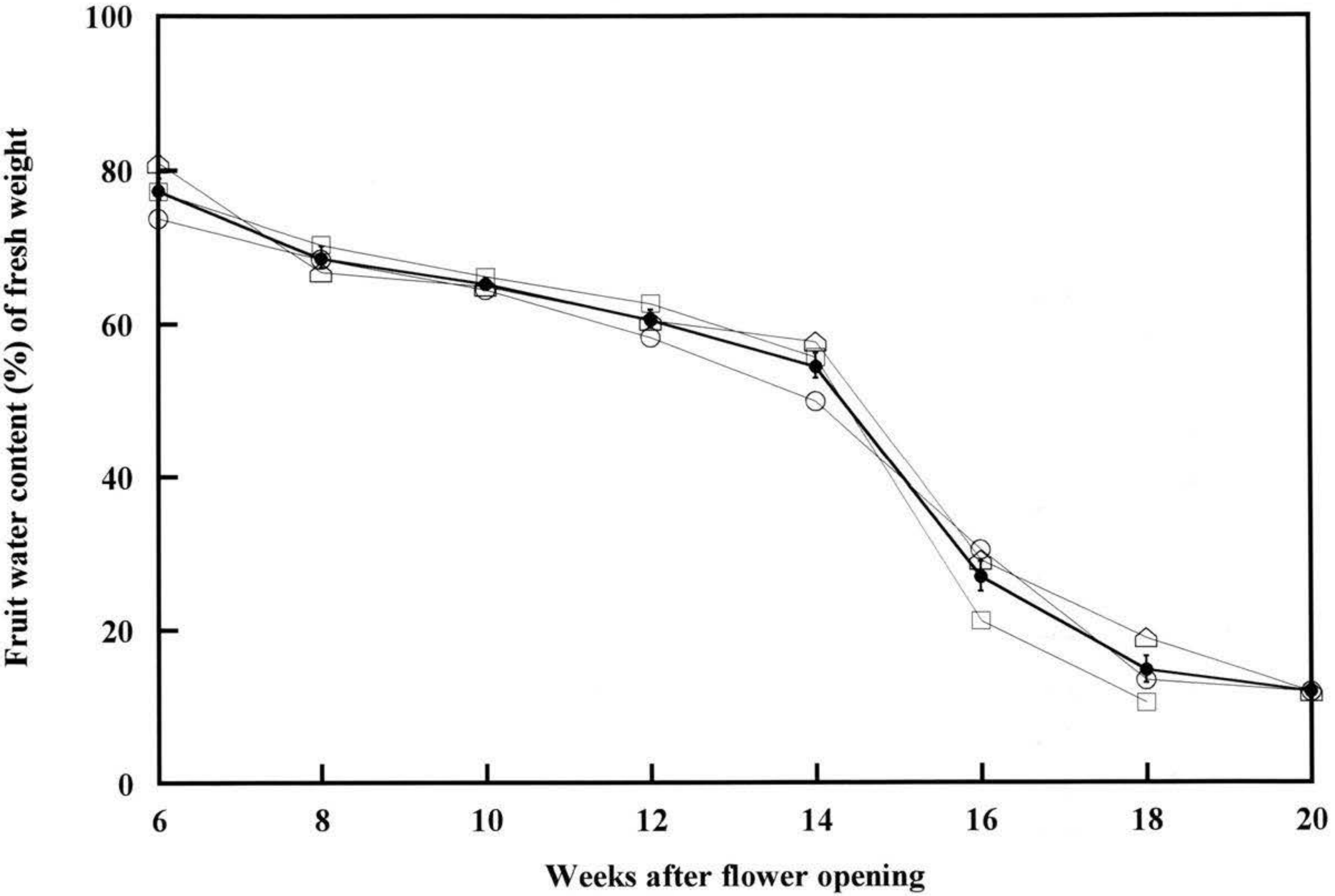

100
80
60
40
20
0
Fruit water content (%) of fresh weight
6
8
10
12
14
16
18
20
Weeks after flower opening

Fig. 64. Percent germination capacity (GC) (A) and seedling dry weight (SDW) (B) of small (diameter <10 mm), medium (diameter 10-14 mm) and large (diameter >14 mm) fruits from clones 5 (□), 12 (▨) and 17 (■). The line with error bars represents the means of the three clones from each fruit size.

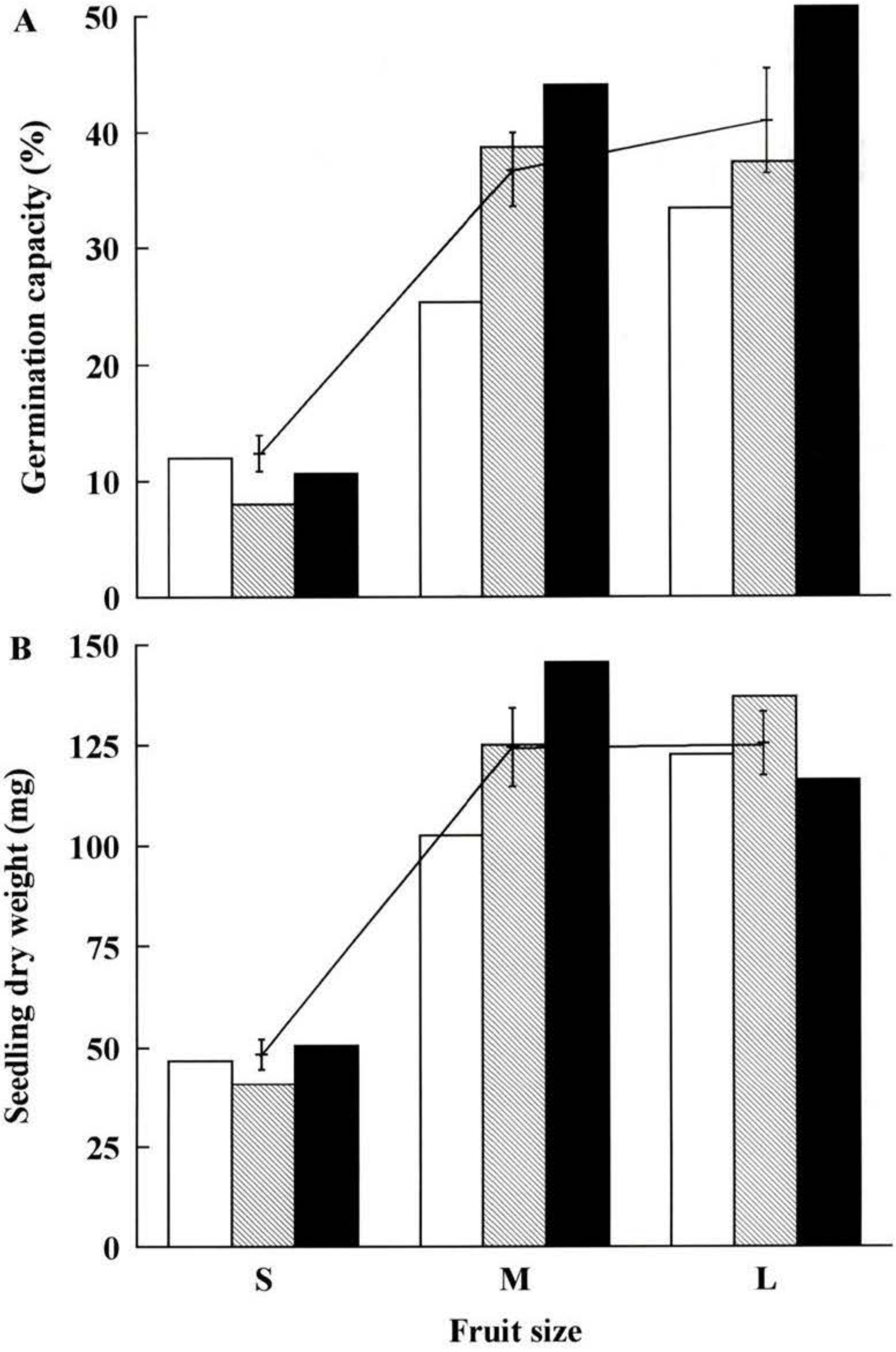

A
B
Germination capacity (%)
0
10
20
30
40
50
Seedling dry weight (mg)
0
25
50
75
100
125
150
S
M
L
Fruit size

Fig. 65. Percent germination capacity (GC) (A), peak value (PV) (B) and germination value (GV) (C) of fruits from maturation stages M-1(16±1 w AFO), M-2 (20±1 w AFO), M-3 (24±1 w AFO) and M-4 (28±1 w AFO) of clones 5 (□), 12 (▨) and clone 17 (■). The line with error bars represents the means of the three clones from each maturation stage.

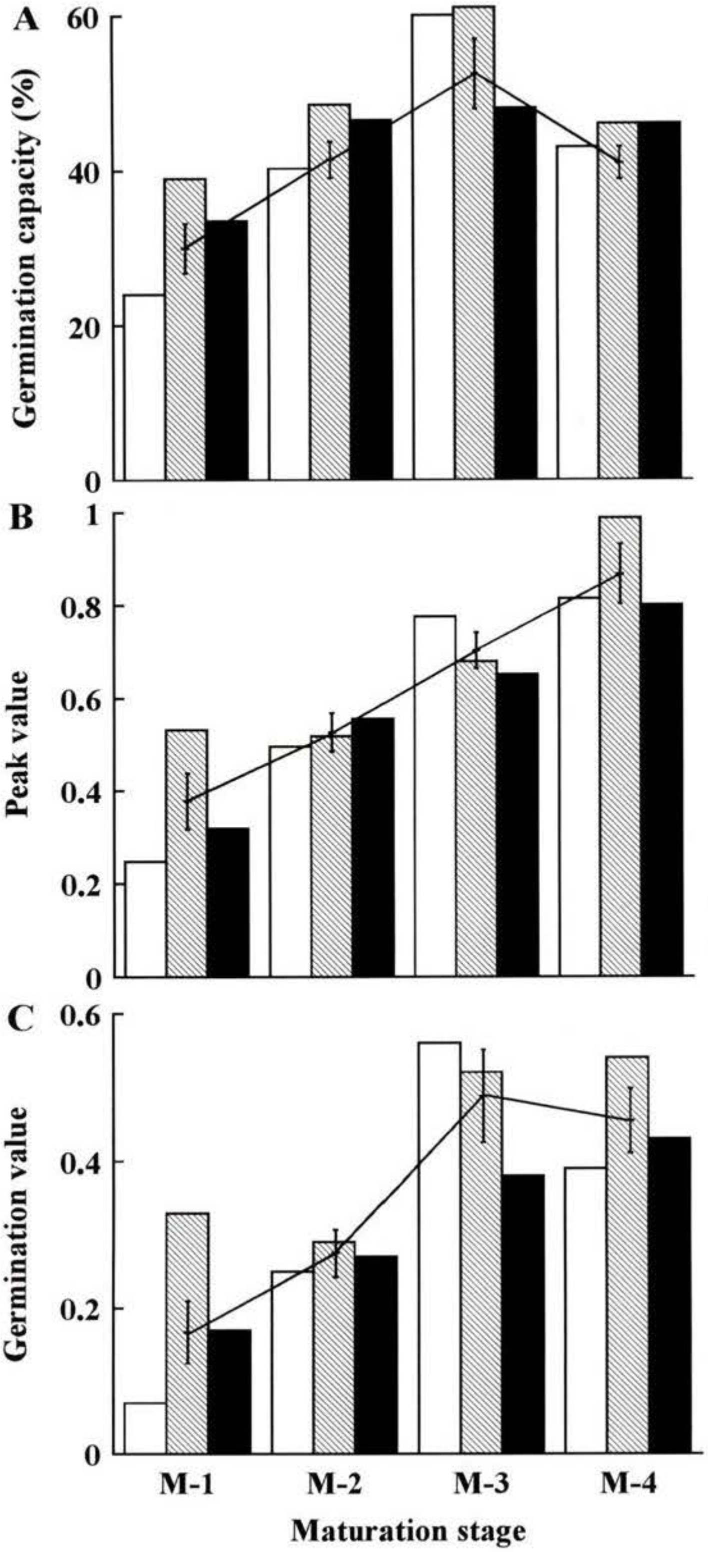

A
Germination capacity (%)
0
20
40
60
B
Peak value
0
0.2
0.4
0.6
0.8
1
C
Germination value
0
0.2
0.4
0.6
M-1
M-2
M-3
M-4
Maturation stage

Fig. 66. Germination rates of fruits from maturation stages M-1 (16±1 w AFO)(-○-), M-2 (20±1 w AFO)(-□-), M-3 (24±1 w AFO)(-◇-) and M-4 (28±1 w AFO)(-△-) of the three clones. T-values, T-1, T-2, T-3, T-4, represent the break of germination curves of fruits from M-1, M-2, M-3, M-4, respectively. Peak values (PVs) are derived from germination percentages at T-value divided by the number of days needed to reach T-values.

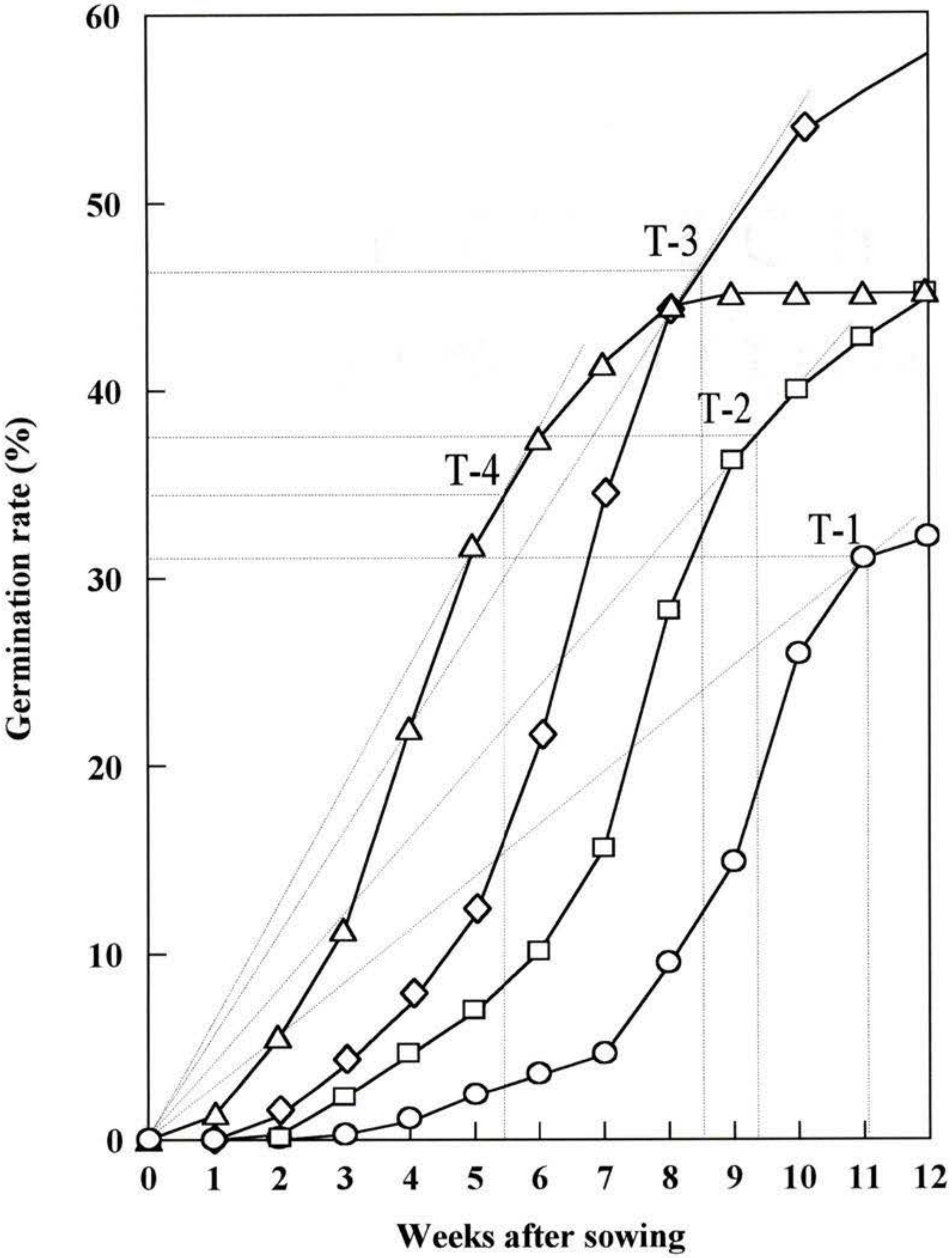
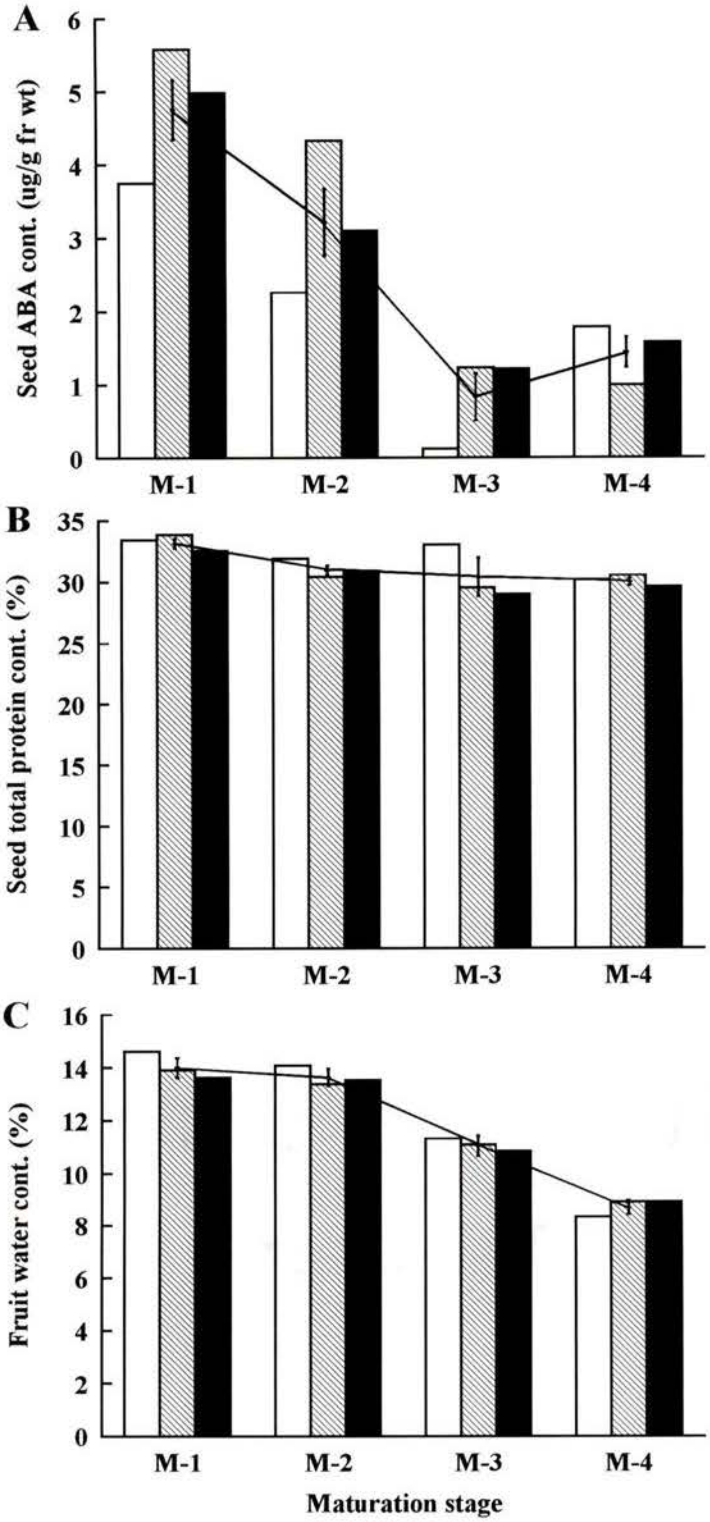

Germination rate (%)
0
10
20
30
40
50
60
Weeks after sowing
0
1
2
3
4
5
6
7
8
9
10
11
12
T-1
T-2
T-3
T-4

Fig. 67. Seed abscisic acid (ABA) content (A), seed total protein content (B) and fruit water content (C) of fruits from maturation stages M-1 (16±1 w AFO), M-2 (20±1 w AFO), M-3 (24±1 w AFO) and M-4 (28±1 w AFO) of clones 5 (□), 12 (▨) and 17 (■). The line with error bars represents the means of the three clones from each maturation stage.


A
Seed ABA cont. (ug/g fr wt)
0
1
2
3
4
5
6
M-1
M-2
M-3
M-4
B
Seed total protein cont. (%)
0
5
10
15
20
25
30
35
M-1
M-2
M-3
M-4
C
Fruit water cont. (%)
0
2
4
6
8
10
12
14
16
M-1
M-2
M-3
M-4
Maturation stage

CHAPTER 5

DISCUSSION

5.1. Phenology, pollination and fruit set

The number of flowering trees and fruit-bearing trees varied among clones as well as among years. The proportion of flowering trees that eventually produce fruit is not the same among years (Figs. 2A and B). The number of fruit-bearing trees correlated with total fruit production of clones 5 and 17. The total fruit production showed that 1989 and 1993 were the good crop years for clones 5 and 17, and the poor crop years were in 1990, 1991, 1992, 1994 and 1995 (Fig. 3A). This indicates there may be up to a 4-year interval between good crop years for these two clones. Although clone 12 had two good crops, 1991 and 1993, the 7-year data varied considerably and did not confirm a 2-year interval. Hedegart (1976) reported that teak in Thailand had a 1-3 year interval between good crop years. The interval was 4-5 years in India (Murthy, 1973a). It is possible that the cycle of good crops do not occur at the same time for all clones. Observations over more years are needed to confirm this conclusion.

Irregularity in fruit production has been suggested to be caused partly by a competition between fruit development and flower initiation and to result from a depletion of carbohydrate reserves following a good crop year (Sedgley and Griffin, 1989). The greater the production in the good crop year, the less is the production in the following year. Some reports also suggest that plant growth regulators such as gibberellins produced by the developing seed inhibit floral initiation (Luckwill, 1980; Stutte and Martin, 1986). It is likely that both carbohydrate levels and plant growth regulators could affect irregularity in tree crops. In the clones used at the CSO in my study, lack of flower initiation was not the most important factor restricting reproduction. Percentage of flowering trees was between 40 to 70% in four of the seven years for which records were available but in only two of these years was fruit production significantly greater than that in the poorest flowering year (Figs. 2 A, B).

Fruit production varied among clones, with clone 17 having a higher fruit-production capacity than clones 12 and 5, which had about the same production capacity. Time of flowering may affect fruit production because the early flowering clone (clone 5) was more likely to be "other-pollen" limited (Casper and Niesenbaum, 1993) reducing cross-pollination. Thus, most flowers in clone 5 may be self-pollinated, eventually resulting in seed and fruit abortion. Secondly, in the beginning of the flowering period there may not be enough flowers open to attract sufficient numbers of pollinators. Therefore, limited numbers of pollinators would result in lack of pollination in clone 5 (early flowering clone), thus, lack of fertilization and fruit set. In general, variation in flowering period may affect pollen availability, pollen quality, and pollinator behaviour. The very poor fruit production in clone 5 appeared to result from a very high abortion rate during the first 72 h AFO, after which only 5% of pistils remained. This is likely due to a lack of pollination or high incidence of self- pollination.

There may be a causal relationship between poor fruit production and the occurrence of peak flowering during the peak rainy season. In clone 5 the peak flowering occurred at the peak rainy season. Heavy rains may cause a high abortion rate either because flowers are knocked off the pedicel or because there are fewer pollinators foraging in the inflorescences. This suggests that treatments to delay flowering until the peak rainy season has passed or eliminating early flowering clones are worth investigating. Early flowering clones may contribute little to seed production. The peak flowering period of clone 17 began toward the end of the peak rainy season, and that of clone 12 after the peak rainy season, yet clone 17 had lower flower abortion than clone 12 (Table 2, Fig. 4). This indicates that heavy rain is not the only factor affecting the abortion rate, rather it is one of a combination of several factors, including the amount of cross pollination. Tangmitcharoen and Owens (1996) reported the occurrence of self-pollination was very high in teak which also may cause fruit abortion. In general, all clones had a very high abortion rate during the first 3 d AFO. This commonly occurs in many tree species and in teak is the time between pollination and early endosperm development. Observations on teak embryogenesis show that a lack of endosperm development or endosperm abortion are very common and may cause seed abortion, therefore, fruit abortion (Section 5.2.).

Fruit production per tree was very low in the 12-year-old CSO. Over the 7-year period, the average fruit production was 0.137 kg/tree for clone 5, 0.174 kg/tree for clone 12 and 0.676 kg/tree for clone 17. A 14-year-old CSO in Northern Thailand produced between 0.3-3 kg of fruits/tree with an average of 0.7 kg/tree (Boonthavee *et al.*, 1992). Fruit production from a 20-year-old SPA in Thailand ranged from 10 to 50 kg/ha, and an 18-year-old seed orchard produced 7 to 10 kg/tree (Kaosa-ard, 1991). The same age SPA in Andhra Pradesh, India produced 2 to 7 kg/tree. In Nigeria a 20-year-old SPA produced between 2 and 2.5 kg of fruits/tree and the same age seed orchards produced 1.7

to 4.1 kg/tree (Suangtho and Lauridsen, 1990). The CSO in Indonesia produces less fruits than those in Thailand and Nigeria. Several possibilities may cause the poor fruit production. First, it may be caused by the fact that trees at the CSO in Indonesia are quite young. Hedegart (1976) indicated that the first large crop was obtained from 20 to 25-year-old trees. Second, the trees in the CSO in Indonesia may grow in a less suitable site, classified as a type 2 site and, therefore, produce less fruits. Third, the trees may put more energy into vegetative growth than reproductive growth. Observations on the vegetative growth are needed to confirm this.

Flowers only have a one-day cycle as in Thailand as well as in Indonesia. Flower abortion in Indonesia started as early as 8 h AFO, but mostly after 12 h AFO. This is much shorter than reported in Nigeria, where unfertilized flowers fall off within 10 d of pollination (Egenti, 1978), and flowers that do not fall off within 14 d usually grow into mature fruits. It seems probable that in Nigeria flowers that fall off within 10 d consist of both unfertilized and fertilized flowers, since fertilization occurs within 16 to 24 h AFO. Those that remain for 14 d AFO may be the vigorous developing young fruits which were successful in competition for substrate.

The number of pistils containing at least one pollen tube in the style increases significantly 16 h AFO and continues to increase until 28 h (Fig. 26A). This suggests that most pollen did not germinate soon after being transferred to the stigma since it is not likely that pollen transfer occurs after 6 h AFO (Bryndum and Hedegart, 1969; Egenti, 1978). By then stigmas are no longer receptive and anthers begin to wither (Tangmitcharoen and Owens, 1996). An *in vitro* study of teak pollen germination showed that for pollen collected on the day the flower opens, 100% germinated within 8 h after being put in 14% sucrose medium (Egenti, 1978). The same sequence may occur *in vivo,* in that pollen grains germinate 8-10 h after being transferred to the stigma. It is also possible that these slow-to-germinate pollen grains were the incompatible ones. A slow germination of incompatible pollen occurs in *Corylus avellana* L. (hazelnut). There, compatible pollen germinated 4 h after pollination and penetrated the style 12 h after pollination (8 h later), whereas incompatible pollen germinated 18 h after pollination and the pollen tubes were distorted and did not penetrate the stigma surface (Hampson and Azarenko, 1993).

The proportion of pollinated pistils per collection was the same for all clones except for the collection 20 h AFO (Fig. 27A). This indicates that the high or intermediate abortion rates of clones 5 and 12 were not entirely caused by lack of pollination. Tangmitcharoen and Owens (1996) reported that the number of pollinated pistils was not the major factor limiting fruit production, but rather the high incidence of self-pollination; thus flower abortion was due to lack of fertilization. It is likely that the pistils which remain 24 h AFO are those with at least one fertilized ovule (compare Figs. 4 and 26 A, B at 24 h AFO). Thus, most of the pistils that abort within 24 h AFO may be unpollinated or unfertilized. This implies that pistils which abort during 48 and 72 h AFO are fertilized. Although fertilized, some of those pistils might not be able to compete with more vigorous fertilized pistils, and may abort due to substrate competition between the growing fruits. In general, poor fruit set (or high fruit abortion) is related to the high substrate requirement for fruit production but limited resource (Ehrlen, 1991; Ramirez, 1993).

The greater fruit set in clone 17 may have been due to a higher proportion of cross-pollinated pistils since its flowering period coincided with the CSO flowering period when more clones were flowering. In general, however, flower abortion of the three clones within the first 3 d AFO was very high, averaging 86.67%, almost the same as in Nigeria (90%) (Egenti, 1978) and slightly lower than in Thailand (98.7%) (Hedegart, 1973).

Pollen-tube abnormalities, such as tube arrest in the style, reversal in direction, irregular growth, and swelling of tube tip in the micropyle, are similar to those observed by Tangmitcharoen and Owens (1996) for teak. The causes of these abnormalities are not clear, although self-pollination, thus, incompatible pollen, may contribute to most of them. Pollen-tube arrest in the style as indicated by swollen tips was also observed in avocado (*Persea americana* Mill.) (Sedgley, 1976) and *Macadamia* spp. (Sedgley, 1983). This phenomenon is common *in vitro* and occurs when the availability of nutrients for tube growth decline and products of pollen metabolism increase (Tupy, 1960). Swelling of tube tips in the style is common in cases of gametophytic self-incompatibility where tubes are inhibited after penetrating the stigma and part of the style (Sedgley and Griffin, 1989). Bending of tube tips was also observed in teak and may be caused by incompatible pollen as reported in self-fertile *Lycopersicon esculentum* cv 'Peto 95 pollinated with *L. peruvianum* acc. LA 1708 pollen which are known to be incompatible (Gradziel *et al.*, 1993) or as in *Rhododendron* (Williams *et al.*, 1986). The difference is that in *L.*

esculentum, pollen-tubes were convoluted and occurred in the central cell, and was termed pollen-tube hypertrophy, whereas in teak the pollen-tube tip bent in the micropyle, before penetrating the embryo sac.

The entry of the pollen tube into the embryo sac via one of the synergids is common in most angiosperms and has been described in considerable detail (Maheswari; 1950; Jensen and Fisher, 1968; Went and Willemse, 1984; Russell, 1993). Sealing off of the micropyle once a pollen tube enters may be the mechanism by which pollen tubes reduce pollen competition.

Although pollen tubes reached the ovary as early as 8h AFO, fertilization did not occur until 16-28 h AFO, with the majority occurring 24 h AFO. At fertilization a pollen-tube penetrates one of the synergids and discharges the two male gametes into the synergid. This time lag is difficult to explain but it also occurs in avocado (Sedgley, 1979). It is possible that the embryo sac is not quite mature or the pollen tubes need to synthesize or absorb more energy to be able to penetrate the synergid.

5.2. Embryogenesis

The cup-like hypostase seems to have an important role in embryogenesis. It serves as a direct connection between the mature embryo sac and the maternal plant. Its dense cytoplasm and thick cell walls suggest several functions, including serving as a barrier tissue for stopping the encroachment of the embryo sac (Venkateswarlu and Rao, 1972), or a connection between the ovule vascular supply with the embryo sac, thus facilitating nutrient transport (Tilton, 1980).

Three antipodals were present in the mature embryo sac when the flowers opened. This is contrary to Pal's (1951) report that teak antipodals degenerated early, even before the embryo sac matures. The antipodals of teak did not proliferate as in some species of the Verbenaceae (Misra, 1937; Tatachar, 1940), but are arranged in a pyramid form and start to degenerate just before fertilization.

The formation of endosperm was similar to that described for teak by Koorders (1896) in which there were two types of endosperm. In my study the terms "early endosperm" and "endosperm proper" (Johri, 1984; Johri *et al.*, 1992) are used to differentiate these two types. The early endosperm is a column of enlarged cells that extent from the micropylar end to the chalazal end of the embryo sac. These were described as feebly developed by Koorders (1896). The endosperm proper develops at the chalazal end of the early endosperm and is that portion of the endosperm where the embryo develops and matures. A micropylar haustorium was not found in this study of teak, but was described in other Verbenaceae, *Lantana indica*, and *Stachytarpheta indica* (Pal, 1951; Tatachar, 1940). It is possible that what was described as the micropylar haustorium was the basal cell which remained at the micropylar end after the apical cell developed into a proembryo. What other investigators described as a chalazal haustorium was observed and was the projection at the distal end of the endosperm proper that became embedded in the hypostase and persisted until the seed was fully developed. It is interesting to note, however, that Pal (1951), who also studied teak, did not report the two types of endosperm. What was reported as endosperm in his study may be what is termed endosperm proper in my study.

The early endosperm may be responsible for nutrient supply for the developing embryo during early stages and provides a channel through which the embryo reaches the endosperm proper. It may also provide nutrients for the lengthening suspensor. As the embryo proper reaches the endosperm proper, the suspensor is anchored at the proximal end of the endosperm proper. The developing embryo is then nourished by the endosperm proper which may withdraw nutrients from the maternal plant through the chalazal haustorium at the hypostase. At that time the early endosperm cells started to vacuolate and then degenerate.

The suspensor, after becoming anchored in the endosperm proper, reaches its maximum length at the heart-shape embryo stage, which is a common feature in angiosperm embryogenesis (Yeung and Meinke, 1993). The suspensor may have an important role in early embryo development. Several functions have been ascribed to it, such as nourishing the embryo proper at the early stages and supplying some important phytohormones as in *Phaseolus coccineus* (Cionini *et al.*, 1976). Removal of the suspensor at the cotyledon stage in *P. coccineus* does not affect the embryo development (Yeung and Sussex, 1979). That is the time when the suspensor starts to degenerate in teak and in *Vicia faba* (Johansson and Walles, 1994). The teak suspensor was always uniseriate and did not become massive as in *Lippia nodiflora,* another member of Verbenaceae (Pal, 1951).

Teak embryo development is of the Solanad type (Pal, 1951), where the first division forms a linear tetrad. This was difficult to verify in this study due to the shrinkage of the material during fixation, although the technique used in this study was similar to the technique used by Pal (1951).

Teak ovules reach their maximum size between 8-11 w AFO (Fig. 61). This coincides with the maximum fruit diameter that is reached between 8-12 w AFO (Fig. 62). At that time the embryos inside the ovules are still developing and have not reached their maximum size. The maximum size of the embryo is reached between 14-16 w AFO when the cotyledons become thick and fill the ovule cavity. At this time fruit water content starts to decline (Fig. 63) indicating the end of the seed development stage and the beginning of the maturation stage.

5.3. Reproductive success

The number of flowers per inflorescence ranged from 1330 to 2000 in the CSO trees used for this study. This was less than reported in Thailand, 1200 to 2700 Fl/Infl (Siripatanadilox, 1974) and much less than reported in India, about 10 000 Fl/Infl (Mathew *et al.*, 1987). The different numbers of flowers per inflorescence may be due to different tree age or vigor. The number of flowers opening each day ranged from 30 to 100 in Indonesia compared to 100 to 300 in Thailand. However, in the same age tree as in Indonesia, 250 flowers opened each day in Nigeria. This indicates that teak trees in the CSO in Indonesia do not flower as vigorously as those in the sites in Thailand and Nigeria. This may be due to a less suitable growth environment at CSO which is classified as a type 2 site (pers. comm.), more vigorous vegetative growth or more juvenile trees.

Flower initiation is not a major constraint in teak fruit production, although it varies among clones. The major constraints are low fruit and seed set, as indicated by low

Fr/Fl and S/O ratios (Table 3). In a practical sense, the low S/O ratio is not a constraint since it is more desirable to have only one seedling per fruit in order to reduce sibling competition and labor for thinning following germination. This leaves low fruit set as the major constraint as occurs in many other tree species (Stephenson, 1981; Trueman and Turnbull, 1994; Yates *et al.*, 1994). Many studies have been conducted and several hypotheses have been suggested (Guitian, 1993) to explain the reasons for low fruit set. In general, fruit set is thought to be resource-limited, thus, any attempts to increase nutrient supply during the flowering period may increase fruit set. In general, most teak fruits occur in the proximal half of the inflorescence. This indicates that most fruits develop from flowers that open early within an inflorescence. Thus, fruit set in teak is not random with respect to flower position. As reported in *Prunus mahaleb* (Guitian, 1994), the first fruits to develop may have an advantage over those which develop later within the same infloresecence and an ovary reserve mechanism acts to ensure constant fruit set within each inflorescence.

Most fruits produced in all clones had only one fully developed seed (Table 5) suggesting a tendency towards a fixed abortion system controlled by the maternal plant. This commonly occurs in other species with dry indehiscent fruits (Casper and Wiens, 1981), although position of the surviving ovule is random in teak. The fixed abortion system reduces sibling competition as fruit is the dispersal unit and the inflated calyx serves as the dispersal vehicle for the enclosed fruit. A fixed abortion system provides opportunity for selection at the zygote level, either through competition among embryos or by direct control from the maternal plant. It is also possible that the development of the early fertilized embryo prevents other ovules from developing. In *Sophora japonica* the early fertilized ovules inihibit the growth of additional pollen tubes by secreting IAA into the style, thus, preventing subsequent fertilization (O'Donnell and Bawa, 1993).

Few data are available on fruit abortion from the time flowers open until fruit maturation. It seems that teak has a double sigmoid curve in this regard, meaning that there are two major times when fruits abort. The first occurs within 72 h AFO, and the second after fruits have reached a certain stage of development. This abortion pattern also occurs in *Gmelina arborea* L., another member of Verbenaceae (Bolstad and Bawa, 1982). Introducing additional pollinators into the CSO to enhance cross-pollination may reduce the first abortion rate, and increased nutrient supply may reduce the second.

5.4. Fruit maturation and germination

Teak fruits in Indonesia took 8 w to develop from flower opening in February until the end of March when they reached maximum size (Fig. 63). They then matured 16 ±1 w later for a total of 24±1 w AFO (Fig. 67C) at which time the fruit water content had declined to about 11%. This period was more precisely delimited than reported in Northern Thailand where flowering occurred in July and it took 50 d (about 7 w) to develop and another 70 to 150 d (10 to 21 w) to mature for a total of about 120 to 200 d (17 to 28 w) (Hedegart, 1976). In Northern India, flowering occurred during September and October and fruit ripening started in November and continued until March when they matured. The fruits fall during April and May, therefore from flowering to maturation was a total of 7 to 8 months (Seth and Kaul, 1978), about the same as in Thailand. In all three locations flowering starts in the beginning of the rainy season and maturation occurs during the dry season.

Teak germination capacity (GC) and seedling dry weight (SDW) were affected by fruit size. The use of fruits larger than 10 mm in diameter increased the GC from 10.22% to 36-40% and the SDW from 48.50 mg to between 124.38 and 125.21 mg (Figs. 64A, B). Thus fruits with diameter >10 mm produced more vigorous seedlings that grew faster than seedlings from smaller fruits regardless of the clone.

The decline in fruit diameter between 14 and 20 w AFO was caused by reduced fruit water content (Figs. 64) as fruits entered the maturation stage. Experiments 1 and 2 (Figs. 63) demonstrate that fruit-water content continue to decline up to 28±1 w AFO. The decline in water content from M-1 (16±1 w AFO) to M-3 (24±1 w AFO) was correlated with an increase in GC from 32.91% to 57.67%. Further decline from M-3 to M-4 (28±1 w AFO) is related to a reduction in GC from 57.67% to 45.00%. It seems that, although teak fruits had dried somewhat at 16±1 w AFO (M-1), physiological maturity was not yet reached. The GC of 57.67% obtained from M-3 is surprisingly high compared to field germination of less than 10% and demonstrates that the 10-year-old CSO can be used as a seed source. Hedegart (1975) suggested that SPA or CSO should not be used as seed sources until the trees are about 20 yrs old due to their low production and low seed quality. More investigations concerning field germination capacity of fruits from young stands are needed.

The almost constant protein content after M-2 onward indicated that seeds reach their physiological maturity at M-2 (20±1 w AFO) because at that time this storage reserve ceases to accumulate, and the seed development phase appears to switch into a germination phase. In the latter phase, ABA and moisture content decline. It is expected that during that phase seeds have their highest viability and vigor. On the other hand, GC and GV reach their maximum at M-3. The increase in GC from M-2 to M-3 may correlate with the decline in ABA content from M-2 to M-3, when it reaches its minimum of 0.84 μg/g fw (Fig. 67A). The decrease in GC from M-3 to M-4 may be caused by factors other than ABA content, such as fruit water content, which reaches its minimum of 8.84% without any drastic changes in ABA or protein content (Figs. 67B, C). In practice, at the CSO fruits are left on trees for four weeks after reaching physiological maturity causing field weathering that results in lower GC. The higher PV obtained from fruits at M-4 means that field weathering speeds up the germination but at the expense of GC. Germination value (GV) determination (Fig. 65C), which is a combined measurement of germination capacity and germination speed, shows that among the maturation stages, M-3 (24±1 w AFO) is the optimum time to collect teak fruits. Although these fruits germinate more slowly than fruits collected at M-4 (Fig. 66), the higher GC obtained from M-3 is more advantageous.

CHAPTER 6

SUMMARY AND CONCLUSIONS

The study on reproductive phenology showed that the time of flowering may affect the fruit production capacity. The low fruit production capacity (clone 5) is related to early flowering, intermediate fruit production capacity (clone 12) with late flowering and high fruit production capacity (clone 17) coincides with the peak CSO flowering period. The time of flowering in relation to season affects flower-abortion rate. Treatments to delay the flowering period until the peak rainy season has passed need to be investigated.

The preemegent reproductive success (PERS) was very low and varied among clones. The PERS of clone 5 (0.1%) was lower than clones 12 and 17 (0.4 and 0.5%, respectively). The major constraints in teak reproduction were low fruit and seed set. However, since one seed per fruit is desirable, that left the low fruit set as the major constraint. The higher fruit set in clone 17 (35.563 Fr/Infl) than clones 12 and 5 (17.875 and 10.375 Fr/Infl) may be caused by a higher incidence of cross-pollination, since the flowering period of clone 17 coincided with the peak CSO flowering period, when more trees were flowering.

The highest open-pollination rate of about 40 % was reached 24 to 28 h AFO and did not vary among clones, except collection 20 h AFO. Despite some abnormal growth, most pollen tubes were able to reach the lower part of the ovary or micropyle 16 to 24 h AFO, but fertilization rate could not be quantified.

Fertilization occurred from 16 to 24 h AFO. Pollen tube entry into the embryo sac is through a synergid. The early endosperm developes soon after fertilization and fills the whole embryo sac within 1 w AFO. Endosperm proper developes in the chalazal end of the early endosperm during 1 and 2 w AFO. The zygote starts to develop into a proembryo 3 d AFO. About 1 to 2 w AFO a club-shape embryo forms and grows into the endosperm proper. A heart-shape embryo developes during 4 and 5 w AFO and cotyledons 5 to 6 w AFO. The ovule develops into its maximum size 8 to 11 w AFO. The seed matures 14 to 16 w AFO. Therefore, the endosperm proper has a more important role in embryo development than early endosperm that degenerated early (2 to 3 w AFO).

Failure in endosperm development was the major cause of seed abortion, thus fruit abortion. Undeveloped early endosperm causes zygote abortion, and a lack of or abnormal endosperm proper development results in embryo abortion. This phenomenon is suggested to be caused by a high incidence of self- pollination. Introduction of additional pollinators may increase cross-pollination rate, thus fruit set.

Although the seed matured from 14 to 16 w AFO, the highest quality fruits were collected 24±1 w AFO, when their water content had declined to about 11% and during that time the fruits reached their maturation phase as indicated by the decline in seed ABA content. The germination capacity was highest (57.67%) among fruits collected at M-3. Clonal variation did not effect the quality of the fruit produced.

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