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Effects of temperature on seed germination in *Pterocarpus macrocarpus*

by

Chaiyasit Liengsiri

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF Master of Science

DEPARTMENT OF FOREST SCIENCE

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Supervisor

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Date January 19, 1987

ABSTRACT

Pterocarpus macrocarpus Kurz is a tropical forest tree species and indigeneous to Thailand. Seeds collected in December 1984 from six different provinces in Thailand were investigated for germihation in response to temperature comprising both constant and day-night alternating temperatures and for genetic variability by electrophoresis of six enzymes encoded by 11 loci.

Different germination patterns were observed among the six stands. The sensitivity and tolerance to temperatures during germination, which were determined by the area within 80% germination isolines, appeared under the influence of ecological climate rather than geographical location. A wide range of temperature tolerance was observed from a stand that experienced both cool and warm climates. Seeds originating in the warmer climate tolerated high germination temperatures better. However, all seed lots were identical in their optimum temperature regime (30/25°C day-night alternation) for maximum total germination with the overall mean of 91.07%. They also shared a wide common range of temperature preference for ≥80% total germination.

A high total germination (≥80%) was obtained over a wide range of alternating temperatures, but was restricted to a narrow range of constant temperatures. Zero germination was obtained at temperatures under 20°C and over 40°C constant regimes. Within a moderate temperature range, 25-35°C, germination was less dependent on both amplitude of

diurnal fluctuation and duration of alternating temperatures.

Populations of *P. macrocarpus* were variable genetically, but no clinal patterns relative to the geographic locations could be observed. Single populations were, on the average, polymorphic at 63.6% of their loci and had 2.0 alleles per locus. The mean expected heterozygosity (20.3%) was higher than observed heterozygosity (12.1%). This discrepancy was attributed to the high level of inbreeding.

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Table of Contents

List of Tables

List of Figures

List of Plates

1. Introduction

1.1 Background of *Pterocarpus macrocarpus* Kurz

Pterocarpus macrocarpus (Syn. *P. Parvifolius* Pierre) is a medium to large size deciduous forest tree belonging to Papilionaceae. The tree is indigenous to Thailand and Burma, and extends to southern Vietnam (Rojo 1977). Its vernacular name is paduak or Burma paduak (Troup 1921) and in Thailand it is called praduu (Smitinand 1980).

Praduu is not a gregarious tree, but grows scattered and in association with other species. It can be found in both mixed deciduous forest and dry deciduous dipterocarp forest at an altitude between 100 and 600 m (Smitinand 1977; Santisuk and Niyomthamma 1983).

Mature trees are 15-30 m high. The bark is greyish brown to dark brown with irregular exfoliated scales. The leaves are imparipinnate comprising 5-10 leaflets arranged alternately with one terminal leaflet. The tree is leafless during the cold and dry season (December to March). The inflorescence is a raceme with yellow perfect and zygomorphic flowers blooming from March to May. The flat indehiscent fruit or samara bearing 1-3 seeds ripens 3-4 months after flowering. The yellowish red to brick red wood is used for furniture, flooring, posts, shafts of carriages and agricultural implements (Troup 1921; Smitinana 1975; Santisuk and Niyomthamma 1983).

1.2 Nursery problems of *P. macrocarpus*

P. macrocarpus is a commercially valuable timber not only in Thailand, but also in the international timber trade (Anonymous 1979). Because of its fire tolerance and pest resistance, it has recently been suggested for plantation and watershed improvement projects in Thailand (Dullayapach and Pureevirojkul 1979).

Nevertheless, the silvicultural information about *P. macrocarpus* is obscure. The literature gives no pertinent information on nursery practice but is largely based on individual observations and experience. Seedling production in nurseries has been impeded and has succeeded only marginally. A large number of seeds, therefore, have to be used in order to meet the requirement of planting stock. A high degree of physical damage to seeds during extraction is also encountered (personal experience). As a result, the cost of seedling production is inevitably high due to extra expenditure for additional seed collections, processing and nursery bed preparation. The limitation of costs, times and space results in a reluctance to define it as a major plantation species.

However, it is possible that these nursery impediments can possibly be overcome if production can be enhanced and the costs can be lowered. As a result, praduu may become a major and desirable plantation species in reforestation projects.

1.3 Temperature and germination of seed

Because seeds are the major propagules used to produce seedlings, seed germination is the most crucial stage for seedling production. Germination of seeds is a complex process involving various physiological and morphogenetic events that result in the transformation of an embryo into a seedling (Berlyn 1972). This process is influenced by various internal and external factors, which can favour or inhibit germination. Some may have profound long-term effects on germination physiology (Bewley and Black 1982). Among the external factors, i.e., water, light, temperature and gases, temperature is obviously one of the important factors.

Seeds of many temperate species generally require a pre-germination treatment by low temperature (1-15°C), i.e., chilling or stratification, to break dormancy (Althen 1971; Edwards 1973; Zasada and Viereck 1975; Wilson *et al.* 1979; Totterdell and Roberts 1979; Barton 1982; Carl 1983; Hopper *et al.* 1985).

The required period of stratification differs depending on the depth of dormancy, the origin of the seeds and/or their maturity (Hellum and Dymock 1986). Fowler and Dwight (1964) studied 11 provenances of white pine (*Pinus strobus* L.) seeds and found that seeds from northern sources required considerably shorter stratification periods than those from southern sources. But the opposite results were obtained in sweet gum (*Liquidambar styraciflua* L.) seeds

(Wilcox 1968).

Corylus avellana L. requires only 30-60 days of stratification (Gosling and Ross 1980), whereas Arkansas oak (*Quercus arkansana* Sarg.) needs up to 120 days to attain the best germination (Wirges and Yeiser 1984). Observed germination was found to increase with increasing chilling period. The period of 6-9 weeks is generally optimal in many species (Bonner and Farmer 1966; Pinfield *et al.* 1974; Barton 1982; Hopper *et al.* 1985; Tanaka *et al.* 1986). Conversely, seeds of desert species require high temperature pretreatment to increase germination (Reese 1961; Capon and Asdall 1967). These requirements of temperature pre-treatment, therefore, relate to the ecological climate prevailing in the habitat of the plants.

Many reserchers have contributed to investigations to characterize germination of seed in responses to temperatures. Haasis (1928) studied the germination energy of several coniferous-tree seeds. Stein (1951) and Franklin and Krueger (1968) reported the germination of true fir and mountain hemlock seeds on snow. Lang (1965) found that *Acer platanoides* L. and *Trifolium repens* L. seeds can germinate on frozen soil or ice.

Agrostemma githago germinated to 50% at temperatures down to 2°C but *Primula forinosa* failed to germinate below about 11°C, even though both possess the same upper temperature range of 27°C (Thompson 1970). Cucumber (*Cucumis sativus* L. CV Long green improved) and mung bean (*Phaseolus*

aureus Roxb.) germination was also inhibited at 10°C (Simon *et al.* 1976). Lettuce (*Lactuca sativa* L.) tested on thermo-gradient bars covering the range of 0-40°C germinated close to 100% over a temperature range from ca. 2°C to 25°C but displayed steeply declining germination between 25 and 30°C and produced very few seedlings above 30°C (Thompson *et al.* 1979). Morrow *et al.* (1982) also reported the high temperature inhibition of germination in jointed goatgrass (*Aegilops cylindrica* Host.), which germinated over 80% at temperatures between 10 and 25°C but only 2% was obtained at 35°C.

Thompson (1973b) found that *Gypsophila perfoliata* L. can germinate in a broad range of temperatures, 2°C to over 40°C, while the leek (*Allium porrum*) is capable of germinating only in the range of 7-23°C. The celery (*Apium graveolens* L.) cultivars Golden Self Blanching and Avon Pearl have the optimum temperature range of 10-15°C and 7-20°C, respectively, for germination (Thompson 1974c). The same author (1974a) also found differences between cultivars of tomato (*Lycopersicum esculentum* Mill.) even though overall optimum temperature range occurred between 26 and 32°C.

The seed of many species has been reported to germinate better under diurnal alternating temperatures than under constant temperatures (Morinaga 1926; Kearns and Toole 1939; Thompson 1969; Young *et al.* 1973; Mayeux and Scifres 1978; Fischer *et al.* 1982). Some appear to require temperature

alteration for germination because constant temperatures depress germination (Thompson 1974b and c; Totterdell and Roberts 1980). Some can germinate over a restricted range of constant temperatures but high germination can be achieved over a wide range of alternating temperature regimes (Ellis and Roberts 1979; Rosa and Corbinean 1986). The requirement for diurnal fluctuating temperatures and for the effective amplitude of temperature fluctuation differs among species (Thompson *et al.* 1977).

However, Everitt (1983b) found no specific temperature requirements for germination of 2 woods legumes, Retama (*Parkinsonia aculeata*) and twisted acacia (*Acacia schaffneri*). Both have similar germination patterns over a wide range of constant and alternating temperatures. Mayeux (1982), and Potter *et al.* (1984) postulated that alternating temperatures did not enhance germination of the species studied while Hylton and Bass (1961) and Everitt (1983a) reported lower germination obtained under alternating rather than constant temperatures.

The temperature requirement of seeds to germinate, therefore, is obviously variable. The widely distributed species can have either different temperatures optima (Stearns and Olson 1958; McWilliams *et al.* 1968; Thompson 1975; Bevington 1986) or similar temperatures optima (Thompson 1973a; Calamassi *et al.* 1984; Ng 1985) for germination. Thompson (1973b) has also investigated and discussed this phenomenon comprehensively.

Moreover, it has been proven that germination characteristics at least in part are also under genetic control. This evidence has been widely discussed by Whittington (1973). Barnett and Farmer (1978) and Bramlett *et. al.* (1983) also reported this evidence when they studied yellow poplar (*Liriodendron tulipifera* L.)and Virginia pine (*Pinus virginiana* Mill.), respectively.

1.4 Study aspects and objectives

Because it is distributed over a wide range of ecological climates and habitats, *P. macrocarpus* may exhibit variable germination characteristics. Optimum temperatures for seed germination may also vary among sources. This might be due to geographical adaptation or genetic variability of the species. However, none of the literature sources revealed relevant evidence for this in *P. macrocarpus*. Questions, therefore, may arise as to what the influences of temperature upon seed germination of this species are. Are there any differences in germination characteristics among populations? Do seeds from different origins posses similar or dissimilar optimum temperature for germination? What are their temperature optima? Is there any genetic diversity among populations? And what is the pattern of this genic variation?

In order to explore these questions, two study aspects were defined:

Study I: Effects of different temperature regimes on seed germination.

This study was conducted to investigate the germination responses among six seed sources to a set of temperature regimes comprising both constant and alternating temperatures.

The objectives of this study were:

1. To determine the influence of different temperature regimes upon seed germination in *P. macrocarpus*.
2. To characterize the pattern of germination responses to temperature for 80% germination for each seed source.
3. To establish the optimum temperature regimes giving the germination capacity of at least 80% in *P. macrocarpus*.

Study II: Genetic variability among populations.

This study was designed to verify whether the sample stands studied were all genetically different.

The objectives of this study were:

1. To determine the level of genic diversity among populations.
2. To determine the pattern of this variation.

The results of both studies are expected to be able to help foresters or nurserymen in raising planting stock for reforestation. Results are also expected to provide information for further research involving seed germination.

2. Materials and methods

2.1 Seed sources and seed collection

Six natural stands were selected from 6 different provinces in Thailand as seed sources for this study. They are Chiang-rai, Tak, Kanchanaburi, Saraburi, Nakhonratchasima and Udonthani (Figure 1). These stands were anticipated to be the most likely representative stands covering the range of different climates and habitats. The geographic locations and climatic information of each stand are presented in Table 1 and Figure 2, respectively.

A total of 25 trees were sampled per stand. They were on average more than 20 years old. Each selected tree was normally located a minimum of 100 m away from its nearest sampled neighbour. This was done to avoid sib-trees. However, some trees beyond these criteria were also sampled in order to obtain the required number of samples.

Mature fruits were collected in December 1984 and extracted in Muak-lek, Thailand, soon after collection. Six hundred extracted seeds were sampled from each tree, kept separate by individual trees in heat sealed plastic bags and shipped by air to the University of Alberta, Canada, for study.

There were many mechanically damaged seeds mixed in each bag (tree). The seeds from each bag, therefore, had to be cleaned. After cleaning, the number of undamaged seeds from each bag varied from about 250 to 500 seeds, and only

22 trees produced 400 and more seeds per tree. Only the seeds from 22 trees per stand were used for study. This was done to avoid the problems of unequal sample size among seed sources and to ensure that enough seeds would be available to be sampled equally from each tree throughout the experiment.

Two hundred and fifty seeds from each of 22 trees in each seed source were initially sampled and pooled for germination study. The rest of the seeds were kept for electrophoretic analyses of genetic variability and for additional pooling for the germination testing. All seeds were kept in air-tight glass containers and stored in the refrigerator at about 4-6°C until used. The pictures and X-radiographs of fruits and seeds are illustrated in Plate 1 (page 26).

Table 1. Geographic locations of six selected seed sources of *P. macrocarpus* in Thailand.

Seed source	Latitude N	Longitude E	Altitude (m)
1. Chiang-rai	19°50'	99°35'	650
2. Tak	16°50'	99°20'	140
3. Kanchanaburi	14°40'	98°35'	100
4. Saraburi	14°30'	101°10'	270
5. Nakhonratchasima	14°25'	101°48'	380
6. Udonthani	17°21'	103°06'	180

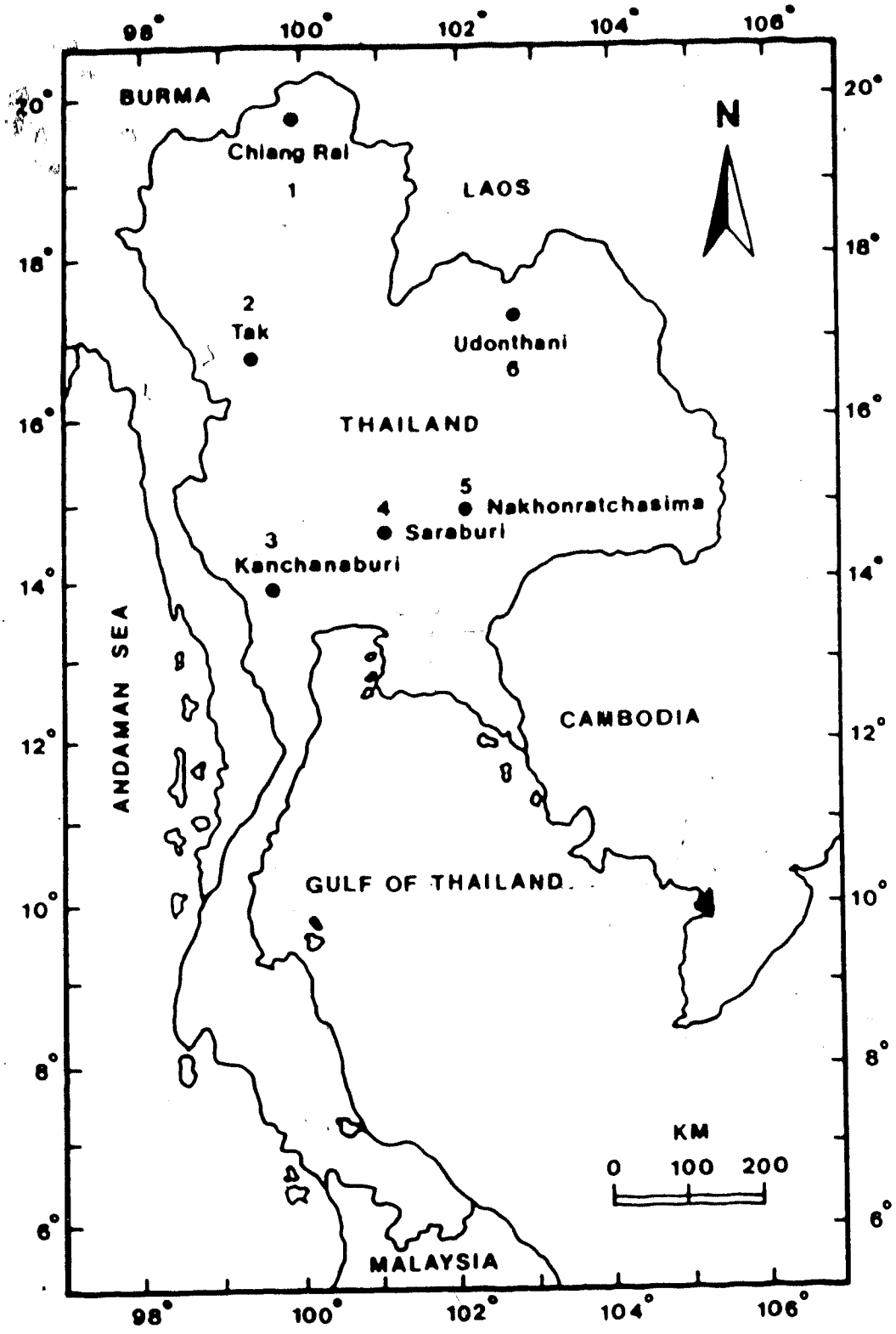


Figure 1. Map of Thailand showing the locations of six selected *P. macrocarpus* seed sources (•).

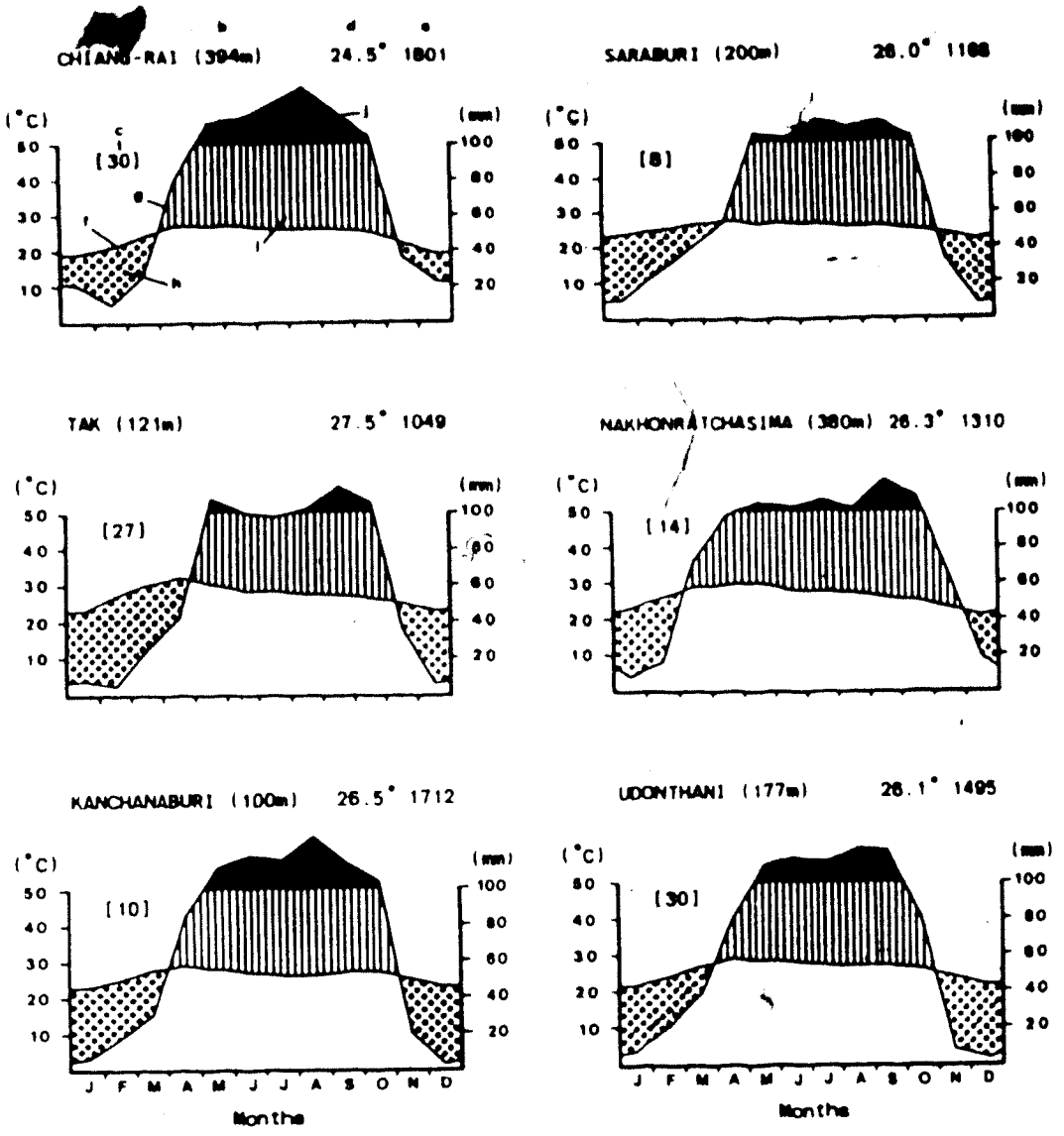


Figure 2. Climatic diagrams (Walter 1973) of seed sources studied; a=station, b=altitude, c=duration of observations (years), d=mean annual temperature (°C), e=mean annual rainfall (mm), f=curve of mean monthly temperature (°C), g=curve of mean monthly rainfall (mm), h=relative period of drought (dotted), i=relative humid season (vertical shading), j=mean monthly rainfall over 100mm (black shading, scale reduced to 1/10).

2.2 Study I: Seed germination

2.2.1 Germination conditions

The germination conditions assigned for the germination test consisted of both constant and day-night alternating temperatures. The latter was the combination of two different temperatures where the day temperature was 8 hours with light and the night temperature was maintained for 16 hours in darkness. The 8 hour photoperiod was also applied to constant temperatures resulting in 8 hour day and 16 hour night periods as those in alternating temperatures. Full light capacity of the equipment used was applied, i.e., four fluorescence and four incandescence lights for growth chambers and eight fluorescence lights for germinators. Average light intensity at growing surface was 13.0 and 95.5 μmol S^{-1} m^{-2} for germinator and growth chamber, respectively. The 13.0 μmol S^{-1} m^{-2} is adequate for germination. Temperature tolerance was controlled to ±1°C. The temperature regimes assigned were presented in Figure 3.

In addition to the 31 common tested temperature regimes presented in Figure 3, some additional tests were carried out for some seed sources (Table 2).

Table 2. Additional temperature regimes required for completion of 80% germination isoline of some seed sources.

Seed source	Day/night alternating temperatures (°C)				
Chiang-rai	15/25	15/35	25/15	30/15	30/50
Tak					30/50
Nakhonratchasima				30/15	

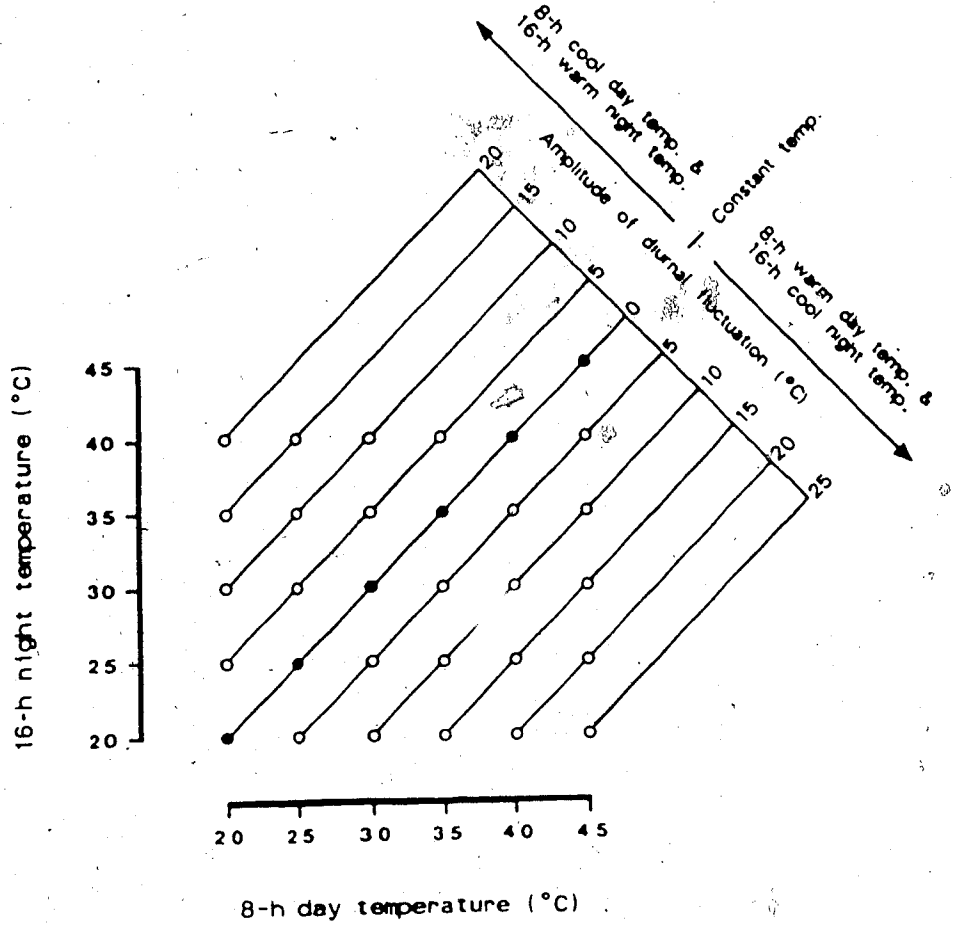


Figure 3. Grid diagram of 31 common temperature regimes tested *Pterocarpus macrocarpus* seed germinability; constant temperature (•), alternating temperatures (o).

2.2.2 Germination test

All germination tests were carried out within controlled environment chambers, germinators (Conviron model G30) and growth chambers (Conviron model E7). Four replications of 50 seeds were used in each treatment. All six seed sources were tested concurrently under the same condition.

Prior to germination, sampled seeds were subject to X-radiography. This was done to determine the number of sound seeds tested and to avoid the cutting test at the end of the test period. The radiation was conducted within a cabinet X-ray machine (Model M110NH, TF1 Corp.CT). The exposure condition was set at 15Kv, 5mA and 25 seconds. The focus-film-distance (the distance between the focal spot and the film surface) was about 47 cm. Seeds were placed on 1 mm thick clear plastic plate which was placed on top of the film. Ready packed Kodak X-Omat TL films, 8x10 inches, were used.

After radiation, seeds were scarified individually by hand with medium grain sand paper. Kimpak (20-ply cellulose paper) was used as a germination medium. Scarified seeds were germinated on moistened Kimpak inside a clear polycarbonate plastic germination box of 28x24x6 cm dimension designed by Wang and Ackerman (1983). Water was supplied regularly to maintain an adequate moisture level and uniformity of germination media and humidity inside the box.

The time course of the germination test was 14 days, and counting and recording the germinants was done daily and at the same time each day. The seed was considered to be germinated once the length of the emerging radicle was equal to or longer than the length of the seed coat. Only normal seedlings were counted as germinating seeds (ISTA 1976). Once counted the germinants were discarded.

The total number of germinants counted over the test-per was used to compute the total germination or germina capacity in a percentage using the formulation below:

$$\text{Total germination (\%)} = \frac{\text{Total germinants over test-period} \times 100\%}{\text{Number of sound seeds tested}}$$

Individual values of total germination and means averaged from four replications were used for further statistical analyses.

2.2.3 Analyses of seed germination

2.2.3.1 Patterns of germination

Germination responses to temperatures expressed as total germination (%) of each stand were plotted on a two-dimensional diagram (grid diagram) with 8-hour day temperature as the abscissa and 16-hour night temperature as the ordinate. The isolines (contour lines) of 90%, 80%, 70% etc. total germination were drawn between similar values calculated by linear

interpolation from means of total germination (%). This approach was similar to those of Thompson (1974b and c) and Adkins *et al.* (1984).

Comparisons of temperature range within an 80% germination area were made. Common regimes for germination of ≥80% were also determined by directly superimposing the 80% contour lines of six sources.

2.2.3.2 Responses to temperature of variable duration

One-way ANOVA was used to compare the germination responses to temperature of variable duration. Comparisons were made between responses under given temperature combinations and variable duration as for example: germination under 35/40°C (8h/16h-day/night) was compared with germination under 40/35°C (8h/16h-day/night) to determine the effects of duration alone at given temperatures, i.e., between 8h and 16h durations for 35°C and for 40°C, and assuming the light effect to be zero. Comparisons were also made among 8h, 16h and 24h temperature regimes (see Appendix 1).

The comparisons were carried out among temperatures along 20, 25, 30, 35 and 40°C axes but not for 45°C due to incomplete data along the 45°C night temperature axis (Figure 3).

2.2.3.3 Constant temperatures

Two-way ANOVA was performed to determine the differences in total germination (%) among seed sources,

among temperature regimes and among their interactions.

Multiple comparisons of means by using Student-Newman-Keuls' test (Steel and Torrie 1980) were also performed in case of existing significant differences, and one-way ANOVA was applied where appropriate. A power parabola (Adams and Hills 1977) was also applied to fit the germination curves.

2.3 Study II: Genetic variability

2.3.1 Tissue preparation

Diploid tissues of emerging radicles were used for analyses. One seed from each of 22 trees per stand was analyzed. Seeds were scarified and germinated for about 2-3 days at a 30/25°C alternating regime.

The preliminary technical survey revealed that germinants with emerging radicles of ca. 5-8 mm possess high enzymic activity and well-resolved enzyme bands on gels. Due to heterogenous germination, 10 seeds per tree were germinated, and one germinant from each family was randomly sampled.

Each sampled individual was dissected and the radicle was ground with a motorized tissue homogenizer in a 0.5 ml auto-analyzing cup with two drops of extraction buffer (Appendix 2). Four 1x12 mm paper wicks (Wattmann No. 3 qualitative paper) were placed in each sample. Standards were also prepared from emerging coleoptiles of corn

(Spancross hybrid) in a similar manner by using Yeh and O'Malley's (1980) extraction buffer. The experience gained suggested that only freshly prepared homogenate be used to attain high resolution.

2.3.2 Electrophoresis

Horizontal starch gel electrophoresis was used by following the general procedures of Conkle *et al.* (1982). The amount of 350 ml of 12.5% (W/V) starch gel (Hydrolysed starch Lot No. 417-1, Connaught laboratories Ltd., Willowdale, Ontario) was made for each of four buffer systems, A, B, C and D (Table 3). After cooking and degassing, the gel was poured into a 7x22x0.9 cm plexiglass mold and left to cool at room temperature. While cooling, the gel was covered with plastic wrap to prevent desiccation.

With a thin-blade scalpel, the cooled gel was cut along the length on a parallel ca. 1.5 cm from the edge. The gel was separated along the cutline. One wick from each sample cup was vertically introduced into this cutline, considered to be the origin and the cathodal end, with the far edge of the mold being the anodal end. Standard and red-food-dye-saturated wicks were also introduced. Loaded gels were then subjected to a direct electric current for electrophoresis (enzyme separation) operated within a refrigerator (ca. 4°C).

The initial current applied to all gels was 300 volts. After the dye bands migrated about 0.5 cm beyond the origin, after approximately 10-15 minutes, the power was shut off and the wicks removed. A bag of crushed ice was then placed on each gel and electrophoresis was continued. The current was then adjusted as required, 300 volts for buffer system A and B, and 200 volts for buffer system C and D. The migrating distance of dye bands was used to determine the completion of electrophoresis (Table 3).

After the completion of electrophoresis, the gels were sliced with monofilament nylon sewing thread into 1 mm thick slices. One slice was then stained for each enzyme system. Top and bottom gel slices were not used. The cathodal gel slab (small portion) was also discarded because cathodal migration of enzymes had not occurred.

Six enzyme systems were stained for 11 loci (Table 3). The enzyme staining recipes and techniques followed those of Siciliano and Shaw 1976, Collier and Murray 1977 and Yeh and O'Malley 1980 (Appendix 2). The well stained gel slices were scored for gene (allele) frequency.

Scoring of allelic frequency at each locus was carried out by a direct count of electromorphs (allozymes) across each individual (tree) used in each population. Putative allozymes (bands) migrating the same distance were considered the same allele. At each locus the most common allele was arbitrarily designated as allele 1 and the others as 2 and 3, etc.

When more than one putative locus was detected in any enzyme system, the most anodal locus, the one which migrated the furthest, was designated as locus 1 and the others given numbers as they decreased in mobility. These loci were identified by their enzymatic symbolic abbreviation followed with a hyphenated numeral; for example *AAT-3* represents the third locus of aspartate aminotransferase (*AAT*). Pictures of some stained gels are illustrated in Plate 1.

The allelic frequency data, the proportion of the number of same allele to the total alleles at each locus, were used for further analyses.

2.3.3 Analyses

Four parameters were computed to estimate the genetic variability among six populations studied:

1. The observed heterozygosity (H_o) at that locus: number of heterozygous individuals/total number of trees.
2. The expected heterozygosity (H_e) at that locus (Nei 1978):

$$H_e = 1 - \sum_k P_i^2$$

Where P_i is the frequency of the ith allele, summed over k alleles.

3. The percentage of polymorphic loci (P), calculated at the frequency of the most common allele is ≤95%.
4. The average number of alleles per locus (A).

Due to the type of tissues assayed, individual trees could not be genotyped. Thus, no attempt was made to investigate within-population variability. All parameters were calculated and compared on a population basis.

Table 3. Enzyme systems assayed in the study of genetic variation in *P. macrocarpus*.

Enzyme[1]	Abbr.	EC code	Buffer system[2]	Dye band front (cm)	No. of loci scored
1. Aspartate aminotransferase	*AAT*	2.6.1.1	B	3	2
2. Colorimetric esterase	*CE*	3.1.1.1	A	2	2
3. Isocitrate dehydrogenase	*IDH*	1.1.1.42	C	3	1
4. Malate dehydrogenase	*MDH*	1.1.1.37	C	3	1
5. Phosphoglucose isomerase	*PGI*	5.3.1.9	A	3.5	2
6. 6-phosphogluconic dehydrogenase	*6PG*	1.1.1.44	D	3	3

[1]See appendix 2 for stain recipes.
[2]Buffer systems (see appendix 2 for formulations):
A. Ridgway *et al.* 1970.
B. Schaal and Anderson 1974.
C. Siciliano and Shaw 1976.
D. Florence 1981 after Namkoong *et al.* 1979.

Plate 1.

A: Fruits and seeds of *P. macrocarpus*.

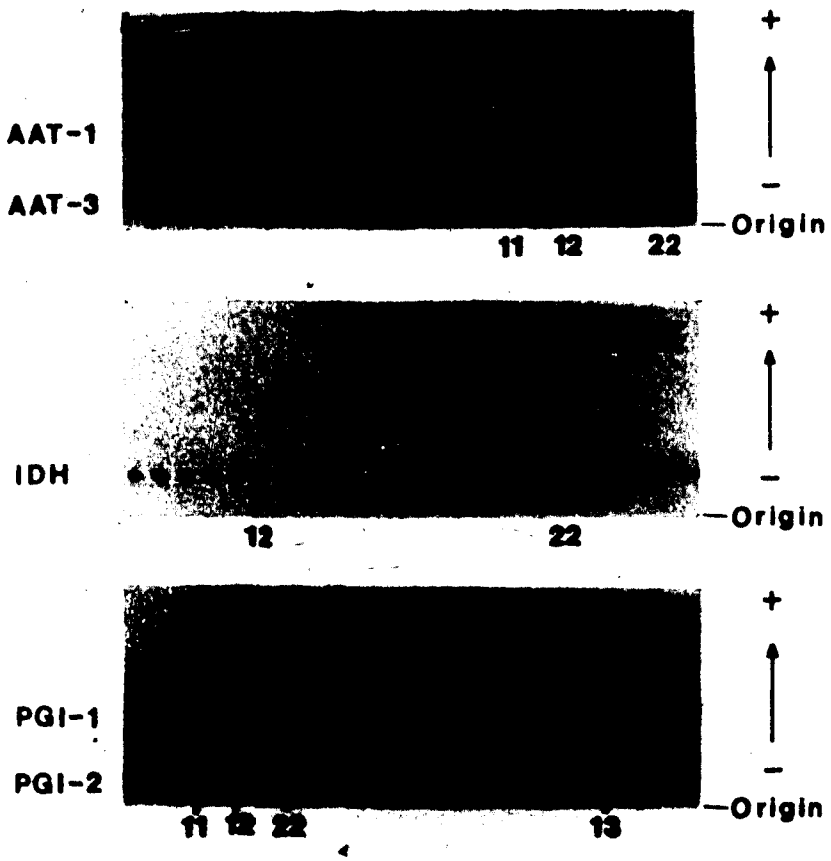
B: X-radiograph of seeds showing sound seeds (not labeled), crack seeds (c) and rotten seeds (r).

C: X-radiograph of fruits showing variation in number of seeds per fruits, 1-3 seeds.

D: *AAT* gel slice showing segregation at both *AAT*-1 and *AAT*-2 loci; numbers denote alleles and genotypes.

E: *IDH* gel slice showing segregation of 3 alleles; numbers denote alleles and genotypes.

F: *PGI* gel slice showing homozygous *PGI*-1 locus and heterozygous *PGI*-2 locus; numbers denote alleles and genotypes.


AAT-1
AAT-3
+
−
Origin
11 12 22
IDH
+
−
Origin
12 22
PGI-1
PGI-2
+
−
Origin
11 12 22 13

3. Results

3.1 Study I: Seed germination

3.1.1 Patterns of germination

The optimum temperature for maximum germination occured under a temperature combination between 30°C (8h) day and 25°C (16h) night for all six sources (Figure 4). The maximum germination varied from 89 to 93% depending on the source.

Germination under hot conditions 45/40°C (day/night) fell to less than 50% and no germination was found under 45°C constant temperatures. Low germination was also observed at cool temperatures of constant 20°C and when the day/night difference exceeded 20°C (45/20°C).

Germination to above 70% was achieved over a wide range of both constant and alternating temperatures (Figure 4). The area within 80% isoline (Figure 5) varied among seed sources and additional tests had to be performed (Table 4).

The germination rate (days to reach 50% of actual total germination; R_{50}) at the optimum temperature (30/25°C) was similar among sources except that the Saraburi lot germinated more quickly than the rest (Table 5).

Germination for seed at the optimun temperature (30/25°C) tapered to zero before the end of the 14th day after having started on day three (Figure 6). This is very rapid germination. There was no obvious difference among seed sources in their total germination (Table 5).

Table 4. Means ± S.D. of total germination (%) for additional tests for completion of 80% isolines.

Seed source	Temperature regimes (°C)				
	15/25	15/35	25/15	30/15	30/5C
Chiang-rai	77.15 ±1.12	80.22 ±3.90	64.62 ±5.09	63.19 ±11.0	39.41 ±6.14
TAK					22.28 ±11.8
Nakhonratchasima				64.35 ±9.02	

Table 5. Means ± S.D. of total germination (%) and R_{50} (days), ANOVA and S-N-K multiple comparisons at optimum temperature regime (30/25°C).

Seed source	Total germination' (%)	R_{50}' (days)
	(ns)	(*)
Chiang-rai	92.44±1.85 a	4.05±0.14 a
Tak	89.10±5.38 a	4.49±2.20 a
Kanchanaburi	88.58±4.28 a	4.40±2.20 a
Saraburi	92.20±1.87 a	3.93±0.19 b
Nakhonratchasima	92.68±3.91 a	4.09±0.24 a
Udonthani	91.42±1.92 a	4.46±0.41 a
Grand mean±S.D.	91.07±3.51	-

'Brackets indicate ANOVA results in each *column*:
(ns): non-significantly different.
(*) : significantly different at P≤0.05.

Each mean averaged from four replications.

Means followed by the same letter in each *column* were not significantly different at P≤0.05 level determined by S-N-K test.

In order to determine the germination patterns in responses to temperature a series of isolines were drawn as seen in Figure 5. Different patterns of temperature tolerance emerged. The northernmost source (Chiang-rai) from the coolest climate gave markedly different isolines compared to the southerly areas. The Tak source, from the hottest climate, showed the greatest tolerance to hot temperatures. The other four sources gave similar germination patterns.

Seed from all sources germinated to 90% except for the Tak and Kanchanaburi which reached only 80% but three northeastern sources, Saraburi, Nakhonratchasima and Udonthani, showed very limited areas of 90% germination, indicating these lots are very specific in their temperature tolerance.

The 80% isolines was used to present patterns of germination and to compare the differences between sources because it was the only complete line that could be drawn for all sources within the tested temperatures. It will be evident from Figuer 5 that the 80% areas are generally oriented along a day temperature axis. Most of the areas fall below the 45° line (diagonal line in Figure 3) meaning that the lots were sensitive to the duration of temperature exposure. The Chiang-rai source is the exception covering areas approximately equally over both the upper and lower parts.

The areas within the 80% isolines were different among sources (Figure 5). The Chiang-rai source had a much larger area than the other five indicating a tolerance to daytime germination temperatures between 15 and 46°C with night temperatures varying between 18 and 40°C. The other five sources germinated similarly within daytime temperatures from 26°C to 42°C (except for Tak at 46°C) and within nighttime temperatures between 22 and 33°C. All sources had a narrower tolerance to night than to day temperatures. This was particularly true for the Tak source.

The constant temperature regimes, at which ≥80% of the seed germinated, ranged from 26°C to 34°C for five sources while the Chiang-rai source tolerated temperatures from 22°C to 36°C.

When superimposing all six areas within the 80% isoline (Figure 7) it can be seen that common temperatures for optimum germination range from 26°C to 40°C for daytime temperatures and from about 22°C to 32°C for nigthtime temperatures.

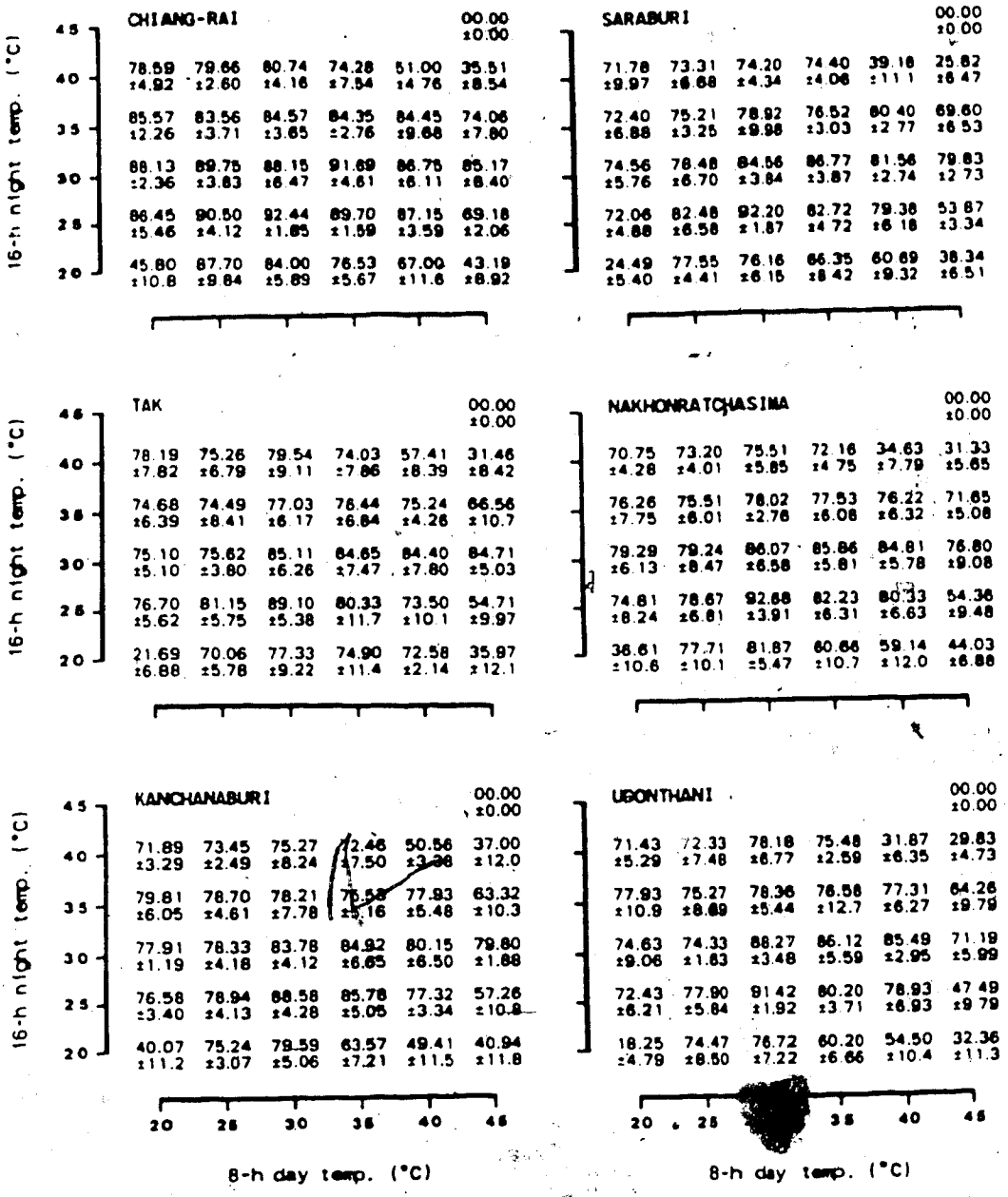


Figure 4. Means±S.D. of total germination (%) of each of 31 common temperature regimes of each of six seed sources.

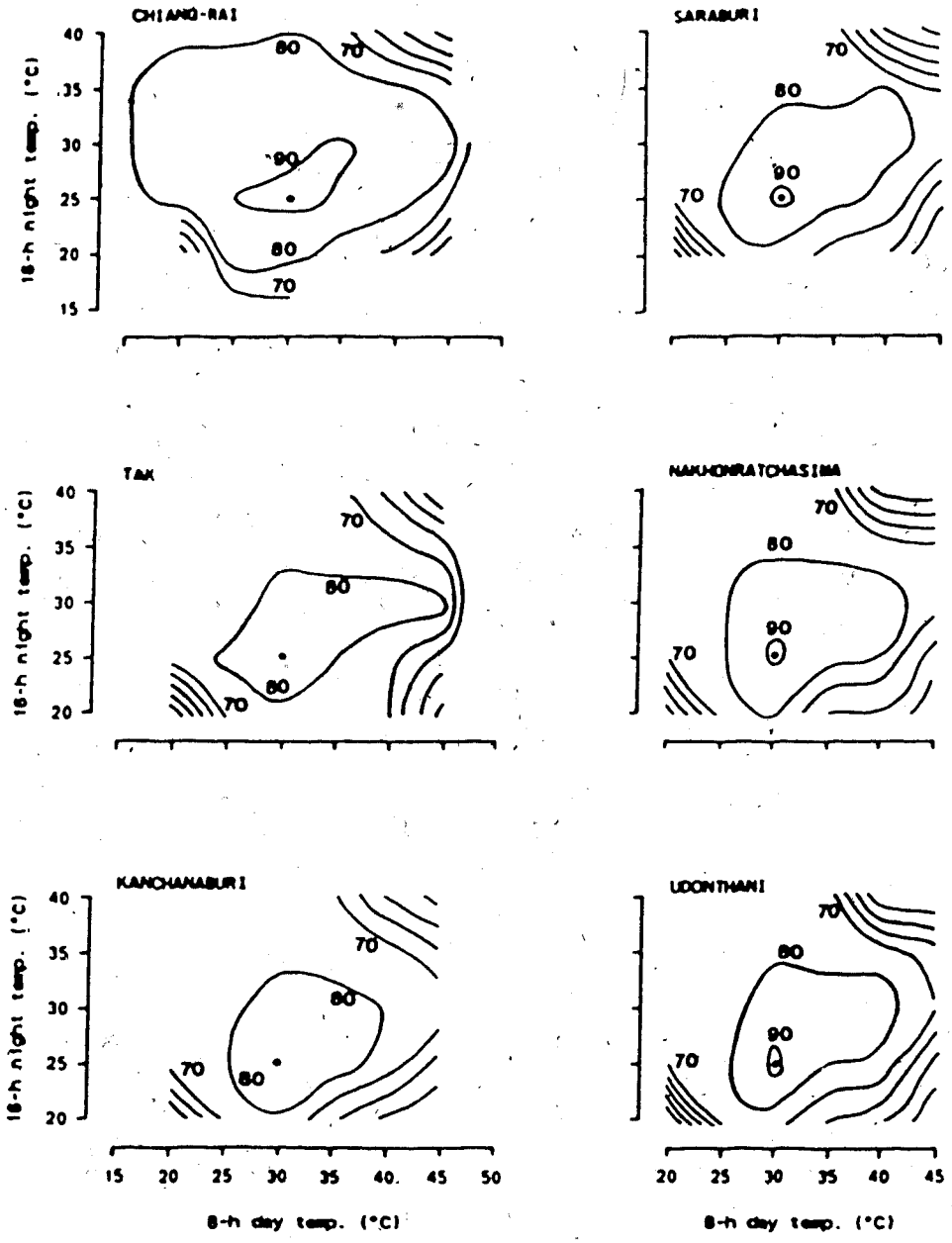


Figure 5. Germination patterns in responses to temperature of each of six seed sources; every source achieved the highest total germination at the 30/25°C regime (●).

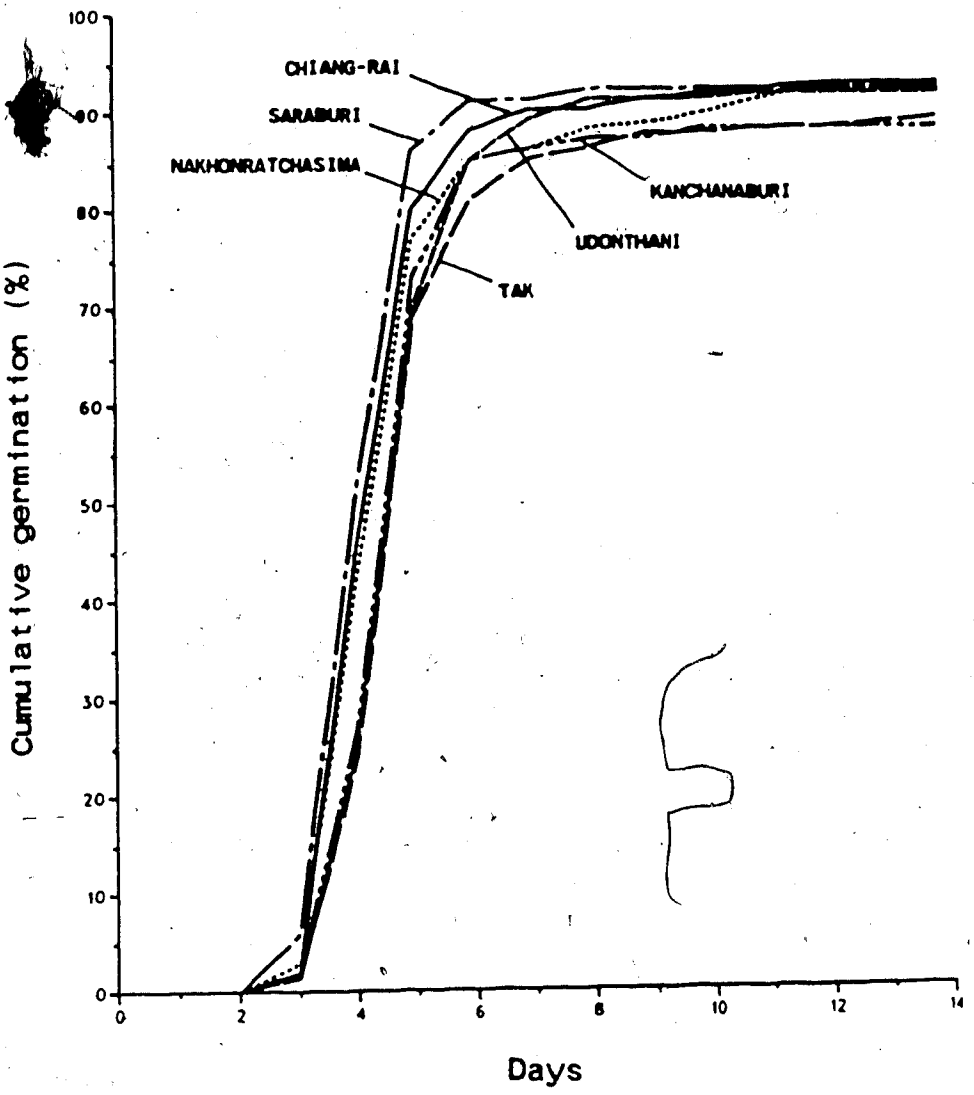


Figure 6. Cumulative germination (%) over testing period of each of six seed sources at the optimum temperature regime of 30/25°C.

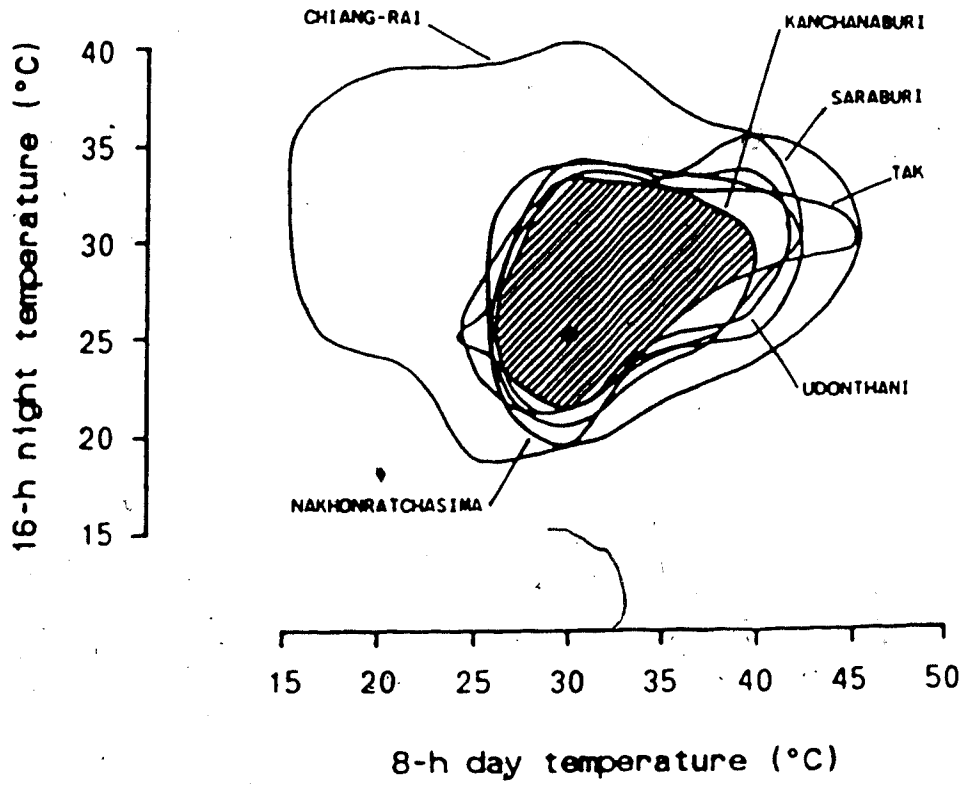


Figure 7. Common area (temperature regimes) for total germination ≥80% among six seed sources (▨); optimum regime for maximum germination (•).

3.1.2 Responses to temperature of variable duration.

Tests were carried out to evaluate the influence of diurnal temperature variations between night and day on germination (Figure 3) under 8h and 16h durations (Figure 8). Tests were also carried out to compare germination over a range of exposure times from 8h through 16h to 24h (Figures 9 and 10). One-way ANOVA was performed to compare all possible pairs (see Appendix 2 for diagram explanation).

The first set of evaluations was carried out to test if the length of exposure to a given temperature (8h vs 16h) gave rise to different responses. It became clear that a 5°C fluctuation between day and night had almost no effect on germination except for the 30/25°C and 25/30°C regimes where the 30/25°C regime gave rise to the highest total germination (Figure 8).

When day-night differences in temperature exceeded 5°C (i.e., 10°C, 15°C and 20°C) a random set of differences in germination emerged (Figure 8). No one particular set of comparisons was common for all sources. This suggests that the day or night may be 8h or 16h. It does not really influence the overall germination as long as diurnal fluctuations do not exceed 15°C. When they reach 20°C, there is significant variation. When the temperatures are cool (20°C) or very hot (40-45°C) the 16h exposure gives less germination than the 8h exposure to such temperatures.

When germination under 8h, 16h and 24h temperature durations was compared (Figures 9 and 10), it was found that every source showed differences between a constant 40°C and all other regimes along the 40°C day and night axes (Figure 9).

The comparison of temperature regimes along the 35°C day and night axes and the constant 35°C temperature were consistently significant for the Chiang-rai source, variably significant for Kanchanaburi, Saraburi and Nakhonratchasima and not significant at all for the Tak and Udonthani sources.

When making comparisons for the 30°C axes the responses were again variable, Udonthani being most sensitive, Tak, Kanchanaburi and Saraburi showing some response and Chiang-rai and Nakhonratchasima showing no response. Because the response to variation between day and night was generally significant it is tempting to postulate that *P. macrocarpus* seeds require variable day/night temperatures to reach their best germination and that constant temperatures are less desirable.

While the above comparisons (Figure 9) were made based on the daytime temperatures, the data in Figure 10 are based on the night time temperatures. Figure 10 data are to a large extent the mirror immage of the data in Figure 9. What this suggests is that extreme temperatures, whether they occur during the day or night , are deleterious for seed germination of *P. macrocarpus*. It is obvious that the

duration of the exposure to high or low temperatures is important rather than the temperature itself being harmful during short (8h) periods, within the temperatures tried.

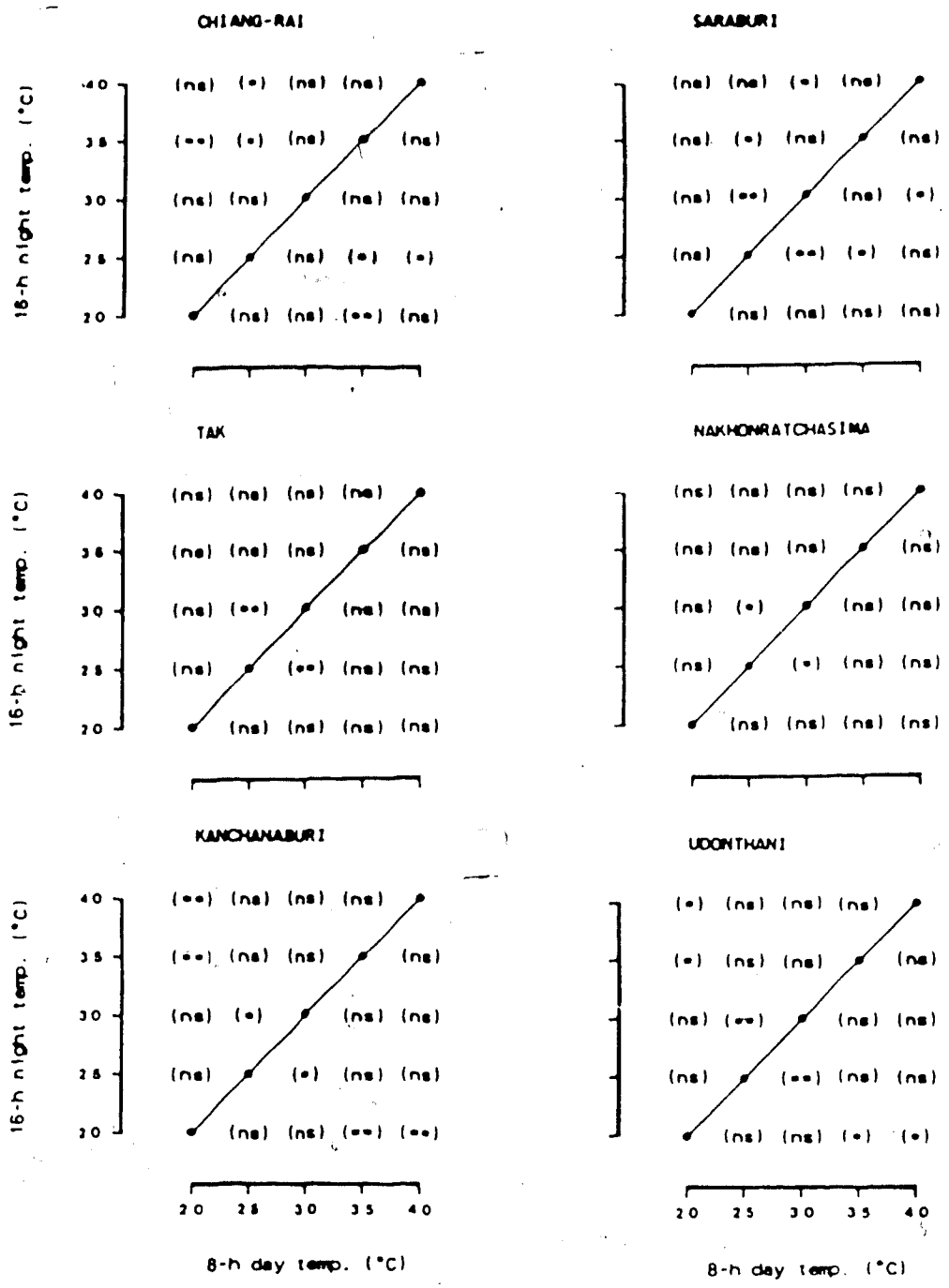


Figure 8. Comparisons of the effects of 8h and 16h durations of temperatures on germination of seed from each of six sources (see Appendix 1 for explanation); (ns): non-significantly different, (*): significantly different at P≤0.05, (**): significantly different at P≤0.01.

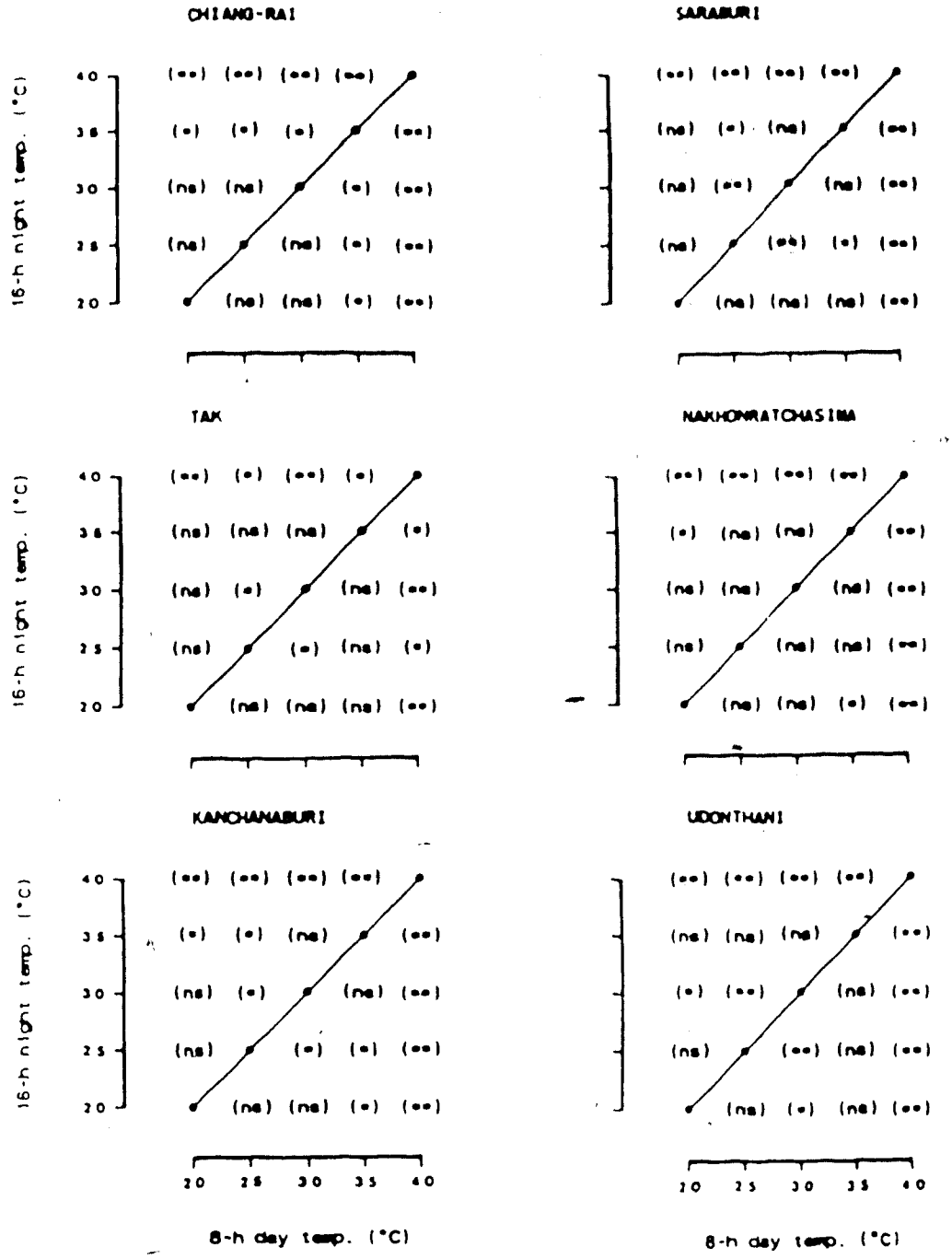


Figure 9. Comparisons of the effects of 8h, 16h and 24h durations of warmer temperature phase of the temperature combinations on germination of seed from each of six sources (see Appendix 1 for explanation); (ns): non-significantly different, (*): significantly different at P≤0.05, (**): significantly different at P≤0.01.

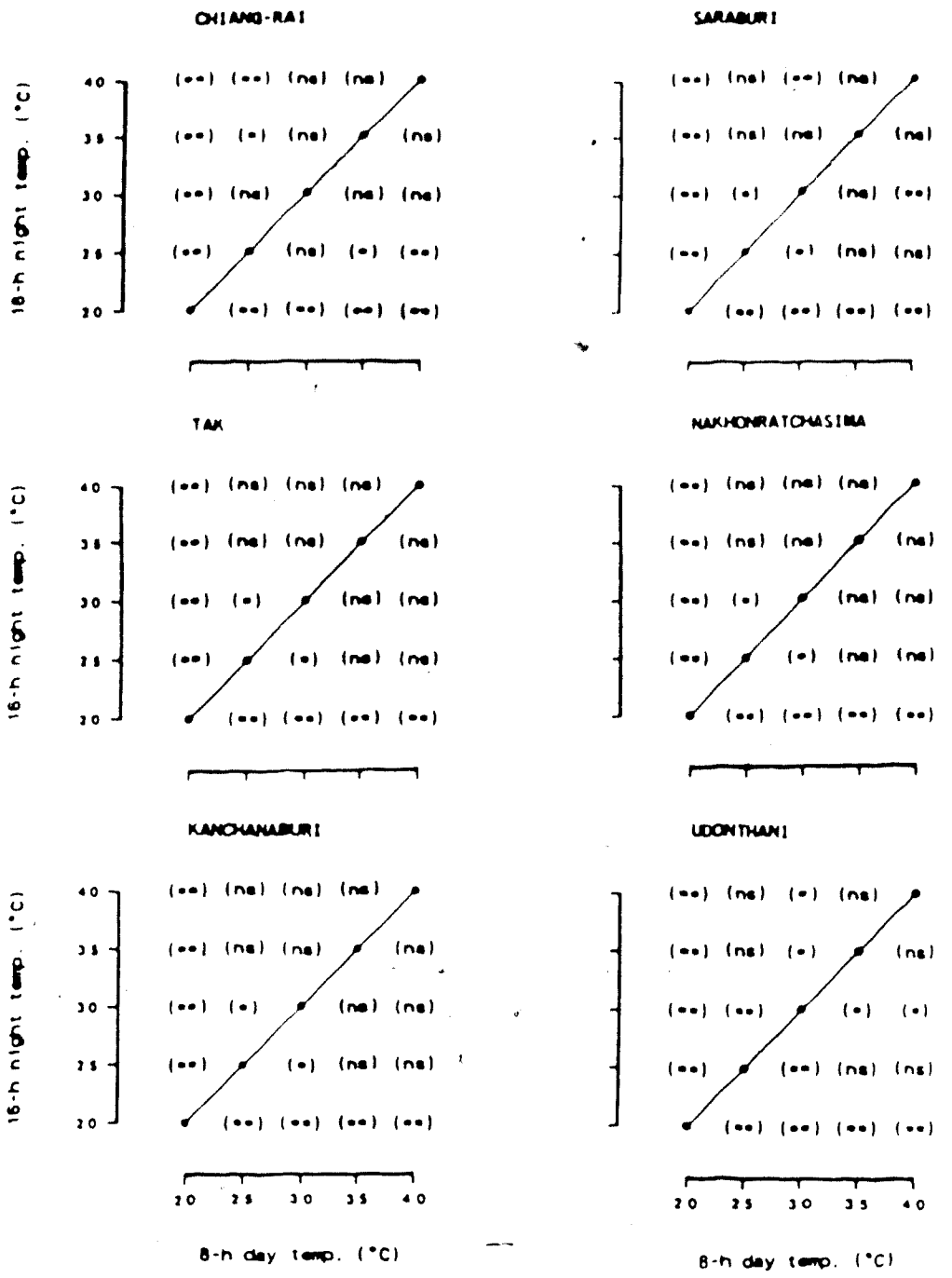


Figure 10. Comparisons of the effects of 8h, 16h and 24h durations of cooler temperature phase of the temperature combinations on germination of seed from each of six sources (see Appendix 1 for explanation); (ns): non-significantly different, (*): significantly different at P≤0.05, (**): significantly different at P≤0.01.

3.1.3 Constant temperatures.

Peak germination was reached under the 25°C constant temperature for Chiang-rai and under the 30°C constant temperature for all the other sources. Peak values ranged between 84 and 91% germination for all lots. Low total germination (less than 57%) was achieved at a 40°C constant temperature and even less total germination (less than 46%) was achieved at a 20°C constant temperature (Table 6). At 45°C constant temperature, no germination occurred.

Table 6. Means ± S.D. of total germination (%) at different constant temperatures of each of six seed sources.'

	Temperature (°C)					
Seed source	20	25	30	35	40	45
Chiang-rai	45.80 ±10.8	90.50 ±4.12	88.15 ±6.47	84.35 ±2.76	51.00 ±4.76	0
Tak	21.69 ±6.88	81.15 ±5.75	85.11 ±6.26	76.44 ±6.84	57.41 ±8.39	0
Kanchanaburi	40.07 ±11.2	78.94 ±4.13	83.78 ±4.12	75.53 ±5.16	50.56 ±3.38	0
Saraburi	24.49 ±5.40	[illegible]	84.56 ±3.84	76.52 ±3.03	39.18 ±11.1	0
Nakhonratchasima	36.6 ±10.6	[illegible]	86.07 ±6.58	77.53 ±6.08	34.63 ±7.79	0
Udonthani	18.25 ±4.79	77.90 ±5.84	88.27 ±3.48	76.58 ±12.7	31.87 ±6.35	0

'8-hour photoperiod was provided as day phase.

Highly significantly differences were found among different temperature regimes, seed sources and their interactions determined by two-way ANOVA (Table 7).

Table 7. ANOVA for seed germinability tested at constant temperatures.

SOV	df	SS	MS	F'
Temperatures (T)	5	141300.504	28260.101	716.062 **
Seed sources (S)	5	1737.948	347.590	8.807 **
T X S	25	3603.903	144.156	3.652 **
Error	108	4262.311	39.466	
TOTAL	143	150904.666		

'(**): significant different at P≤0.01 level.

In order to determine which sources were different from their neighbours, a one-way ANOVA was performed and an S-N-K test was also used (P≤0.05) to determine which sources were different (Table 8).

There were significant differences among seed sources at 20°C constant temperature (Table 8). Chiang-rai, Kanchanaburi and Nakhonratchasima sources had 46%, 40% and 37% germination, respectively. The other three, Tak, Saraburi and Udonthani, had only 22%, 25% and 18%, respectively.

At the hot end of the scale, i.e., 40°C constant temperature, seedlots were also different in their germination (Table 8). Tak had 57%, Chiang-rai had 51% and Kanchanaburi had 51% germination while the other three, Saraburi, Nakhonratchasima and Udontani, had 39%, 35% and 32% germination, respectively, obviously significantly less than the first three (Table 8).

It would therefore seem that the Chiang-rai and Kanchanaburi sources were adapted to cooler conditions, that

the Tak source is adapted to the warmest conditions and does poorly under cold conditions and that the remaining three sources, Saraburi, Nakhonratchasima and Udonthani, were intermediate between these two groups (Table 8). All lots responded similarly in germination at constant temperatures between 25 and 35°C.

Table 8. ANOVA results and multiple comparisons of means by S-N-K test among six seed sources in each temperature regime.

	Temperature (°C)[1]					
Seed source	20	25	30	35	40	45
	(**)	(ns)	(ns)	(ns)	(**)	(ns)
Chiang-rai	45.8a	90.5a	88.2a	84.4a	51.0ab	0a
Tak	21.7bc	81.2a	85.1a	76.4a	57.4a	0a
Kanchanaburi	40.1a	78.9a	83.8a	75.5a	50.6ab	0a
Saraburi	24.5bc	82.5a	84.6a	76.5a	39.2bc	0a
Nakhonratchasima	36.6ab	78.7a	86.1a	77.5a	34.6c	0a
Udonthani	18.2c	77.9a	88.3a	76.6a	31.9c	0a

[1]Brackets indicated results of ANOVA among seed sources at each temperature:
(ns): non-significant.
(**): significantly different at P≤0.01 level.
Means followed by the same letters in each column were not significantly different at P≤0.05 level determined by S-N-K test.

Temperature had an obvious effect on germination within sources (Table 9) and it is clear from this table that cold (20°C) had a more deleterious effect on Tak, Saraburi and Udonthani than on the others but even in Kanchanaburi the cold (20°C) had a worse effect than did the heat (40°C). In all cases, however, temperature between 25 and 35°C solicited similar responses.

Table 9. ANOVA results and multiple comparisons of means by S-N-K test among temperatures for each of six seed sources.

Seed source[1]		Temperature (°C)					
		20	25	30	35	40	45
Chiang-rai	(**)	45.8b	90.5a	88.2a	84.4a	51.0b	0c
Tak	(**)	21.7c	81.2a	85.1a	76.4a	57.4b	0d
Kanchanaburi	(**)	40.1c	78.9a	83.8a	75.5a	50.6b	0d
Saraburi	(**)	24.5c	82.5a	84.6a	76.5a	39.2b	0d
Nakhonratchasima	(**)	36.6b	78.7a	86.1a	77.5a	34.6b	0c
Udonthani	(**)	18.2c	77.9a	88.3a	76.6a	31.9b	0d

[1]Brackets indicated the ANOVA results among temperatures of each individual seed source:
(**): significantly different at P≤0.01 level.
Means followed by the same letters in each row were not significantly different at P≤0.05 level determined by S-N-K test.

Curves were plotted to describe each relationship between constant temperatures and total germination (Figure 11). The curves were fitted using the approach of Adams and Hills (1977).

The individual line formulae are:

Chiang-rai:

$$Y^{1.3} = [-3.538095+6.400262X^{0.03}-2.983997(X^{0.03})^2]\times 10^7$$

$R^2 = 0.902$
Maximum germination = 93.28% at 28.55°C

Tak:

$$Y^{1.3} = [-2.885618+5.579274X^{0.01}-2.696804(X^{0.01})^2]\times 10^8$$

$R^2 = 0.902$
Maximum germination = 86.57% at 29.5°C

Kanchanaburi:

$$Y^2 = [-4.156985+5.948776X^{0.1}-2.12457(X^{0.1})^2]\times10^4$$

$R^2 = 0.925$
Maximum germination = 84.52% at 28.92°C

Saraburi:

$$Y^{1.9} = [-8.503961+15.90396X^{0.02}-7.435394(X^{0.02})^2]\times10^7$$

$R^2 = 0.941$
Maximum germination = 87.68% at 28.74°C

Nakhonratchasima:

$$Y^{1.4} = -3098.15+278.42X^{0.33}-5.3423(X^{0.33})^2$$

$R^2 = 0.915$
Maximum germination = 88.22% at 29.85°C

Udonthani:

$$Y^{1.1} = [-40.197+16.93897X^{0.47}-1.723375(X^{0.47})^2]\times10^2$$

$R^2 = 0.945$
Maximum germination = 90.84% at 29.60°C

All correlation coefficients (R^2) were high varying only between 0.90 and 0.95 suggesting very close curve fits.

Maximum germination varied between 84 and 93% for all sources when respective temperatures were between 28.6 and 29.8°C. Germination dropped to zero between 42 and 44°C and between 18.1 and 19.6°C for all sources.

Chiang-rai: $Y^{1.1} = [-3.538095 + 6.400262X^{0.01} - 2.983997(X^{0.01})^2]x10^1$; $R^2 = 0.902$

Tak: $Y^{1.0} = [-2.885618 + 5.579274X^{0.01} - 2.696804(X^{0.01})^2]x10^0$; $R^2 = 0.902$

Kanchanaburi: $Y^3 = [-4.156985 + 5.948776X^{0.1} - 2.12457(X^{0.1})^2]x10^4$; $R^2 = 0.925$

Saraburi: $Y^{1.9} = [-8.503961 + 15.90396X^{0.01} - 7.435394(X^{0.01})^2]x10^1$; $R^2 = 0.941$

Nakhonratchasima: $Y^{1.6} = -3098.15 + 278.42X^{0.05} - 5.3423(X^{0.05})^3$; $R^2 = 0.915$

Udonthani: $Y^{1.1} = [-40.19715 + 16.93897X^{0.01} - 1.723375(X^{0.01})^2]x10^1$; $R^2 = 0.945$

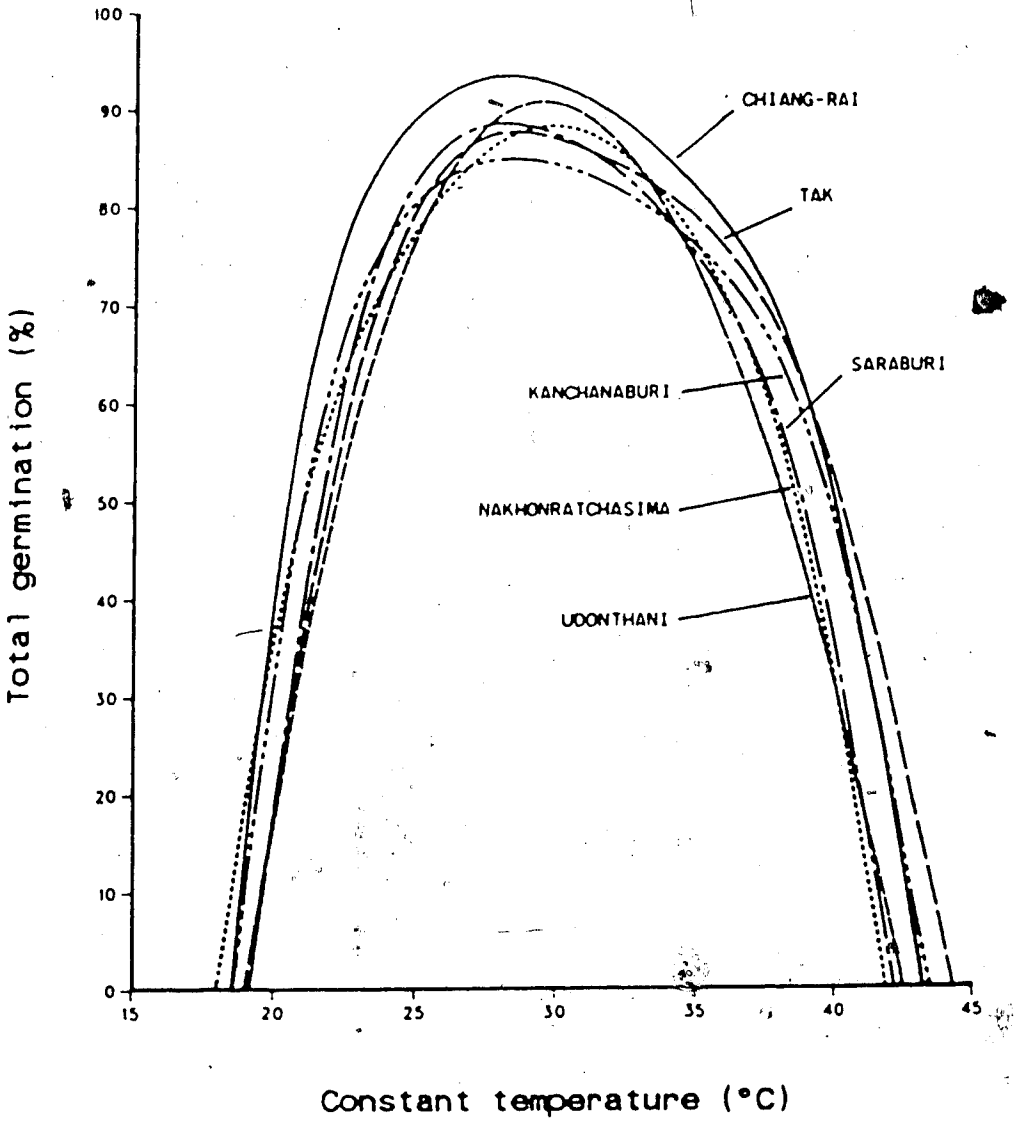


Figure 11. Total germination (%) at different constant temperatures of each of six seed sources.

3.2 Study II: Genetic variability

Eleven putative loci coding for 6 enzymes were assayed to evaluate genic diversity among six *P. macrocarpus* populations. Only one locus (*PGI-1*) was monomorphic for all populations (Table 10). The remaining 10 loci (*AAT-1*, *AAT-3*, *CE-1*, *CE-2*, *IDH*, *MDH*, *PGI-2*, *6PG-1*, *6PG-2* and *6PG-3*) were polymorphic in at least some of the populations. Allele frequencies and both expected and observed heterozygosities at each locus for each population are presented in Table 10. Four genetic parameters were also computed to measure the variability among populations (Table 11). These are the percentage of loci polymorphic (P) at the 95% criterion, average number of alleles per locus (A), and expected and observed heterozygosity, H_e and H_O, respectively.

Allele frequencies varied considerably among populations at each locus (Table 10). With the exception of 3 loci, *AAT-1*, *AAT-3* and *6PG-2*, the same allele (allele 1) was predominant at each locus in all populations with a frequency of over 0.8 except for *IDH* in the Kanchanaburi population (0.50) and for *PGI-2* in the Nakhonratchasima population (0.773). There was, however, appreciable variation in frequency of this common allele at each locus among populations. No single predominant allele among population appeared in three previously mentioned loci, *AAT-1*, *AAT-3* and *6PG-2*. Of 10 polymorphic loci, the Tak and the Saraburi populations were polymorphic in all loci, whereas the other four populations were monomorphic at

at least one locus and *CE-1* was the only common monomorphic locus in all these four populations (Table 10).

There was a substantial level of genetic variation among populations determined by four genetic parameters (Table 11). Single populations of *P. macrocarpus* were polymorphic, on the average, at 63.6% of their loci, ranging from 54.5% in the Chiang-rai, Kanchanaburi and Udonthani populations to 81.8% in the Tak populations. These polymorphic loci segregated for 2 or 3 alleles (Table 10). The mean number of alleles per locus varied from 1.8 in the Kanchanaburi and Udonthani populations to 2.4 in the Tak population, and the overall mean averaged over 6 populations was 2.0 (Table 11).

Both expected and observed heterozygosities were also variable among populations (Table 11). In general, means of expected heterozygosity (H_e) in each population were relatively higher than those of observed heterozygosity (H_o) with the exception of the Chiang-rai population in which both values were similar. The values ranged from 15.7% in the Chiang-rai population to 24.6% in the Tak population, with an overall population mean of 20.3%.

In contrast, the Chiang-rai population had a higher mean H_o than that of the other five populations. These means varied from 7.0% in the Saraburi population to 16.1% in the Chiang-rai population and the overall population mean was 12.1%.

Table 10. Allele frequencies, expected and observed heterozygosities (H_e and H_o, respectively) at each of 11 loci in six *P. macrocarpus* populations.

Locus	Allele	Population					
		Chiang-rai	Tak	Kancha-naburi	Sara-buri	Nakhon-ratcha-sima	Udon-thani
AAT-1	1	0.909	0.727	1.000	0.409	0.659	0.705
	2		0.023			0.023	
	3	0.091	0.250		0.591	0.318	0.295
	H_e	0.165	0.408	0.000	0.483	0.464	0.416
	H_o	0.000	0.091	0.000	0.000	0.045	0.045
AAT-3	1	0.432	0.523	0.182	0.773	0.818	0.795
	2	0.568	0.477	0.818	0.227	0.182	0.205
	H_e	0.491	0.499	0.298	0.351	0.298	0.326
	H_o	0.500	0.409	0.364	0.182	0.182	0.318
CE-1	1	1.000	0.932	1.000	0.909	1.000	1.000
	2		0.068		0.091		
	H_e	0.000	0.127	0.000	0.165	0.000	0.000
	H_o	0.000	0.136	0.000	0.000	0.000	0.000
CE-2	1	0.909	0.841	1.000	0.909	0.818	0.932
	2	0.091	0.136		0.091	0.182	0.068
	3		0.023				
	H_e	0.165	0.274	0.000	0.165	0.364	0.127
	H_o	0.182	0.273	0.000	0.000	0.091	0.136
IDH	1	1.000	0.955	0.500	0.977	0.977	0.977
	2			0.205			
	3		0.045	0.295	0.023	0.023	0.023
	H_e	0.000	0.086	0.621	0.045	0.045	0.045
	H_o	0.000	0.091	0.409	0.045	0.045	0.045
MDH	1	0.977	0.887	0.955	0.909	0.955	1.000
	2		0.068				
	3	0.023	0.045	0.045	0.091	0.045	
	H_e	0.045	0.207	0.086	0.165	0.086	0.000
	H_o	0.045	0.227	0.091	0.182	0.000	0.000

Table 10. (concluded)

Locus	Allele	Population					
		Chiang-rai	Tak	Kancha-naburi	Sara-buri	Nakhon-ratcha-sima	Udon-thani
PGI-1	1	1.000	1.000	1.000	1.000	1.000	1.000
	H_e	0.000	0.000	0.000	0.000	0.000	0.000
	H_o	0.000	0.000	0.000	0.000	0.000	0.000
PGI-2	1	0.932	0.841	0.864	0.818	0.773	0.886
	2	0.068	0.159	0.045	0.182	0.227	0.114
	3			0.091			
	H_e	0.127	0.267	0.243	0.298	0.351	0.202
	H_o	0.045	0.045	0.091	0.000	0.273	0.227
6PG-1	1	0.977	0.887	0.818	0.955	0.909	0.955
	2		0.068	0.182	0.045	0.091	0.045
	3	0.023	0.045				
	H_e	0.045	0.207	0.298	0.086	0.165	0.086
	H_o	0.045	0.136	0.000	0.000	0.000	0.091
6PG-2	1	0.454	0.795	0.636	0.773	0.705	0.682
	2	0.523	0.205	0.364	0.091	0.068	0.118
	3	0.023			0.136	0.227	0.204
	H_e	0.520	0.326	0.463	0.376	0.447	0.480
	H_o	0.773	0.136	0.000	0.318	0.409	0.318
6PG-3	1	0.909	0.818	0.886	0.886	0.864	0.818
	2	0.068	0.159	0.114	0.114	0.136	0.182
	3	0.023	0.023				
	H_e	0.169	0.305	0.202	0.202	0.235	0.298
	H_o	0.182	0.091	0.227	0.045	0.182	0.182

Table 11. Genetic variability in *P. macrocarpus* populations measured by four genetic parameters; percentage of loci polymorphic (P), averaged number of alleles per locus (A), percentage of expected and observed heterozygosities (H_e and H_o, respectively).

Population	P[1]	A[2]	H_e[2]	H_o[2]
Chiang-rai	54.5	1.9±0.701	15.7±18.5	16.1±25.2
Tak	81.8	2.4±0.674	24.6±14.3	14.9±11.5
Kanchanaburi	54.5	1.8±0.751	20.1±21.0	10.7±15.5
Saraburi	72.7	2.0±0.447	21.2±14.9	7.0±10.8
Nakhonratchasima	63.6	2.0±0.632	22.3±17.4	11.2±13.5
Udonthani	54.5	1.8±0.603	18.0±17.6	12.4±12.2
Means±S.D.	63.6 ±11.5	2.0±0.635	20.3±17.3	12.1±14.8

[1]The frequency of the most common allele is ≤0.95.
[2]Means±S.D.

4. Discussion

4.1 Study I: Seed germination

4.1.1 Patterns of germination

Populations of *P. macrocarpus* exhibited different germination behavior in response to temperature, as determined by the 80% isoline (Figure 5). These differences were rather obvious in a north-south direction, but seemed to be influenced by the local ecological climate of the sources (Figure 2). McWilliams *et al.* (1968), Thompson (1970 and 1975), and Bevington (1986) also found similar results.

Seeds of the northernmost source, Chiang-rai, experienced both cool and warm temperatures (Figure 2) and might be expected to be more tolerant within a wider temperature range than the others (Figure 5). Seeds from this source germinated well in both cool and warm temperatures. The Tak source encountered somewhat warmer temperatures (Figure 2) and was therefore adapted well to the high temperature regimes for germination (Figure 5). The other four are only slightly different from each other in their mean annual temperatures (Figure 2). They expressed only slightly different germination patterns, and tolerated relatively narrow temperature ranges (Figure 5). The geographic and climatic differentiation of germination in response to temperature is common and has been reported for other species distributed over a wide geographic range

(Stearns and Olson 1958; Thompson 1970, 1973b and 1975; Thompson and Cox 1978).

Despite different patterns, all sources shared the large common core of temperature preference for ≥80% germination and they all had an identical optimum temperature regime (30/25°C) for maximum germination (Figure 7). There is a greater correspondence among the six seed sources in their total germination during the short warm period than during the long warm period (Figures 3 and 7).

The deviation of the 80% contour lines from the common temperature core (Figure 7) of each source might be explained by either the adaptive survival in a particular ecological climate (Thompson 1973b; Mayer and Poljakoff-Mayber 1982) or the influence of temperature during seed development and maturation (Heide *et al.* 1976; Wurzburger and Koller 1976). Even though the mean annual temperatures differ among sites, they are similar during those months when seeds deve[illegible]ay to September (Figure 2). This commonality may explain why all lots have such a large common temperature preference area for germination (Figure 7).

In addition, individual parent trees might pass on their climatic adaptation to their progenies resulting in different sensitivity and tolerance to temperatures for germination. As pointed out by Lindauer and Quinn (1972) the adaptive germination response results in

genetic-environmental interactions in the natural growth habitat of a species.

4.1.2 Responses to temperature of variable duration

Diurnal fluctuation in temperature has been reported to break dormancy and improve seed germination in many species (Thompson 1974b and c; Totterdell and Roberts 1980). The effectiveness of the amplitude of the temperature fluctuation also affects germination (Thompson 1969; Ellis and Roberts 1979). Thompson *et al.* (1977) have investigated the diurnal fluctuating temperature requirement of several species and the values range from 1°C to 9°C. Young and Evans (1986) also reported a minimum requirement of 15°C diurnal fluctuation for optimum germination of white horehound (*Marrubium vulgare*) seed. Totterdell and Roberts (1980) have also tried to find the optimum period for day and night temperatures using different lengths of exposure.

Amplitude of diurnal fluctuations in temperature, between 8h and 16h, does not influence germination in *P. macrocarpus* consistently or significantly. Only random variations proved significant within the test matrix (Figure 8) and only at the optimum temperature of 30/25°C did it matter how long the exposure was.

However, when constant temperature regimes, i.e., a 24h duration and null amplitude, were introduced into the comparisons, only those including 20°C or 40°C constant regimes showed significant differences in all sources

(Figures 9 and 10). Comparisons including 25, 30 or 35°C constant regimes were significantly different merely in some cases for some sources but this was not common for all sources.

The results suggested that within the mild temperature range, 25-35°C, germination was independent of both duration and amplitude of the diurnal cycle. Basically, the common area of temperature preference for ≥80% germination (Figure 7) agreed well with this result.

The seeds of *P. macrocarpus* germinate better under day/night alternating than constant temperatures. The difference between day and night needs to be 5°C or more. The seeds of *P. macrocarpus* will therefore germinate best during May to August when these temperatures conditions are met and soil moisture is also avialable (Figure 2). The coincidence of both favorable temperature conditions and available soil moisture in the field predetermine the survival of *P. macrocarpus*.

4.1.3 Constant temperatures

That constant temperature inhibits seed germination has been reported for many species (Thompson 1969, 1974b and c; Totterdell and Roberts 1980). Some seeds are capable of germinating over a restricted range of constant temperatures but germination commonly is improved by alternating temperatures (Thompson 1974c; Ellis and Roberts 1979). Germination of *P. macrocarpus* was also restricted to a

fairly narrow range of constant temperatures (Figure 11). The optimum constant temperatures for maximum germination computed from regression equations range from 28.6°C to 29.8°C, similar to that of *Gmelina arborea*, another tropical tree of similar habitats (Ng 1985).

Once the temperature increased or decreased from the optimum, germination declined sharply. Germination was low at both 20°C and 40°C and within a few degrees beyond these values, germination fell to zero (Figure 11). Optimum temperatures seemed to occur at the middle range (28-32°C) of constant temperatures tested. At this temperature transition in membranes is more effective and influences seed germination and subsequent growth (Hendricks and Taylorson 1979). They also pointed out that factors favorable to germination increase at constant temperatures above 20°C, but at higher temperatures, above 29°C, loss of endogenous constituents or leakage from organelles could be one of the limiting factors to germination. Such factors may explain why seeds of *P. macrocarpus* germinated well only over a narrow range of constant temperatures (28-32°C) and germination decreased beyond this range.

The germination response to temperature, and dispersal of seed at the right time for germination influence survival, distribution and population size of the species (Thompson 1970; Koller 1972; Harper 1977; Mayor and Poljakoff-Mayber 1982). That fruits of *P. macrocarpus* are ripe during the cool months (December to January) and still

hang on the tree for a few months (Troup 1921) may reflect a mechanism for avoiding the unfavorable cool temperatures for germination. Shedding of fruits occurs sometime prior to the period during which germination is favored (May to August) thus allowing the species to establish itself.

4.2 Study II: Genetic variability

The six populations of *P. macrocarpus* studied were quite variable genetically. The level of this genic diversity appeared random. No clinal patterns relative to geographic locations were observed. This may not be surprising, since relatively few populations were sampled. The results here were similar to those reported for *Sequoiadendron giganteum* [Lindl.] Buch. (Fins and Libby 1982), *Pinus nigra* Arnold (Nikolić and Tucić 1983) and *Pinus sylvestris* L. (Gullberg *et al.* 1985), but dissimilar to those reported for lodgepole pine (*Pinus contorta* Dougl. spp. *Latifolia* Engelm.) (Yeh and Layton 1979) and Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) (Yeh and O'Malley 1980).

The populations of *P. macrocarpus* were highly polymorphic (63.6%). This value is similar to that (67.7%) reported from 20 coniferous species by Hamrick *et al.* (1981); however, it was higher than that reported for some coniferous species, e.g., *Pinus resinosa* Ait. (Fowler and Morris 1977), *Picea sitchensis* (Bong.) Carr. (Yeh and El-Kassaby 1980), *Thuja plicata* Donn ex D. Don (Copes 1981),

Pinus contorta Dougl. var. *latifolia* Engelm. and *P. banksiana* Lamb. (Dancik and Yeh 1983).

The average number of alleles per locus of this species (2.0) was moderate and similar to that reported for knobcone pine (*Pinus attenuata* Lemm.) (Conkle 1981) and some tropical species (Torres *et al.* 1978), and only slightly lower than that (2.29) summarized from 20 coniferous species (Hamrick *et al.* 1981).

Individuals of *P. macrocarpus* were more highly heterozygous than some other conifers, e.g., *Pinus ponderosa* (O'Malley *et al.* 1979), *Picea sitchensis* (Bong.) Carr. (Yeh and El-Kassaby 1980), *Pseudotsuga menziesii* (Mirb.) Franco (Yeh and O'Malley 1980), *Pinus contorta* Dougl. (Wheeler and Guries 1982), *Pinus rigida* Mill. (Guries and Ledig 1982). This high heterozygosity might be due to the small number of loci assayed, which possibly caused an inflated value as previously reported (Lundkvist and Rudin 1977; Lundkvist 1979; Nikolić and Tucić 1983). Lewontin (1974) has suggested a large number of loci be screened in order to determine the heterozygosity more precisely. However, tropical species may have higher genic variability than do temperate species (Nevo 1978). This author also pointed out that habitat generalists (widespread, broad-niched) are typically more variable genetically than habitat specialists (geographically restricted, narrow-niched). *P. macrocarpus* may be considered as a habitat generalist because it is widely distributed in different forest types and climates,

and thus could maintain a high genetic variability.

The discrepancy between expected and observed heterozygosities, 20.3% and 12.1%, respectively, might be due to a high level of inbreeding (Table 10). In other words, at many loci fewer heterozygotes were found than would be expected from the frequencies of the alleles in the population if random mating were occurring. *P. macrocarpus* has perfect flowers. The pollination mechanism of this species is believed to rely on insects as pollinators. The movement of pollinators among adjacent flowers within the crown or between adjacent crowns of related neighbours may result in a high proportion of selfing and inbreeding progeny. Thus, a large number of homozygous individuals would be expected (Levin and Kerster 1968). The sampled trees of *P. macrocarpus* were located a minimum of 100 m apart from each other. It is not likely that pollination among sampled trees occurred. The high level of homozygous individuals observed might well support the evidence of selfing or inbreeding among clustered relatives (siblings). However, a study of the mating system is essential to demonstrate this evidence. This inbreeding effect on a relatively high level of observed homozygous individuals was also reported in *Eucalyptus kitsoniana* (Leuhm) Maiden (Fripp 1982) and giant sequoia (*Sequoiadendron giganteum* [Lindl.] Buch.) (Fins and Libby 1982).

The most likely explanation for the existence of population differentiation in *P. macrocarpus* might be the

selection pressures that proceed over environmental heterogeneity (Yeh and Layton 1979; Nikolić and Tucić 1983; Knowles and Grant 1985; O'Reilly *et al.* 1985). Nevo (1978) also pointed out that natural selection is the major determinant of genetic population structure and differentiation and also referred to the environmental variation hypothesis, i. e., physical (climate, chemical) and biological (food, competition) variables are major determinants of genetic variation. As trees and stands are scattered and distributed over different forest types, geographic locations and climates, individual trees encounter different local habitats. Different alleles or genotypes which are favoured by a particular locality may give rise to individual adaptability within that microsite, thus maintaining themselves in the community (Hamrick *et al.* 1981). The selection pressures may also be acting from generation to generation due to changes in or fluctuation of environment. As a result a wide variety of genotypes exist and differentiation among genotypes occurs on many of sites.

5. Conclusions and recommendations

5.1 Conclusions

1. Germination patterns are different among *P. macrocarpus* populations and are believed to be under control of the local climate prevailing in their habitats.
2. Sources are sensitive to temperature for germination and show great variability. Nevertheless, all sources gave maximum germination at one identical temperature regime (30/25°C).
3. Germination within the range of 25°C to 35°C was remarkably similar for all seed sources regardless of whether the day or night was 8h or 16h long.
4. Alternating temperatures were better than constant temperatures for seed germination. A high germination (≥80%) could be obtained over a wide range of alternating temperatures but was restricted to a narrow range of constant temperatures, and zero germination was reached at constant temperatures just under 20°C or just over 44°C regardless of the seed sources.
5. Populations of *P. macrocarpus* were variable genetically, but no clinal patterns relative to geographic origins could be observed.

5.2 Recommendations

1. Seeds should be germinated under 30/25°C day-night alternating temperatures to reach the highest total germination regardless of origins.
2. Germination performed under variable environments, e.g., lathhouse, shade under trees, should be avoided during the cool months (December to February). If necessary, monitoring of the temperature of the seedbed and the surrounding atmosphere should be done. Likewise, seeds should not be exposed to high temperatures, over 40°C, for prolonged periods during germination or to temperature below 20°C.
3. Seedling vigor classes should be developed to determine the time course of germination required in nursery practice.
4. The mating system of *P. macrocarpus* should be investigated in order to design a seed orchard and to test whether it is necessary to maintain a broad genetic base. Meanwhile, a provenance trial should also be performed to define seed zones.

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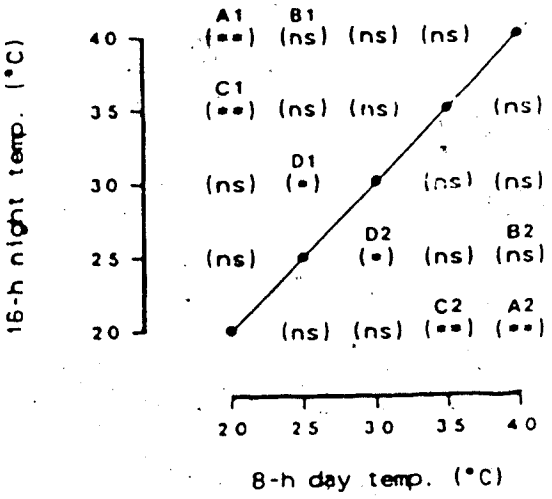
Appendix 1

A. Explanation for comparisons between 8h and 16h durations and for Figure 8



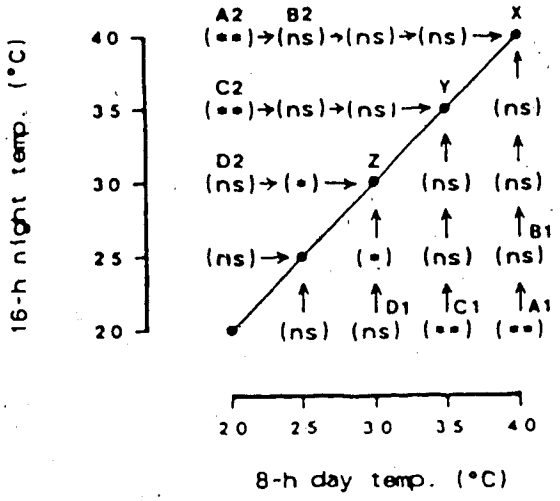
(ns): non-significant different
(*): significant different at P≤0.05
(**): significant different at P≤0.01

Each comparison was made between two alternating temperature regimes composed of the same pair of combined temperatures but different durations, 8 and 16 hours. The regime A1 (20/40°C) was compared to regime A2 (40/20°C) and significant differences were found. The regime B1 (25/40°C) was compared to B2 (40/25°C) and they were not significantly different. There were also significant differences in the comparisons between C1 (20/35°C) and C2 (35/20°C) and between D1 (25/30°C) and D2 (30/25°C).

Likewise, other comparisons were also carried out in a similar manner throughout the diagram. The upper and lower halves of diagram are symmetical, hence, the results of the

comparisons can be read from either part.

B. Explanation for comparisons between 8h, 16h and 24h durations and for Figure 9



(→): direction of comparisons
(ns): non-significant different
(•): significant different at P≤0.05
(••): significant different at P≤0.01

The comparisons were made, as directed by the arrows, between two alternating temperature regimes composed of the same pair of combined temperatures but different durations (8 and 16 hours) and a constant temperature regime which was similar to the warmer temperature phase of alternating regimes. The constant regime represented the 24-h duration and also emphasized which temperature phase in the alternating regimes was compared.

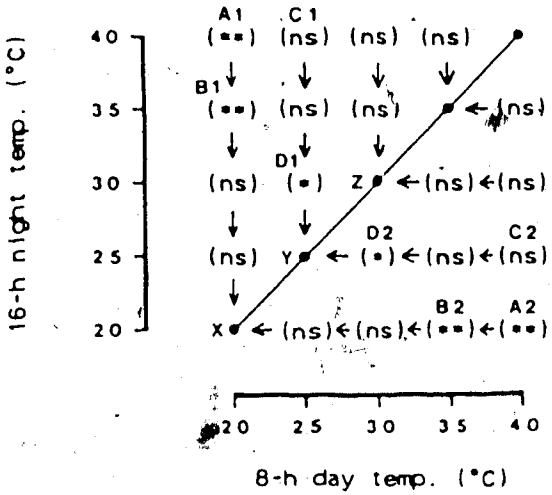
For example, the regime A1 (40/20°C), A2 (20/40°C) and X (40/40°C) were compared for 8h, 16h and 24h durations for 40°C when combined with 20°C in alternating ones, and

significant differences were found. When 40°C was combined with 25°C in alternating regimes, there were no significant differences, i.e., comparisons between B1 (40/25°C), B2 (25/40°C) and X (40/40°C).

There were significant differences in the comparisons between C1 (35/20°C), C2 (20/35°C) and Y (35/35°C), but no significant differences existed in the comparisons between D1 (30/20°C), D2 (20/30°C) and Z (30/30°C).

Likewise, similar comparisons were also applied to other pairs throughout the diagram. Both upper and lower halves of the diagram are symmetrical; thus, the results can be examined from either part.

C. Explanation for comparisons between 8h, 16h and 24h durations and for Figure 10



(→): direction of comparisons
(ns): non-significant different
(*): significant different at 0.05
(**): significant different at 0.01

The comparisons were made as directed by the arrows to test the effects of 8h, 16h and 24h durations. In each instance, two alternating temperature regimes comprising the same pair of combined temperatures but different durations (8 and 16 hours) and a constant temperature. The constant temperature regime represented the 24-hour duration. It was similar to the cooler temperature phase of the alternating regimes, and it also indicated which temperature phase in the alternating regimes was emphasized.

For examples, A2 (20/40°C), A2 (40/20°C) and X (20/20°C) were compared to test 8h, 16h, and 24h durations for 20°C, and there were significant differences. There were also significant differences in the comparisons of

B1 (20/35°C), B2 (35/20°C) and X (20/20°C). However, there were no significant differences in the comparisons between C1 (25/40°C), C2 (40/25°C) and Y (25/25°C) to test 8h, 16h and 24h durations for 25°C when it was combined with 40°C. However, when 25°C was combined with 30°C significant differences were found, i.e., among D1 (25/30°C), D2 (30/25°C) and Y (25/25°C).

A similar procedure was applied to other comparisons throughout the diagram. Both upper and lower halves are symmetrical; therefore, the results can be investigated from either side.

Appendix 2

A. Extraction buffer
(Modified formula of Forest Genetics Lab., Univ. of Alberta, Edmonton, Alberta, Canada)

0.1M Tris-HCl pH 7.5	80 ml
Ascorbic acid	0.2 g
Cysteine	0.095 g
Tween	1 ml
10% $MgCl_2$	2 ml
10% $CaCl_2$	2 ml
Sucrose	17.1 g
2-mercaptoethanol	3 drops

Adjust pH to 7.5 at room temperature with 1 M tris and add up to 100 ml with distilled water before adding 2-mercaptoethanol.

B. Buffer formulations used to run gels
(4 buffer systems)

1. Ridgway *et al.* 1970
(Initial current 300 volts, after dewicking 300 volts)

Electrode buffer (pH 8.1):

0.06 M lithium hydroxide	10.07 g/4 l
0.30 M boric acid	74.16 g/4 l

Adjust pH to 8.1 at room temperature with boric acid or lithium hydroxide if necessary.

Gel buffer:

0.03 M trizma base	72.60 g/2 l
0.005 M citric acid (anhydrous)	19.20 g/2 l
1% electrode buffer	200 ml/2 l

Adjust pH to 8.5 at room temperature with trisma base or citric acid if necessary.
When pouring gels dilute 1:10 with distilled water and mix with 12.5% (w/v) starch.

2. Schaal and Anderson 1974
(Initial current 300 volts, after dewicking 300 volts)

Electrode buffer (pH 8.1):

0.31 M boric acid	76.64 g/4 l
0.063 M NaOH	10.08 g/4 l

Adjust pH to 8.1 at room temperature with NaOH or boric acid if necessary.

Gel Buffer:
0.08 M trizma base.....19.36 g/2 l
Adjust pH to 8.65 at room temperature with 1 M citric acid. Check pH immediately before mixing with 12.5% (w/v) starch and readjust if necessary, no dilution necessary.

3. Siciliano and Shaw 1976
(Initial current 300 volts, after dewicking 200 volts)

Electrode Buffer (pH 7.0):
0.13 M trizma base.....62.97 g/4 l
0.043 M citric acid (anhydrous).....33.04 g/4 l
Adjust pH to 7.0 at room temperature with 10 N NaOH or conc. HCl.

Gel Buffer:
Dilute 24 ml of electrode buffer with distilled water to total volume of 350 ml and mix with 12.5% (w/v) starch.

4. Florence (1981) after Namkoong *et al.* (1979)
(Initial current 300 volts, after dewicking 200 volts)

Electrode Buffer (pH 7.0):
0.125 M trizma base.....60.54 g/4 l
Adjust pH to 7.0 at room temperature with 1 M citric acid (anhydrous).

Gel Buffer:
0.05 M L-histidine-HCl.....41.92 g/4 l
1.40 mM EDTA.....1.60 g/4 l
Adjust pH to 7.0 at room temperature by using 1 M tris. When pouring gels dilute 1:5 with distilled water and mix with 12.5% (w/v) starch.

C. Stain buffer formulations

1. 0.2 M Tris-HCl pH 8.0:
Trizma base.....24.22 g
Distilled water.....1 litre
Adjust pH to 8.0 at room temperature with conc. HCl.

2. 0.2 M Tris-maleate pH 6.4:
   0.2 M tris.....60 ml (approx.)
   (Trizma base.....12.11 g/500 ml H_2O)
   0.2 M maleic acid.....40 ml (approx.)
   (Maleic acid.....11.61 g/500 ml H_2O)
   When using mix 2 solutions together and adjust pH to 6.4 at room temperature with appropriate one.

D. Stock solutions for stain components

Fast blue BB salt
conc.: 10 g/100 ml H_2O
G-6-PDH (Glucose-6-phosphate hehydrogenase)
conc.: 5000 units/100ml 0.005 M citrate
(citric acid 0.96 g/l H_2O) pH 7.5
$MgCl_2$ conc.: 10 g/100 ml H_2O
MTT [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide]
conc.: 1 g/100 ml H_2O
NAD (β-nicotinamide adenine dinucleotide)
conc.: 1 g/100 ml H_2O
NADP (β-NAD phosphate)
conc.: 1 g/100 ml H_2O
NBT (Nitro blue tetrazolium)
conc.: 1 g/100 ml H_2O
PMS (Phenazine methosulfate)
conc.: 1 g/100 ml H_2O

E. Stain recipes

Enzyme staining recipes and procedures were used directly from or modified slightly from Siciliano and Shaw (1976), Collier and Murray (1977) and Yeh and O'Malley (1980).

1. Aspartate aminotransferase (*AAT*) E.C. 2.6.1.1 (Siciliano and Shaw 1976)

   Stain buffer: 0.2 M Tris-HCl pH 8.0.....50 ml
   Fast blue BB salt.....2 ml
   AAT substrate solution.....50 ml
   α-ketoglutaric acid.....146 mg
   L-aspartic acid.....532 mg
   Polyvinylpyrrolidone.....20 g
   EDTA.....0.2 g
   Sodium phosphate dibasic.....5.68 g
   Distilled water to 200 ml
   Add fast blue BB salt just before staining.
   Incubate gels at 37°C in dark and leave overnight with stain solution.

2. Colorimetric esterase (*CE*) E.C. 3.1.1.1
(Collier and Murry 1977)

Stain buffer: 0.2 M Tris-maleate pH 6.4.....100 ml
α-naphthyl acetate.....40 mg
Fast blue BB salt.....2 ml
Incubate at 37°C in the dark for approx. ½ hr. and keep in ca. 4°C fridge overnight with stain solution.

3. Isocitrate dehydrogenase (*IDH*) E.C. 1.1.1.41
(Yeh and O'Malley 1980)

Stain buffer: 0.2 M Tris-HCl pH 8.0.....90 ml
NADP.....2 ml
$MgCl_2$.....2 ml
MTT.....2 ml
PMS.....2 ml
DL-isocitric acid.....400mg
Incubate at 37°C in the dark.

4. Malate dehydrogenase (*MDH*) E.C. 1.1.1.37
(Yeh and O'Malley 1980)

Stain buffer: 0.2 M Tris-HCl pH 8.0.....[illegible]0 ml
DL-malic acid.....50 ml
0.5 M DL-malic acid (67.01 g/l H_2O)
in 0.2 M tris (24.22 g/l H_2O)
Adjust pH to 7.0 at room temperature
with conc. NaOH.
NAD.....4 ml
NBT.....2 ml
PMS.....2 ml
MTT.....2 ml
Incubate at 37°C in the dark.

5. Phosphoglucose isomerase (*PGI*) E.C. 5.3.1.9
(Yeh and O'Malley 1980)

Stain buffer: 0.2 M Tris-HCl pH 8.0.....92 ml
NADP.....2 ml
$MgCl_2$.....2 ml
MTT.....2 ml
PMS.....2 ml
G-6-PDH.....2 ml
Fructose-6-phosphate.....50 mg
Incubate at 37°C in the dark.

6. 6-phosphogluconic dehydrogenase (*6PG*) E.C. 1.1.1.44 (Yeh and O'Malley 1980)

Stain buffer: 0.2 M Tris-HCl pH 8.0	20 ml
NADP	4 ml
$MgCl_2$	2 ml
MTT	4 ml
PMS	6 ml
Phosphogluconic acid (Na, salt)	20 mg

Incubate at 37°C in the dark.

Note: All pH values were measured at room temperature (ca. 20-22°C).

Appendix 3

A. Total germination (%) data of 31 common temperature regimes.

Temp. Regime (°C)	Rep.	Seed source: Chiang-rai	Tak	Kancha-naburi	Sara-buri	Nakhon-ratcha-sima	Udon-thani
20/20	1	34.69	18.75	30.00	22.45	36.73	18.00
	2	38.30	14.00	46.94	20.41	32.65	16.00
	3	55.10	24.00	31.25	30.61	51.06	25.00
	4	55.10	30.00	52.08	24.44	26.00	14.0
Mean±S.D.		45.80 ±10.8	21.69 ±6.88	40.07 ±11.2	24.49 ±5.40	36.61 ±10.6	18.25 ±4
20/25	1	84.00	70.83	72.00	69.77	63.64	73.
	2	92.00	79.17	79.17	72.34	83.33	
	3	89.80	83.33	76.00	78.72	77.27	64
	4	80.00	73.47	79.17	67.39	75.00	26
Mean±S.D.		86.45 ±5.46	76.70 ±5.62	76.58 ±3.40	72.06 ±4.88	74.81 ±8.24	72.43 ±6.21
20/30	1	86.96	74.42	78.95	67.39	75.00	83.33
	2	86.96	68.18	78.26	81.40	75.00	76.00
	3	91.67	79.54	78.26	75.56	84.00	61.90
	4	86.96	78.26	76.19	73.91	79.17	77.27
Mean±S.D.		88.13 ±2.36	75.10 ±5.10	77.91 ±1.19	74.56 ±5.76	79.29 ±6.13	74.63 ±9.06
20/35	1	88.00	83.33	70.83	68.75	69.56	62.50
	2	83.33	77.08	84.00	77.08	70.83	84.00
	3	86.96	70.21	82.61	79.17	86.36	78.26
	4	84.00	68.08	81.82	64.58	78.26	86.96
Mean±S.D.		85.57 ±2.26	74.68 ±6.93	79.81 ±6.05	72.40 ±6.88	76.26 ±7.75	77.93 ±10.9
20/40	1	79.31	66.67	72.41	62.07	76.92	76.92
	2	81.48	83.33	67.86	85.71	67.86	73.08
	3	71.43	82.76	71.43	68.96	70.37	64.28
	4	82.14	80.00	75.86	70.37	67.86	71.43
Mean±S.D.		78.59 ±4.92	78.19 ±7.82	71.89 ±3.29	71.78 ±9.97	70.75 ±4.28	74.43 ±5.29

A. (continue)

Temp. Regime (°C)	Rep.	Seed source: Chiang-rai	Tak	Kancha-naburi	Sara-buri	Nakhon-ratcha-sima	Udon-thani
25/20	1	77.55	68.75	77.08	77.55	67.35	65.31
	2	94.00	62.50	70.83	71.43	74.47	74.00
	3	81.25	75.51	77.55	79.59	91.49	74.00
	4	98.00	73.47	75.51	81.63	77.55	86.00
Mean±S.D.		87.70 ±9.84	70.06 ±5.78	75.24 ±3.07	77.55 ±4.41	77.71 ±10.1	74.43 ±8.50
25/25	1	86.00	80.00	72.92	77.55	72.92	78.00
	2	88.00	85.42	81.63	78.72	74.00	79.59
	3	94.00	85.71	81.63	92.00	80.00	70.00
	4	94.00	73.47	79.59	81.63	87.76	84.00
Mean±S.D.		90.50 ±4.12	81.15 ±5.75	78.94 ±4.13	82.48 ±6.58	78.67 ±6.81	77.90 ±5.84
25/30	1	91.67	76.19	73.91	70.83	66.67	73.33
	2	84.00	70.73	76.09	82.98	85.11	73.33
	3	91.67	75.56	83.33	75.00	82.98	76.74
	4	91.67	80.00	80.00	85.11	82.22	73.91
Mean±S.D.		89.75 ±3.83	75.62 ±3.80	78.33 ±4.18	78.48 ±6.70	79.24 ±8.47	74.33 ±1.63
25/35	1	78.00	69.39	80.85	77.78	83.67	77.55
	2	85.42	81.63	79.59	72.73	75.51	82.98
	3	85.11	65.31	72.00	78.26	73.47	77.78
	4	85.71	81.63	82.35	72.09	69.39	62.79
Mean±S.D.		83.56 ±3.71	74.49 ±8.41	78.70 ±4.61	75.21 ±3.25	75.51 ±6.01	75.27 ±8.69
25/40	1	81.82	74.07	76.00	81.48	67.86	76.92
	2	76.19	70.37	72.22	69.23	76.67	61.54
	3	79.17	71.43	75.00	75.86	72.41	73.08
	4	81.48	85.18	70.59	66.67	75.86	77.78
Mean±S.D.		79.66 ±2.60	75.26 ±6.79	73.45 ±2.49	73.31 ±6.68	73.20 ±4.01	72.33 ±7.48

A. (continue)

Temp. Regime (°C)	Rep.	Seed source: Chiang-rai	Tak	Kancha-naburi	Sara-buri	Nakhon-ratcha-sima	Udon-thani
30/20	1	90.00	78.72	81.25	81.25	89.58	80.85
	2	88.00	71.43	84.78	70.00	77.08	83.67
	3	78.00	69.39	79.59	71.74	81.63	75.00
	4	80.00	89.80	72.73	81.63	79.17	67.35
Mean±S.D.		84.00 ±5.89	77.33 ±9.22	79.59 ±5.06	76.16 ±6.15	81.87 ±5.47	76.72 ±7.22
30/25	1	92.00	83.33	82.98	93.75	95.65	90.00
	2	90.00	93.62	91.84	93.75	91.84	93.88
	3	93.88	85.71	92.00	91.30	87.50	92.00
	4	93.88	93.75	87.50	90.00	95.75	89.80
Mean±S.D.		92.44 ±1.85	89.10 ±5.38	88.58 ±4.28	92.20 ±1.87	92.68 ±3.91	91.42 ±1.92
30/30	1	83.33	93.63	82.98	86.27	90.91	91.49
	2	95.74	82.98	84.78	82.00	91.30	88.89
	3	82.22	85.11	78.72	80.85	77.27	89.36
	4	91.30	78.72	88.64	89.13	84.78	83.33
Mean±S.D.		88.15 ±6.47	85.11 ±6.26	83.78 ±4.12	84.56 ±3.84	86.07 ±6.58	88.27 ±3.48
30/35	1	86.96	76.60	81.25	91.11	76.47	78.00
	2	88.00	70.08	83.67	66.67	80.08	81.63
	3	83.33	76.34	81.25	79.17	80.60	82.98
	4	80.00	85.11	66.67	78.72	74.92	70.83
Mean±S.D.		84.57 ±3.65	77.03 ±6.17	78.21 ±7.78	78.92 ±9.98	78.02 ±2.76	78.36 ±5.44
30/40	1	76.67	75.86	72.00	75.00	82.14	78.57
	2	85.18	83.33	72.00	80.00	75.00	68.96
	3	77.78	68.96	69.56	70.37	76.92	80.00
	4	83.33	90.00	87.50	71.43	68.00	85.18
Mean±S.D.		80.74 ±4.16	79.54 ±9.11	75.27 ±8.24	74.20 ±4.34	75.51 ±5.85	78.18 ±6.77

A. (continue)

Temp. Regime (°C)	Rep.	Seed source: Chiang-rai	Tak	Kancha-naburi	Sara-buri	Nakhon-ratcha-sima	Udon-thani
35/20	1	75.51	81.25	70.83	69.39	62.50	52.10
	2	74.55	60.00	54.35	64.00	60.42	62.18
	3	84.63	72.34	67.39	76.00	46.81	68.00
	4	71.43	86.00	61.70	56.00	72.92	58.50
Mean±S.D.		76.53 ±5.67	74.90 ±11.4	63.57 ±7.21	66.35 ±8.47	60.66 ±10.7	60.20 ±6.66
35/25	1	91.30	80.00	91.30	85.71	73.91	76.60
	2	90.00	64.00	85.42	87.76	81.25	80.85
	3	87.50	89.58	87.23	79.17	88.64	85.11
	4	90.00	87.76	79.17	78.26	85.11	78.26
Mean±S.D.		89.70 ±1.59	80.33 ±11.7	85.78 ±5.05	82.72 ±4.72	82.23 ±6.31	80.20 ±3.71
35/30	1	87.27	89.13	78.00	82.61	83.67	78.26
	2	92.00	88.00	84.00	87.23	86.00	91.49
	3	97.96	73.47	83.67	91.84	93.75	87.23
	4	89.58	88.00	94.00	85.42	80.00	87.50
Mean±S.D.		91.69 ±4.61	84.65 ±7.47	84.92 ±6.65	86.77 ±3.87	85.86 ±5.81	86.12 ±5.59
35/35	1	86.25	76.47	68.29	79.59	78.26	71.11
	2	85.36	69.05	78.26	72.34	82.98	93.62
	3	80.25	85.54	80.00	77.08	80.00	63.83
	4	85.54	74.70	75.56	77.08	68.89	77.78
Mean±S.D.		84.35 ±2.76	76.44 ±6.84	75.53 ±5.16	76.52 ±3.03	77.53 ±6.08	76.58 ±12.7
35/40	1	80.00	71.43	65.38	72.41	65.38	76.67
	2	68.96	68.96	80.00	75.86	75.00	73.33
	3	66.67	70.00	66.67	70.00	72.41	78.57
	4	81.48	85.71	77.78	79.31	75.86	73.33
Mean±S.D.		74.28/ ±7.54	74.03 ±7.86	72.46 ±7.50	74.40 ±4.06	72.16 ±4.75	75.48 ±2.59

A. (continue)

Temp. Regime (°C)	Rep.	Seed source: Chiang-rai	Tak	Kancha-naburi	Sara-buri	Nakhon-ratcha-sima	Udon-thani
40/20	1	58.33	74.00	43.75	69.39	65.31	62.00
	2	60.00	73.47	44.68	53.19	41.30	40.00
	3	66.00	69.39	42.55	52.08	66.67	54.00
	4	83.67	73.47	66.67	68.09	63.26	62.00
Mean±S.D.		67.00 ±11.6	72.58 ±2.14	49.41 ±11.5	60.69 ±9.32	59.14 ±12.0	54.50 ±10.4
40/25	1	83.67	62.00	73.47	83.67	75.51	72.00
	2	92.00	76.00	77.08	82.00	76.00	85.71
	3	85.42	86.00	81.63	70.21	89.80	84.00
	4	87.50	70.00	77.08	81.63	80.00	74.00
Mean±S.D.		87.15 ±3.59	73.50 ±10.1	77.32 ±3.34	79.38 ±6.18	80.33 ±6.63	78.93 ±6.93
40/30	1	93.75	92.00	83.67	79.59	89.00	89.58
	2	83.67	79.59	87.50	81.63	77.08	85.71
	3	89.58	90.00	75.51	79.59	89.13	83.33
	4	80.00	76.00	73.91	85.42	83.67	83.33
Mean±S.D.		86.75 ±6.11	84.40 ±7.80	80.15 ±6.50	81.56 ±2.74	84.81 ±5.78	85.49 ±2.95
40/35	1	88.00	76.00	85.71	83.67	77.55	79.59
	2	90.00	79.59	77.08	77.08	83.33	81.63
	3	70.00	69.39	76.00	81.25	68.00	68.00
	4	89.80	76.00	72.92	79.59	76.00	80.00
Mean±S.D.		84.45 ±9.68	75.24 ±4.25	77.93 ±5.48	80.40 ±2.77	76.22 ±6.32	77.31 ±6.27
40/40	1	48.00	50.00	53.19	28.57	43.48	30.00
	2	50.00	56.25	50.00	31.91	29.17	24.00
	3	58.00	69.39	53.06	42.86	27.08	38.78
	4	48.00	54.00	46.00	53.06	38.77	34.69
Mean±S.D.		51.00 ±4.76	57.41 ±8.39	50.56 ±3.38	39.18 ±11.1	34.63 ±7.79	31.87 ±6.35

A. (continue)

Temp. Regime (°C)[1]	Rep.	Seed source: Chiang-rai	Tak	Kancha-naburi	Sara-buri	Nakhon-ratcha-sima	Udon-thani
45/20	1	32.65	18.75	28.57	36.17	40.42	36.73
	2	52.00	46.80	54.35	39.13	40.91	16.00
	3	39.13	37.50	34.04	31.25	40.42	34.69
	4	48.98	40.81	46.81	46.81	54.35	42.00
Mean±S.D.		43.19 ±8.92	35.97 ±12.1	40.94 ±11.8	38.34 ±6.51	44.03 ±6.88	32.36 ±11.3
45/25	1	69.39	63.26	43.75	56.00	54.17	36.73
	2	72.00	53.06	65.31	51.02	44.90	55.32
	3	67.35	61.22	66.67	57.45	67.35	56.25
	4	68.00	41.30	53.33	51.02	51.02	41.67
Mean±S.D.		69.18 ±2.06	54.71 ±9.97	57.26 ±10.8	53.87 ±3.34	54.36 ±9.48	47.49 ±9.79
45/30	1	92.00	83.33	77.08	82.98	85.42	79.59
	2	88.00	80.00	81.25	77.55	76.09	66.00
	3	87.76	91.84	80.00	77.55	81.25	68.00
	4	72.92	83.67	80.85	81.25	64.44	71.17
Mean±S.D.		85.17 ±8.40	84.71 ±5.03	79.80 ±1.88	79.83 ±2.73	76.08 ±9.08	71.19 ±5.99
45/35	1	79.59	58.33	50.00	68.00	77.08	72.34
	2	70.83	56.25	70.45	66.67	74.00	50.00
	3	81.25	76.09	60.46	79.17	65.31	67.35
	4	64.58	75.56	72.34	64.58	70.21	67.35
Mean±S.D.		74.06 ±7.80	66.56 ±10.7	63.32 ±10.3	69.60 ±6.53	71.65 ±5.08	64.26 ±9.79
45/40	1	40.82	30.00	42.86	27.03	37.50	26.00
	2	37.50	20.41	19.15	27.66	31.25	36.73
	3	40.82	35.42	44.68	31.91	23.91	28.00
	4	22.92	40.00	41.30	16.67	32.65	28.57
Mean±S.D.		35.51 ±8.54	31.46 ±8.42	37.00 ±12.0	25.82 ±6.47	31.33 ±5.65	29.83 ±4.73
45/45	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
	4	0	0	0	0	0	0
Mean±S.D.		0±0	0±0	0±0	0±0	0±0	0±0

[1] 8-16h day/night alternating temperatures

B. Total germination (%) data of additional tests for completion of 80% germination isolines.

Seed source	Rep.	Temperature regime (°C)[1]				
		15/25	15/35	25/15	30/15	50/15
Chiang-rai	1	77.55	81.33	71.43	58.33	44.90
	2	78.00	81.08	64.58	62.50	36.73
	3	77.55	83.78	63.26	53.19	44.00
	4	75.51	74.67	59.18	78.72	32.00
Mean±S.D.		77.15 ±1.12	80.22 ±3.90	64.62 ±5.09	63.19 ±11.0	39.41 ±6.14
Tak	1					6.38
	2					33.33
	3					20.83
	4					28.57
Mean±S.D.						22.28 ±11.8
Nakhonrat-chasima	1				60.00	
	2				68.75	
	3				54.17	
	4				74.47	
Mean±S.D.					64.35 ±9.02	

[1] 8-16h day/night alternating temperatures