Domestication of *Chukrasia*

K. Pinyopusarerk and A. Kalinganire

CSIRO Forestry and Forest Products

PO Box E4008 Kingston

ACT 2604 Australia

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Preface

Major areas of the world's tropical forests have been destroyed this century and tropical forests in Southeast Asia continue to be lost at alarming rates. Associated with the decline of forest cover is a concomitant loss in species and reduction of genetic diversity within surviving species. Urgent measures are required to conserve and sustainably use the remaining genetic resources. These goals can be achieved by bringing economically important species, traditionally used by local people, into cultivation by the process of domestication.

Efficient domestication has two key ingredients. Firstly, willing national and international collaboration so that essential resources can be brought to the task: the genetic resources of natural populations, the capacity to establish and manage field trials, and facilities for coordination. Secondly, strategic plans are needed to guide the overall effort; these identify priorities and provide the framework for collaborative activity, and must be dynamic, evolving as the project progresses.

ACIAR has, therefore, supported a project that fosters the development of domestication strategies for commercially important indigenous species in Southeast Asia. Tree species of the Meliaceae family, such as *Chukrasia*, provide highly-prized timbers and are of economic and social importance in many tropical countries. The contribution of these species to the forest sector can be enhanced through a well-designed domestication program.

This technical report demonstrates how domestication strategy is developed for forest trees, using *Chukrasia* as a model species. A suite of collaborative activities related to the domestication process has been initiated. The results from field trials and related research activities are still preliminary in some respects and therefore the strategy recommended is far from complete, and should be reviewed as further information is generated from the on-going research.

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K. Pinyopusarerk
A. Kalinganire
CSIRO Forestry and Forest Products, Canberra

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I. Introduction

Definitions of Domestication

Domestication has been defined and interpreted differently by various plant scientists. In very broad terms 'plant domestication' is the process of taking a wild plant species and bringing it under management and cultivation. Harlan (1975) views domestication as human-induced change in the genetics of a plant to conform to human desires and agroecosystems. Janick *et al.* (1982) define domestication as a two-stage process in plants: the bringing into cultivation of wild plants and subjecting them to different management or selection. Leakey and Newton (1994) consider domestication as a process which involves the identification and characterisation of germplasm resources; the capture, genetic selection and management of genetic resources; and the regeneration and sustainable cultivation of the species in managed ecosystems. Wiersum (1996) sees domestication as a process of naturalisation of plant species towards specific human-induced growing conditions, during which an increased adoption for specific uses normally takes place. Midgley and Turnbull (2002) regard domestication as a challenging process of exploration and manipulation of the wild genetic resource to derive uses and products for maximum social benefit. Thus, domestication is an on-going process in which genetic characteristics and cultivation practices are continuously refined. In genetic terms, domestication is an accelerated and human-induced evolution and it integrates the four key processes of the identification, production, management and adoption of tree genetic resources (Simons 1996).

Humans have engaged in plant domestication for several thousand years, with varying degrees of conscious effort, mainly for food crops, ornamentals, shelter and religious purposes (Leakey 1998). In contrast to the domestication of most agricultural crops, the principal domestication of trees has occurred in the last one hundred years and only a few species are significantly changed from their wild state. Amongst tree species, by far the greatest effort has been placed on those with edible parts, notably the selection of better fruit and nut varieties (Maghembe *et al.* 1994; Ladipo *et al.* 1996; Evans 1999; Leakey *et al.* 2000; Atangana *et al.* 2001; Thomson *et al.* 2001). Recent concerns about tropical deforestation have overshadowed the very positive progress that is being made to rebuild forest resources of indigenous species throughout the tropics. Many species with high potential for the production of a wide range of products have been identified. In general, however, there has been little effort to formally domesticate and improve forestry tree species in most tropical countries due to limited financial and skilled human resources. Until relatively recently, foresters have collected seeds from the wild and used them without selection (Libby 1973). The work of provenance selection, an early stage in the domestication process, gained international importance in the 1960s. In Australia, progress in domestication has been significant for selected species in the genera *Acacia*, *Araucaria*, *Eucalyptus*, *Macadamia, Melaleuca* and *Pinus*. The range is expanding through work developing from farm-based forest resources and emerging opportunities for horticulture.

The Process of Domestication

Domestication of tree species is a multi-faceted process in which a progressively closer interaction between people and plant resources takes place (Wiersum 1996). It has a clear beginning (the wild plant); this is followed by human intervention via propagation, selection and manipulation, and leads

to enhanced human benefit in the form of production of plantations (Libby 1973). Thus domestication entails a continuum of activity and progress, and involves both manipulating and cultivating plants for specific uses. The process may be affected by biological, policy, market and social factors. Strategies for domestication will vary with tree species and many other factors, such as the value of the products, the extent of intraspecific variation, and the beneficiaries of domestication.

Midgley (1995) presented a thematic diagram showing major steps in the domestication process for forest tree species, reflecting experience of the CSIRO Australian Tree Seed Centre (Fig. 1). An essential first step is to decide which species to domesticate.

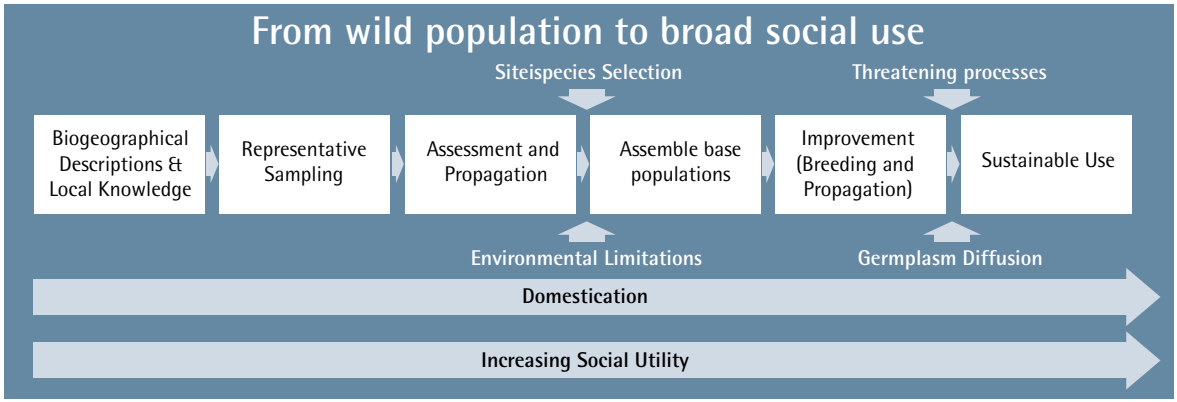
Bio-geographic description and assembly of local knowledge

Domestication of lesser-known tree species should start with the biogeographical description and assembly of local knowledge, carefully examine both scientific and non-scientific records. National herbaria generally provide useful first-hand information on locality of occurrence and flowering and fruiting time which tends to vary between localities. Information on cultivation practices and utilisation by local communities is very valuable.

All this background is essential for planning and undertaking the subsequent steps of the domestication process.

Representative sampling

Domestication implies the collection of seeds or plants, ideally from the entire natural range of the species (Leakey *et al.* 1982). Within the domestication process, different stages may require evolving strategic options for collection. Such strategies are shaped by both biological and non-biological factors including species distribution, reproductive biology, taxonomy and patterns of seeding, ownership of trees, access to genetic resources, obligations to international treaties, and social acceptability of the collection techniques. However, as most of these factors may be unknown at the early stages of domestication, the collection strategy adopted is generally a compromise, influenced by these factors and especially the realities of available resources. The sampling should aim to provide sufficient germplasm for repeat trials as it is common for requests to be received from those who have



■ Figure 1. The process of tree domestication

become aware of a species' promise via results and the literature. An example of this from Australia comes from the screening of species for salt-affected lands where some little-known species and provenances, e.g. of *Acacia stenophylla*, have performed well in trials but insufficient seed has been collected and so the results cannot be followed up by farmers (N.E. Marcar, CSIRO Forestry and Forest Products 1996 pers. comm.).

The current practice by the Australian Tree Seed Centre is to maintain the separate identity of seed from each parent tree. Seed collections aim to sample unrelated trees and provide adequate representation of the genetic variability of the population. For initial investigation of provenance variation, a ten-tree collection per population is usually sufficient to obtain a reliable estimate of provenance performance. More trees, i.e. 50 or more, are sampled to obtain large quantities of seed after provenance trials have indicated the best performing provenances (Gunn 2001). However, the number of trees sampled may have to be less in populations with a restricted distribution.

Assessment and propagation

Results from nursery research and growth assessments of field trials will provide baseline information for an appropriate domestication strategy. Well-designed and replicated provenance trials at multiple locations generally form part of the process of assessment of species.

Studies on differences in seedling morphology in glasshouse experiments provide reliable early information on the extent of the geographic variation in forest tree species, e.g. *Acacia auriculiformis* and *Eucalyptus urophylla* (Pinyopusarerk *et al.* 1991, 1993). In addition, molecular techniques (e.g. isozyme analysis) can be applied to problems such as the estimation of mating system parameters and can efficiently provide estimates of the amount of genetic variation within populations and the extent of genetic diversity between them. Studies of both seedling morphology and isozymes will complement information from growth studies to elucidate geographical patterns of variation.

Assembly of base populations

Assembly of base populations for tree improvement is an important stage in the domestication process. The term 'base population' is used to describe a representative genetic sample of the useful provenances (those which have displayed positive traits in trials or other plantings). Normally, such populations will include progeny from more than a hundred parent trees. An example is *Acacia crassicarpa* which has been widely assessed in provenance trials in many countries. These trials have indicated that the Papua New Guinea provenances are superior in growth, form and survival to those from northern Australia (Harwood *et al.* 1993). Base populations for this species initially included the then-available 150 open-pollinated families from natural stands in Papua New Guinea; other families were added as they became available.

Tree improvement

This stage will generally use the base populations and strategic additions as the basis for further work. Selection for tolerance to threatening processes such as pathogenic fungi has become the focus for work on *Eucalyptus camaldulensis* in Thailand and Vietnam. Some provenances of this species appear to be more tolerant than others to a suite of foliar pathogens. There are indications from Thailand that genetic variation in *Casuarina equisetifolia* tolerance to casuarina blister blight (*Trichosporium vesiculosum*) exists and this presents new challenges for collection of seed and germplasm assessment. Despite an increasing trend to clonal technology in large-scale tree plantations, clonal forestry is a genetic dead-end unless it is well supported by an on-going breeding program.

Stands of genetically uniform trees, while providing improved productivity and facilitating efficient management, can be susceptible to attacks by pathogens such as rust diseases of *Populus* and *Citrus* (Simons *et al.* 1994).

The desired outcome of the process of domestication is the use of improved germplasm (seed or vegetative propagules) for general social benefit. Compared with seed diffusion in agriculture, seed diffusion in forestry is more complicated due to the widespread planting of unimproved seed of indeterminate origin; continued use of seed from these trees undermines efforts to utilise improved seed (Simons 1992). A key action for forestry is to determine the best routes through which to move improved germplasm to both farmers and large-scale growers so that uptake and social benefit can be improved.

Domestication of tree species is a highly variable process. For most multipurpose tree species it may simply consist of identifying suitable seed sources and developing appropriate propagation and cultural practices. In widely planted and economically important timber species, investment in the full domestication process may be warranted, involving systematic sampling and characterisation of genetic variation, development of optimal propagation and silvicultural techniques, and intensive breeding, including use of molecular genetics technologies (Midgley and Turnbull 2002).

The Need for Domestication of *Chukrasia*

Chukrasia is a valuable multipurpose tree of the Meliaceae family, distributed mainly in South and Southeast Asia. The most important product obtained from *Chukrasia* is timber, which is prized for high-grade cabinet work, decorative panelling, furniture, musical instruments and interior joinery such as doors, windows and light flooring. It is also used for railway sleepers, boat building and general construction. Flowers contain a red and yellow dye, bark and leaves contain commercial gums and tannins, and the astringent bark has medicinal uses.

Chukrasia is a priority tree for plantation forestry and genetic conservation in many tropical countries including Lao PDR, Thailand and Vietnam. It has been planted as a shade tree for coffee plantations in India, and shown promise as an agroforestry tree in China, Sri Lanka and Vietnam (Kalinganire and Pinyopusarerk 2000). It has grown successfully in plantations in Australia, China, Myanmar, South Africa, Sri Lanka and Vietnam. Despite this, *Chukrasia* largely remains undomesticated and very little is known about genetic variation among its natural populations. Even the systematic classification of *Chukrasia* species is unclear.

Past and current exploitation of *Chukrasia* has not been sustainable. In Thailand, for instance, many natural stands of *Chukrasia* have been lost through uncontrolled logging. Domestication of *Chukrasia* is crucial for the development of an alternative resource to assure the supply of high-quality timber into the future. A collaborative project, 'Development of domestication strategies for commercially important species of Meliaceae', involving forestry research institutions in Australia, Lao PDR, Malaysia, Thailand and Vietnam is therefore being implemented with support from the Australian Centre for International Agricultural Research (ACIAR). *Chukrasia* is an ideal tree for a pilot study in the domestication of indigenous species in the region because of its proven economic and social importance in Southeast Asia, and the sustained pressure on natural populations for timber over many years. In addition, the natural occurrence of *Chukrasia* encompasses the partner countries in Southeast Asia, making it suitable for development of a cooperative pilot pan-regional domestication strategy.

II. Current Status of Chukrasia Domestication

A coordinated domestication program for *Chukrasia* commenced in 1999 as part of an ACIAR-supported project. Research partners in this project are:

- National Agriculture and Forestry Research Institute, Lao PDR
- Forest Research Institute, Malaysia
- Royal Forest Department, Thailand
- Forest Science Institute, Vietnam
- CSIRO Forestry and Forest Products, Australia

The project addresses two related constraints to the development in the forestry sector of many tropical countries in Southeast Asia. These are: (i) the lack of strategies for, and action on, the domestication of important indigenous forest trees, and (ii) the limited scientific capacity of the national forestry research institutes to perform this work.

The project focuses on *Chukrasia*, which is being overexploited in the wild but is not widely grown commercially in plantations. It was realised during the development stage of the project that information available on this genus was very limited and far from that adequate for formulating an appropriate domestication strategy. With this in mind, a suite of activities relevant to the domestication process as shown in Fig. 1 was initiated to enhance knowledge of this tree and to provide baseline information required for strategy development. These activities were structured to maximise interaction between partner institutions, and incorporate formal and informal training on relevant subjects, thus addressing the second constraint listed above as well as fostering increased understanding of domestication techniques.

In the sections that follow, progress in developing a domestication strategy for *Chukrasia* is outlined.

Biogeographical Descriptions and Assembly of Local Knowledge

Existing information on *Chukrasia* was collated through various databases, e.g. TREECD, CAB Inter-national abstracts, and published and unpublished reports in many libraries. Additional useful information and reports were obtained from personal contacts with plant scientists, foresters and taxonomists in countries where *Chukrasia* was reported to occur naturally. It was found that available information on *Chukrasia* was mainly on taxonomy, wood properties and uses, with less on silviculture and almost none on genetic improvement. Field observations conducted in some countries such as China, Lao PDR, Thailand and Vietnam revealed different habitats occupied by the species. This information was synthesised in a monograph (Kalinganire and Pinyopusarerk 2000), thus making it accessible prior to the intensive domestication process and providing a useful first-hand account for people interested in planting *Chukrasia*. A summary of information extracted from the monograph is presented here.

Nomenclature

The genus Chukrasia A. Juss. belongs to the Meliaceae family. It is a member of the subfamily Swietenioideae within the tribe Swietenieae Benth. & Hook., which includes other important genera such as Entandrophragma, Khaya, Lovoa and Swietenia (Pennington and Styles 1975; Mabberley 1995). The name of the genus is adapted from the Bengali name, chikrassee (Mabberley and Pannell 1989).

Chukrasia A. Juss. is a distinctive genus comprising possibly one or two species: *C. tabularis* A. Juss. and *C. velutina* (M. Roemer) C. DC. (de Candolle 1878; Brandis 1921; Pennington and Styles 1975; Mabberley 1995). The former specific name is derived from the Latin *tabularis* (flattened), in reference to the flat seeds (Mabberley and Pannell 1989). The latter name is derived from the Latin *velutinus* in allusion to the fine short erect hairs on the leaves. Both taxa are recognised in India (Anon. 1974), Sri Lanka (Bandara 1999) and Thailand (Smitinand 1980; Gardner *et al.* 2000). Some botanists, however, consider *C. velutina* to be a variant of *C. tabularis* (Ho and Noshiro 1995).

■ **Table 1. Synonyms of Chukrasia tabularis A. Juss.**

Synonym	Reference
Swietenia trilocularis [Roxb. ex Buch.-Ham]	Journey Madras 1: 184. 1807
Cedrela sp. Wall.	Num. List n. 4892. 1831/2
Swietenia sotrophola Buch.-Ham. ex Wall	Num. List 214, n. 1269. 1831/2
Swietenia chikrassa Roxb.	Fl. Ind. 2: 399. 1832
Chickrassia tabularis (A. Juss.) Wight & Arn.	Prod 1: 123. 1834
Chikrassia nimmonii R. Graham ex Wight	Ind. Bot. 148. 1840
Chikrassia trilocularis (G. Don f.) M. Roemer	Fam. Natur. Monogr. 1:135. 1846
Chikrassia velutina M. Roemer	Fam. Natur. Monogr. 1:135. 1846
Sapindus multijugus Wall.	Num. List n. 8099. 1847
Chikrassia tabularis var. b, Thw.	Enum. Pl. Zeyl. 61. 1858
Melia tomentosa sensu Kurz	Rep. Andam. Vi. 1867
Swietenia villosa Wall. ex Kurz	J. Asiat. Soc. Bengal 42, 2: 65. 1873
Swietenia velutina Wall. ex Kurz	J. Asiat. Soc. Bengal 42, 2: 65. 1873
Chukrasia velutina (M. Roemer) C. de Candolle	de Candolle & de Candolle, Mon. Phan. 1: 727. 1878
Chukrasia velutina var. *macrocarpa* C. de Candolle	de Candolle & de Candolle, Mon. Phan. 1: 727. 1878
Chikrassia tabularis var. *genuina* Theob.	Mason, Burma, ed. 3, 2: 586. 1883
Chikrassia tabularis var. *velutina* (M. Roemer) Theob.	Mason, Burma, ed. 3, 2: 586. 1883
Plagiotaxis chickrassa [Wall. ex] Kuntze	Rev. Gen. Pl. 1: 110. 1891
Plagiotaxis velutina [Wall. ex] Kuntze	Rev. Gen. Pl. 1: 110. 1891
Chukrasia tabularis var. *attopeuensis* Pierre	Fl. For. Cochinch. 5: t. 357C. 1896
Chukrasia velutina var. *dongnaiensis* Pierre	Fl. For. Cochinch. 5: t. 357C. 1896
Chukrasia velutina var. *microcarpa* Pierre	Fl. For. Cochinch. 5: t. 357C. 1896
Chukrasia tabularis var. *velutina* (M. Roemer) Pellegrin	Lecompte, Fl. Gén. Indochine 1: 780. 1911
Chukrasia tabularis var. *dongnaiensis* (Pierre) Pellegrin	Lecompte, Fl. Gén. Indochine 1: 780. 1911
Chukrasia tabularis var. *microcarpa* (Pierre) Pellegrin	Lecompte, Fl. Gén. Indochine 1: 780. 1911
Dysoxylum esquirolii Lévl	Cat. Pl. Yunnan 176: 1916
Chukrasia nimmonii (R. Graham ex Wight) Merr. & Chun	Sunyatsenia 1: 61. 1930
Chukrasia tabularis var. *quadrivalvis* Pellegrin	In Lecompte, Fl. Gén. Indochine suppl. 721. 1946
Chukrasia chickrassa (Roxb.) Schultze-Motel	Kulturpfl. Beih. 4: 209. 1966

On the basis of leaf structure, leaf pubescence and floral characteristics, Kurz (1873) and de Candolle (1878) treated *Chukrasia* as two separate species, namely *C. tabularis* and *C. velutina*. However, using the same traits, Pellegrin (1908) maintained one species (i.e. *C. tabularis*) with four different varieties (*attopeuensis* Pierre, *velutina* King, *microcarpa* Pierre and *dongnaiensis* Pierre). A fifth variety, *quadrivalvis* Pellegrin, was later added by Pellegrin (1950). The variety name, *dongnaiensis*, appears to have implied that the species might be distributed in Dong Nai province in the south of Vietnam, but Le Dinh Kha (Forest Science Institute of Vietnam 2000 pers. comm.) confirms that *Chukrasia* does not occur naturally in southern Vietnam. Mabberley (1995) made the most recent revision by retaining *C. tabularis* as the sole species in the genus, and suggested that the different morphological forms were merely ecotypes in seasonal forests. Table 1 shows a comprehensive list of 29 synonyms of *C. tabularis*, and reflects the on-going revision of the systematic classification in the genus *Chukrasia*.

Due to the uncertainty of the systematic classification of the species, the generic name '*Chukrasia*' is used throughout here in reference to either or both species of the genus. The specific names *Chukrasia tabularis* and *Chukrasia velutina* are used where particular reference is made to the respective taxa.

Botanical description

Chukrasia is deciduous, medium to large tree up to 40 m tall, with a bole branchless for up to 25 m and reaching over 120 cm in diameter at breast height. The stem is generally straight with large convex buttresses to 150 cm from ground. *Chukrasia velutina* is reported to be a smaller tree than *C. tabularis* (Gardner *et al*. 2000).

The bark surface is brown to dark brown, smooth in seedlings, becoming fissured vertically and scaling or cracking into rectangular blocks with age. The inner bark is red-brown or pinkish; sapwood straw; heartwood yellow to reddish brown. Two distinctive bark types have been observed on trees growing naturally in Thailand. The bark of trees which occur in mixed deciduous forest is more deeply fissured and corky than that of those which occur in semi-evergreen forest. The deeply fissured bark type is known locally as *C. velutina*. The smoother bark type is similar to that generally described for *C. tabularis* found in other countries such as China, Lao PDR, Malaysia and Vietnam.

Leaves are both pinnate and bipinnate. The first 7-9 leaves are pinnate with a terminal spike. As the seedling grows, the leaves become either paripinnate with a terminal spike, imparipinnate or bipinnate, 30-50 cm long at maturity. Leaflets are deeply lobed to laciniate in juveniles. Mature leaflets are very variable in shape from obliquely ovate to oblong, more or less asymmetric or even falcate; base obtuse to rounded distally; apex acute to acuneate; subglabrous adaxially; numerous long pointed and simple hairs abaxially with foveola (small pits) in axils between the midrib and secondary veins. There are 6-20 pairs of entire leaflets arranged alternately but the first two pairs are generally opposite, the sub-apical the largest 10-17.5 x 3.5-6.5 cm, the most proximal as small as 4 x 2.2 cm, petiolules 2-8 mm long.

Inflorescence are axillary thyrses, sometimes appearing terminal, 10-30 cm long; primary branches to 16 cm, secondary to 4 cm, bearing fascicles of sweetly-scented flowers. Flowers c.1.5 cm long, unisexual or bisexual. Axes short-pubescent; bracts 2-7(-10) mm, narrowly triangular, often caducous, bracteoles similar but smaller; pedicels 3-4 mm, articulated with pseudopedicels 2 mm long, continuous with calyx. Calyx green, pink or red; 4-5 lobes, 2.5-3.5 mm diameter; lobes obtuse. Petals 4 or 5, free, contorted and much longer than calyx in bud, reflexed in open flowers; 12-20 mm long, narrowly oblong to subspathulate, creamy green or yellowish, often tinged pink, subglabrous

or puberulous. Staminal tube broadly cylindrical, somewhat narrowing distally, margin entire to crenulate; anthers attached to margin; glabrous, colour as petals; anthers 1 mm long, oblong. Disc in male flowers stipitate, scarcely distinguishable from the base of the pistillode; in female flowers narrowly cushion-shaped. Ovary flask-shaped, 3-5-locular, each locule with numerous ovules; style slender; style-head capitate with 3-5 stigmatic ridges; densely pubescent. Pistillode scarcely distinguishable from the pistil; loculi and rudimentary ovules well-developed.

Fruit (capsule) is woody, brown, ovoid or ellipsoid, 2.5-5 cm long and 1.8-4 cm diameter, slightly mucronate at tip, dehiscent by 3-5 valves from the apex, the valves splitting into an outer and inner bifid layer; columella with 3-5 sharply angled ridges, extending to apex of capsule; seed-scars conspicuous.

Seed is flat with a brown membranous wing twice the length of the seed, the whole 0.8-1.8 cm long and 0.4-1.0 cm wide; 60-100 per locule arranged in layers, alternately head to toe.

Qualitative aspects of development

Seedling germination is epigeal. The leafy cotyledons are unequal-sided. The radicle emerges from the end of the seed opposite to the wing; the hypocotyl arches slightly at first and in straightening raises the cotyledons above ground. Juvenile leaflets are deeply lobed, becoming entire when mature. Seedlings start to develop mature leaves 5-6 months after germination.

Young saplings tend to develop a small and sparse crown. As the tree grows the crown becomes deeper and denser but still maintain a good length of branchless bole. Mature trees usually have a clear bole for more than half of total height. The development of a clear bole indicates good natural pruning.

Trees are typically deciduous. In the cooler parts of the range they are usually leafless during the winter, when conditions are dry and cool.

The age to first flowering is 5-6 years. The flowering pattern is very irregular and varies from country to country (Table 2). The frequency of flowering in *Chukrasia* is little known but trees in Lao PDR appear to produce a good seed crop every second year. *Chukrasia* fruits (capsules) turn from green to brown when mature. About six months elapse from flowering to seed maturity. Once the fruit capsule has dehisced the winged seed is disseminated by wind. The empty capsules hang for some time on the tree after opening, and can be easily seen when trees are leafless. Eight kilograms of fruit yield one kilogram of clean seed. The number of seeds per kilogram ranges from 71 000 to 160 000; viability averages 64 000 seeds kg^{-1} in tests by the Australian Tree Seed Centre.

Table 2. Times of flowering and fruit maturation of Chukrasia in its natural range (after Kalinganire and Pinyopusarerk 2000)

Country	Flowering time	Fruit maturation
Cambodia	July	December-February*
China	March-June	August-January
India	April-May	December-March
Indonesia	July-August*	March
Lao PDR	May-June	November-January
Malaysia	June-August*	January-March
Sri Lanka	April-May	November-January
Thailand	June-August	January-March
Vietnam	April-July	November-January

Notes: * indicates that these months are estimated

Silviculture and management

Natural regeneration of *Chukrasia* is good where there are gaps in or near the edge of the forest, but is sparse in closed evergreen forest. It is a pioneer species capable of colonising bare land and can tolerate some degree of shade in the early stages. It coppices well and produces root suckers.

Chukrasia has been established successfully in monocultures in Vietnam (Fig. 2). The planting densities vary from 2500 to 3000 stems ha^{-1}(Nguyen 1996). A lower density of 1100 stem ha^{-1} is used on more fertile land. Plantation-grown trees have generally shown good stem form with clear bole more than half of total tree height.

Figure 2. A 15-year-old Chukrasia plantation at Moc Chau, northen Vietnam

Growth

Seedlings usually attain a height of 1-2 m in the first two years but faster growth is obtained under favourable conditions. In India, heights of 2.7-5.5 m were recorded after two years and 8.5-9.1 m after 5 years (Troup 1921). In Vietnam, 5-year-old trees attained a mean height of 5.7 m on site class 3 as compared to 9.3 m on site class 1 (Nguyen 1996). In Yezin, Myanmar, *Chukrasia* attained 12 m in height and 15 cm in diameter at breast height at 16 years of age and were relatively poor in form (Fig. 3). In the same area *Pterocarpus macrocarpus*, *Albizia lebbek*, *A. procera* and *Acacia catechu* grew faster than *Chukrasia* (C. Harwood, CSIRO Forestry and Forest Products 2000 pers. comm.). It appears that sites for plantations of *Chukrasia* should be fertile with deep and well-drained soils, and good site preparation and tending are necessary to ensure satisfactory growth.

Insect pests

Like many species of the family Meliaceae (e.g. *Swietenia* and *Toona* spp.), *Chukrasia* is susceptible to attack by a shoot-tip borer, *Hypsipyla robusta*. The impact of this borer can be very severe. The larvae of *Hypsipyla* feed on the young apical shoots. Most damaged shoots will die, causing multiple leaders to develop with subsequent loss of stem form and thus commercial value (Fig. 4). In Vietnam, farmers have planted *Chukrasia* in farmlands and damage by shoot borers is much less than that in pure-species planting. The diversity of plant species in farmer's gardens may hinder location of *Chukrasia* trees by the adult *Hypsipyla* moth.

Figure 3. Chukrasia plantation at Yezin, Myanmar

Figure 4. Chukrasia tree develops multiple leaders after being attacked by Hypsipyla shoot tip borers

Uses

The wood of *Chukrasia* is of considerable economic importance especially in Southeast Asia. Major uses are fine furniture, turnery, doors, windows and light flooring. The wood has variable resistance to termite attack.

The wood is moderately hard to hard, moderately heavy to heavy but low in stiffness. The grain is straight, sometimes irregularly interlocked and sometimes wavy, producing a roe figure, with moderately fine but uneven texture. The timber is durable under cover but not in contact with the ground. Tests in Malaysia showed *Chukrasia* wood is difficult or very difficult to saw, but elsewhere (probably with different *Chukrasia* ecotypes or species) it is easy to saw and work by hand or machine. The wood takes a very high polish but it is preferable to polish it after allowing the natural colour to develop to a suitable shade. Nailing, screwing and gluing properties are good. It can be peeled and sliced into veneers which can be glued satisfactorily to produce decorative plywood.

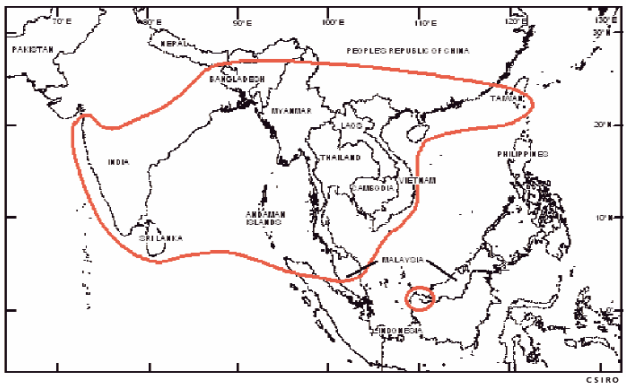
It is planted to provide shade for coffee plantations in India, and is used in agroforestry systems in China and Vietnam. It is also used as an ornamental tree in parks and avenues.

Ecogeographic Surveys

Distribution

Natural occurrence

Chukrasia is usually found scattered in evergreen dipterocarp rainforest, moist semi-evergreen forest and mixed deciduous forest at altitudes from 20 m to 1500 m asl. This distribution extends from India, Sri Lanka and the east and southeast of southern China to Indochina, Myanmar, Thailand, Peninsular Malaysia (not in the south), Sumatra (north but rare) and the western tip of Borneo (Sarawak, Malaysia and West Kalimantan, Indonesia) (Fig. 5) (Anderson 1980; Ho and Noshiro 1995; Mabberley 1995; P. Clegg, Raja Garuda Mas International Forest Service, Indonesia 2000 pers. comm.). This natural distribution range lies between latitude 1° and 25°N and longitude 73° and 120°E. The species is also believed to occur in Bangladesh, Nepal and Pakistan (Mabberley 1995). However, Sahibzada Hafeez (Punjab Forestry Research Institute 1997 pers. comm.) confirms *Chukrasia* is not native in Pakistan.



■ Figure 5. Natural distribution of Chuckrasia

Location of introductions

Chukrasia has been introduced to many countries, outside its natural range, where it is grown as a timber tree. It has been tested in Africa (Cameroon, Nigeria and South Africa) and in Central America (Puerto Rico and Costa Rica) (Streets 1962; Ho and Noshiro 1995). Recently, it has been introduced for trial planting in Argentina (M. Henson 2002 pers. comm.) and Paraguay. Small plantations can be found in Hawaii, South Africa and in countries of natural occurrence. In Australia, it is an aggressive species that produces large seed crops and may form pure stands in disturbed forest. It has become naturalised on parts of the Atherton Tablelands where it was initially established in plantations (Hyland and Whiffin 1993).

Climatic requirements

Rainfall

Over most of its natural distribution, *Chukrasia* occurs in areas with a mean annual rainfall of 1100-3800 mm with a few dry months (Streets 1962; Anon. 1974; Ho and Noshiro 1995; Mabberley 1995; Wasuwanich 1999). Some areas in Myanmar (e.g. in Yezin) where *Chukrasia* is distributed have a mean annual rainfall around 1000 mm (C. Harwood, CSIRO Forestry and Forest Products 2000 pers. comm.). In Sri Lanka, Bandara (1999) reported that *C. tabularis* occurs in areas with high rainfall (1500-1800 mm a year) while *C. velutina* occurs in areas under dry conditions with an annual rainfall below 1500 mm.

Temperature and humidity

In the natural range the mean annual temperature lies between 20° and 27°C. In Vietnam, Nguyen (1996) and Le and Phi (1999) reported the mean annual maximum temperature range to be 18.9-32.4°C and the mean annual minimum temperature range 12.7-23.2°C. The absolute minimum temperature is –3°C and the absolute maximum shade temperature is 42°C (Anon. 1974; Nguyen 1996; Le and Phi 1999). The coldest area in Vietnam where *Chukrasia* occurs is Sapa, Cau Bang, which is 1500 m asl. Although the species can tolerate some frost, damage to leaves and young terminal buds may occur. The seedlings are less damaged by frost than might be expected for a tropical species (Troup 1921; von dem Bussche 1982a, b).

In India, the mean relative humidity in January varies from 45% to 90% and in July from 70% to 100% (Anon. 1974). In Vietnam, it varies from 78% to 85% in July to less than 20% in December-January (Nguyen 1996).

Light

Chukrasia is light demanding. However, young seedlings in natural regeneration may tolerate some degree of shading. It is a dominant tree occurring mostly in the top canopy in natural forests (Anon. 1974; Nguyen 1996).

Soil requirements

Chukrasia occurs on red-yellow, brown-red and brown-yellow soils derived from basalt, limestone, schist and mica schistose. It is most common on limestone (Ho and Noshiro 1995). The species is usually found on deep, fertile and well-drained soils in the plains and on the hills (Anon. 1974; Nguyen 1996; Le and Phi 1999). These soils have a good ability to retain moisture, having 3-4.7% humus in the surface layer; 0.1-3% nitrogen, 7-11 mg/100 mg soil for P_2O_5, and 4-10.5 mg/100 mg soil for K_2O. It does not grow well where hard pans underlay lateritic soils or on barren hills. It is usually absent from heavy-textured and waterlogged soils.

Phytosociology

Chukrasia is a gregarious species capable of invading gaps in the forest. It is common in former shifting cultivation areas and occasionally occurs as a colonist of bare land, including road cuttings (Appanah and Weinland 1993; Ho and Noshiro 1995).

In Vietnam, the most common associates of *Chukrasia* include species of *Aglaia, Artocarpus, Cinnamomum, Dillenia, Elaeocarpus, Erythrophloeum, Garcinia, Gironniera, Knema, Litsea, Markhamia, Parapentace, Pasania, Styrax* and *Vatica* (Nguyen 1996). In Thailand common associates are species of the genera *Anogeissus, Berrya, Erythrina, Dillenia, Garuga, Haldina, Kydia, Lagerstroemia, Pterocarpus, Tectona, Terminalia, Vitex* and *Xylia* (Wasuwanich 1999).

In Lao PDR, *Barringtonia, Canarium, Cratoxylon, Crypteronia, Dillenia, Holarrhaena, Sapium, Shorea, Sterculia, Vitex* and *Wrightia* species are associates of *Chukrasia* in secondary rainforest (Vidal 1960). In the mixed-deciduous forest, most common associates are species of *Bombax, Dracaena, Lagerstroemia, Pahudia, Parkia, Pterocarpus* and *Tetramyxis*. In the rain forest, *Arenga, Aphanamyxis, Artocarpus, Cananga, Capparis, Chisocheton, Diospyros, Elaeocarpus, Eugenia, Ficus, Garcinia, Haplophragma, Hymenodictyon, Lagerstroemia, Miliusa, Nephelium, Polyalthia, Pometia, Protium, Terminalia, Tetrameles, Trewia, Sapindus, Schleichera* and *Xerospermum* species are common associates.

Assembly of Germplasm

Domestication implies the collection of seeds or plants, ideally from the entire natural range of the species, and in time, the selection, propagation and breeding of variants best suited to the needs of man (Leakey *et al.* 1982). For forestry, the first steps are usually taken through provenance testing, where seeds collected from several locations are compared, often outside the species' natural range. Thus, the availability of high-quality and well-documented seed is a prerequisite for any research underpinning a domestication program (Booth and Turnbull 1994; Leakey and Newton 1994).

Coordinated range-wide provenance seed collection of *Chukrasia* took place following the completion of ecogeographic surveys which revealed new information on the species' distribution. The collection involved detailed planning and a substantial investment of resources from ACIAR and all partner countries.

The concept of provenance

The term 'provenance' refers to the geographical area and environment in which parent trees grow and within which their genetic constitution has been developed through natural selection. The idea of provenance implies that genetic patterns of variation are associated with the ecological conditions in which the species evolved (Turnbull and Griffin 1986) and that morphological or other traits can be recognised to characterise them. Ideally, each provenance should be composed of a community of potentially interbreeding trees of similar genetic constitution and of significantly different genetic constitution from other provenances, and where possible may be defined by means of boundaries (Barner 1975). However, delineating provenance boundaries may be difficult for species that occur over an extensive area. It is generally accepted that the boundaries of provenances are set in an arbitrary way during initial sampling of a given species.

The purpose of the *Chukrasia* seed collection was to assemble representative provenances from throughout the natural distribution for investigation of genetic variation of the species. The aim was firstly to collect seed from all countries where the species was known to occur, and secondly to collect seed from different geographical areas within each country. The collection was not exhaustive and further collections may be desirable in due course.

Sampling trees within a provenance

The sampling techniques for *Chukrasia* provenance seed collection followed the guidelines adopted by the Australian Tree Seed Centre which closely match those prescribed by FAO.

- For each provenance, seed was collected from a minimum of ten trees, or less for small populations.
- To minimise the probability of sampling closely related trees, seed was collected from trees at least 100 m apart from each other.

Trees above average in vigour were selected for collection. Isolated trees or trees which showed signs of disease were avoided. No particular attention was given to selecting and collecting from supposed plus trees in natural stands since the role of environmental and competition effects on such trees is unknown.

Collection time and method

Seed collection time for *Chukrasia* varies with locality but is mainly between October and March. Collection was made from the trees, not from off the ground, to avoid uncertainty of the source. Collection from over-mature fruits was avoided as germination tends to be poor and the resultant

seedlings have high mortality (Nguyen 1996). After collection, the capsules were dried in the sun for 2-3 days to promote dehiscence, and the winged seeds were separated by threshing. Special care was taken to protect them from being blown away by wind.

It is desirable to collect an equal amount of seed from each tree within a population. For the *Chukrasia* collection, 100 g of seed was collected from each tree. This amount is sufficient to meet the immediate need for investigation of genetic variation (e.g. provenance trials and isozyme analysis), and to provide for modest future use (e.g. setting up breeding populations). The seed from each tree has been kept separated, and bulked only when needed, for example to represent a provenance origin.

Documentation and registration

It is essential that all information relevant to the seed collection site and trees sampled is recorded at the time of collection. The information recorded included species name, location name (precise location, state and country), latitude, longitude, altitude, aspect, slope, soil information and associated vegetation.

All *Chukrasia* seed from the collection was forwarded to the CSIRO Australian Tree Seed Centre in Canberra, Australia for registration and viability tests. Each seedlot was given a unique number following the ATSC database system.

So far, 32 seedlots comprising 296 individual trees have been collected from nine countries, i.e. Australia, China, India, Lao PDR, Malaysia, Myanmar, Sri Lanka, Thailand and Vietnam. Samples have yet to be obtained from Bangladesh, Cambodia, Indonesia and Nepal. Apart from Australia, where seed was collected from a naturally regenerated stand of unknown origin, collections were made from natural populations. Details of the origin of the seedlots are given in Table 3. These genetic resources are currently reserved for use by regional and international research groups interested in domestication of *Chukrasia*. The access to this material is by contacting the Officer in Charge, CSIRO Australian Tree Seed Centre, PO Box E4008, Kingston, ACT 2604, Australia.

Seed storage behaviour of *Chukrasia*

The ability of seed to retain its viability is an important factor during the domestication process. Many species which are candidates for domestication have seed problems which hinder both research (e.g. provenance testing) and commercial plantation forestry. Rapid loss of seed viability soon after collection is common in many species, and can affect seedling production and jeopardise planting and other programs involving plant propagation from seeds. Viability loss is often caused by the physiology of seed *per se*. Proper handling and storage can improve longevity of seed in the short to medium term.

The requirements for the storage of Meliaceae species such as mahogany, khaya and neem are well understood, but not so for *Chukrasia*. The seed of *C. tabularis* was reported to retain viability for less than 3 months (von dem Bussche 1982b; Rai 1985) or less than one year (Dent 1948). In India *C. velutina* seed was reported to maintain viability for 5 months (Anon.1974) but in Thailand Wasuwanich (1999) found *C. velutina* seeds kept in sealed jars under cool room conditions (2-4°C) for 25 months had a germination capacity of 83-87%.

Seed storage trial of Chukrasia

There is a need for medium- to long-term storage of *Chukrasia* seed from range-wide provenance collection for use in future domestication activities, especially for breeding programs and gene conservation. An experiment was therefore conducted at the Australian Tree Seed Centre to determine the best storage temperature for *Chukrasia* seed.

Seeds of *C. velutina* collected in Thailand and *C. tabularis* collected in Vietnam were tested for

germination capacity after being subjected to three different storage regimes: at room temperature (23°C), in the cold room (4°C) and in the freezer (-16°C) (Pinyopusarerk *et al.* 2001). It was found that seeds which were stored under these conditions for up to 40 months maintained a relatively high level of viability irrespective of the storage temperature regime. However, seeds of *C. tabularis* from Vietnam deteriorated more rapidly when

■ Table 3. Origin of Chukrasia seedlots from range-wide collections

No	CSIRO seedlot	Location	Country	Latitude (° ¢N)	Longitude (° ¢E)	Altitude (m)	No. of parents
1	20186	Atherton	Australia	17 18#	143 43	850	8
2	20030	Sanya, Hainan Island	China	18 10	109 30	45	10
3	20031	Jianfengling, Hainan Island	China	18 42	108 49	65	10
4	20071*	Dehra Dun	India	n/a	n/a	n/a	n/a
5	20105	Pak Baeng, Oudomxay	Lao PDR	20 45	101 53	750	8
6	20204	Nam Bak, Luang Prabang	Lao PDR	20 35	102 30	800	10
7	20123	Tampin Forest Reserve	Malaysia	2 28	102 14	350	2
8	20124	Ulu Tranan Forest Reserve	Malaysia	3 44	101 49	360	4
9	20099	Moeswe Pyinmana	Myanmar	19 57	95 58	209	10
10	20100	Ledagyi Leway	Myanmar	19 50	95 57	220	10
11	20101	Popa Kyaukpadaung	Myanmar	20 53	95 10	180	10
12	20102	Khin Aye Pale	Myanmar	21 56	94 53	155	10
13	20170*	Higurukaduwa	Sri Lanka	6 57	81 09	610	3
18	20319	Balangoda	Sri Lanka	6 38	80 41	500	9
14	20320	Mahiyangane	Sri Lanka	7 19	80 00	150	10
15	20321	Randenigala	Sri Lanka	7 19	80 02	600	5
16	20322	Walapane	Sri Lanka	7 00	80 00	1000	6
17	20323	Hikurukaduwa	Sri Lanka	6 57	81 09	750	10
19	20117	Khao Bin, Ratchaburi	Thailand	13 35	99 40	230	30
20	20118	Mae Phrik, Lampang	Thailand	17 29	99 17	180	12
21	20119	Kamphaengphet	Thailand	16 20	99 16	180	10
22	20120	Obluang, Chiang Mai	Thailand	18 13	98 30	300	12
23	20121	Prachuap Khiri Khan	Thailand	12 05	99 36	250	10
24	20122	Phu Wiang, Khon Kaen	Thailand	16 44	102 20	230	27
25	20194	Uttaradit	Thailand	17 36	100 03	500	11
26	20381	Thung Salaengluang, Phitsanulok	Thailand	16 51	100 30	550	5
27	20384	Huay Khakhaeng, Uthai Thani	Thailand	15 38	99 13	350	5
28	20032	Gia Lai	Vietnam	14 14	108 35	750	7
29	20033	Hoa Binh	Vietnam	20 25	105 28	100	10
30	20034	Son La	Vietnam	20 50	104 45	900	6
31	20035	Thanh Hoa	Vietnam	20 21	105 08	50	10
32	20036	Tuyen Quang	Vietnam	22 00	105 10	75	9

S latitude for seedlot from Australia
* bulk collection

stored at room temperature. After 40 months of storage, seeds of *C. velutina* had a mean germination capacity of 69, 72 and 79% respectively for room temperature, cold room and freezer storage while those of *C. tabularis* had a mean germination capacity of 29, 59 and 59% respectively. The results are thus contrary to earlier reports where *C. tabularis* seed was found to lose all viability within three months to one year. The robust storage behaviour of *C. velutina* seed as reported in Thailand was confirmed by this study. In fact the seeds of *C. velutina* used in this study were already 3 years old before being subjected to this storage experiment for another 40 months. The seeds can thus be stored beyond six years while maintaining a high capacity for germination. Tests before and after the 40-month experimental period showed that moisture content of seeds of both taxa remained fairly stable at 8%. The results suggest *Chukrasia* has orthodox seeds that can be stored for long period provided the seed has a low moisture content and temperature is controlled. For long-term maintenance of viability of *Chukrasia* seed, storage in closed containers in a cold room or freezer is recommended.

Propagation

Effective propagation techniques are essential for successful domestication of *Chukrasia*. Selected genotypes may be captured for use in cultivation by seed and vegetative propagation techniques (e.g. rooted cuttings and tissue culture). The advantage of seedlings in cultivation is that they are cheap and easy to produce, while their genetic variability and the irregularity in flowering and fruiting in some species may be disadvantages. Vegetative propagation offers opportunity to rapidly overcome the limitations to domestication imposed by long generation times and irregular flowering and fruiting of *Chukrasia*. Vegetative propagation is also a way of quickly capturing and utilising genetic variation to increase the productivity of plantations and the quality for forest products (Ahuja and Libby 1993). However, planting material produced by vegetative propagation is generally more expensive than seedlings. Which option to use will depend on the biological and economic factors mentioned above.

Seedling propagation

Propagation of *Chukrasia* from seeds is relatively easy. The seeds are sown broadcast at the rate of about 10 to 20 g m^{-2} in raised beds of fine river sand under shade. Generally, no seed pre-treatment is required. However, to promote uniform germination seeds should be rinsed in tap water for 5 minutes prior to sowing. Germination usually takes place in 1 week and may continue for up to 6 weeks. In tests in Malaysia, 35% of the seeds sown germinated in 1-2.5 weeks, reaching 78% in 4 weeks (Ng 1992). In India, 80%-90% of the seeds germinated within 4 weeks (Rai 1985). Under glasshouse conditions where ambient temperature was maintained around 25°C, 90% of the seeds germinated within 4 weeks but some germinated 10 weeks after sowing (Kalinganire *et al.* 2002).

The germinated seedlings are ready for pricking out into containers when they have produced two pairs of leaves including cotyledons, about 4-6 weeks after germination. A friable, well-drained potting mix is recommended, with complete fertiliser incorporated in the mix or applied regularly in aqueous solution. The recommended mix for *Chukrasia* species in Thailand is coconut husk, burnt rice husk and river sand in a 3:2:1 ratio (Royal Forest Department 1999). In South Africa, pots are filled with a mixture of sandy soil, semi-decomposed pine bark and compost (von dem Bossche 1982b). During the first 2-3 weeks after pricking out, seedlings should receive about 50% shade and after that 25% shade. Excessive watering in the first few weeks under 50% shade can cause damping off. Healthy potted seedlings attain a stem height of 30 cm, suitable for transplanting into the field, within 4-6 months. For the last two

weeks before planting out, seedlings should be 'hardened off' by gradually reducing the watering and exposing them to full sunlight.

Pests and diseases can be responsible for loss of plants in nurseries if left unchecked. Keeping the nursery area clean and regular checking will allow fast corrective action if a disease does break out in the nursery, so that losses may be minimised. For *Chukrasia* species, special attention should be paid to damping-off which is caused by a fungus, *Rhizoctonia solani*, in conditions of excessive moisture. Suitable watering regimes and light sowing reduce the risk. The use of Bordeaux mixture at 1% to control outbreaks gave good results in Vietnam (Nguyen 1996).

Snails and slugs can cause serious damage to young seedlings in nurseries. Baits may be used to reduce the damage.

Vegetative propagation

The principal reason for using vegetative propagation is to take advantage of its ability to capture and fix desirable traits, or combinations of traits, of individual trees. With interest and desire for higher yields and better products, vegetative propagation becomes a useful tool in domestication of forest trees. It results in the formation of clones, each of which retains the genetic traits of the original tree from which cuttings or scions were collected (Leakey and Newton 1994). Therefore, vegetative propagation is important in tree domestication for the multiplication of limited seed material and for the production of genetically uniform stock for planting. However, vegetative propagation does not in itself generate genetically improved material. Only when some form of genetic selection is employed in tandem with propagation will it result in improvement (Leakey and Simons 2000).

A range of approaches can be utilised including grafting, stem cuttings, hardwood cuttings, marcotting (air-layering), suckering, and *in-vitro* techniques such as meristem proliferation, organogenesis and somatic embryogenesis. Some species may not be amenable to vegetative propagation, but most (probably over 90%) tropical trees can be propagated by juvenile stem cuttings (Leakey *et al.* 1990). The *Chukrasia* domestication project has focused on leafy stem cuttings and *in-vitro* tissue culture. Progress made in developing practical protocols for the vegetative propagation of *Chukrasia* species is discussed here.

Vegetative propagation by rooting of leafy cuttings

The technique of rooting 1- or 2-node leafy stem cuttings under mist conditions, which is being used successfully by the Research Centre for Forest Tree Improvement in Vietnam for *Eucalyptus* and *Acacia* species, is working well for *Chukrasia*, giving a high strike and well-formed root systems. The studies to date have focused on examining factors such as the effects of different rooting hormones (indole acetic acid (IAA) and indole butyric acid (IBA)), provenances, progenies, age of stockplants and season of harvesting the cuttings.

Neither IAA nor IBA auxins (0.5 – 2.0 ppm) increased the rooting percentage of leafy cuttings taken from 1-year-old *Chukrasia* seedlings as both treated and un-treated cuttings material rooted almost 100% under mist conditions. However, the material treated with these auxins started to root within a shorter time (2-3 weeks) than the untreated material (average more than 4 weeks). In addition, treated cuttings had a greater number of roots per cutting (mean 8.2, 7.4 and 4.7 respectively for IAA, IBA and untreated) and longer roots (mean 9.4, 9.3 and 8.2 cm respectively for IAA, IBA and untreated). When rooting index (root number multiplied by root length) is taken into consideration the rooting capacity of cuttings treated with IAA or IBA is at least twice that of the untreated material.

The rooting percentage was highest (96%) for the cuttings from 1-year-old seedlings compared to 65%-70% for coppiced shoots from 5-year-old trees and rejuvenated shoots from grafts of 20-year-old trees. Cuttings can be successfully rooted throughout the year but better results have been achieved in spring and summer than in other seasons. Further studies are warranted if *Chukrasia* is to be propagated on a commercial scale: for example information is required on treatments which should be applied to both stockplants and cuttings to obtain consistently high rooting success. In summary, this work has identified useful practical methods, but there is a need to ensure that the techniques are sufficiently robust for future large-scale application.

Micro-propagation by meristem proliferation

As with the cuttings, the existing techniques used in the laboratory of the Research Centre for Forest Tree Improvement in Vietnam are working satisfactorily for *Chukrasia* at the sterilisation, proliferation and rooting phases. The potential multiplication rate is about ten-fold per vegetative generation.

Sterilization and initial culture

The sterilisation of explants is a prerequisite for tissue culture propagation. Young healthy shoots (10-20 cm), each with an axillary bud, were used in the experiments. Shoot tips of the explants were removed and then sterilized. Nodal segments were washed in tap water to get rid of all contaminating factors, then soaked in detergent solution and washed twice by autoclave distilled water. This was followed by surface-sterilisation with $HgCl_2$ 0.1% for 1, 3, 5, 10, 15 and 20 minutes. Finally they were rinsed thoroughly 5-6 times in distilled water. This process was repeated throughout the year to determine the optimum season for bud activity. The results showed the bud activity of cleaned explants to be 14%-38% when sterilised for 1-5 minutes, increasing to 47%-80% when sterilised for 10-20 minutes. With shorter sterilisation time, the number of contaminated explants was high but these explants showed surprising shooting ability (7%). April-August (spring and summer) favoured higher bud activity than other months.

Conditions of culture and shoot proliferation

After sterilisation, explants were cultured in MS medium (Murashige and Skoog 1962) with 0.5 mg L^{-1} benzylaminopurine (BAP). The MS medium consisted of macro-elements, micro-elements and vitamins and supplemented with 3% sucrose, agar and growth regulators (cytokinins and auxins). All cultural media were adjusted to pH 5.8 before autoclaving for 20 minutes at 121°C. Cultures were maintained under a 12-hour photoperiod (3000 lux) at 25-28°C.

The shoots appeared after 10-15 days of culture. When the newly formed shoots reached 10-15 mm in length they were sub-cultured on MS basal medium supplemented with plant growth regulators and other components. This is an important step of in-vitro propagation and the efficiency is judged by the rate of formation of shoots.

Effect of benzylaminopurine (BAP) and kinetin (K) on shoot formation

The effect of different concentration of BAP or K on shoot induction was investigated. The results indicate that the media containing BAP gave higher rates of shoot formation (5-13 shoots per explant) and kinetin was less effective. When BAP was used as a main growth regulator factor in combination with K and IBA auxin, the combinations of BAP and K were more efficient than those of BAP and IBA. The shoot multiplication rate was highest, 9-10 shoots per sample, with the formula 1.5 mg L^{-1} BAP and 0.1 mg L^{-1} K.

Root induction

The final stage of micropropagation is rooting of plantlets. At this stage, shoots are ready to be rooted, isolated and elongated. Shoots that were

20-25 mm in length were cut for rooting. The rooting media were MS basal with different auxin concentrations.

IBA auxin is a well-known rooting hormone widely applicable to numerous species as a root-inducing chemical. Different IBA concentrations, 0.5, 1.0 and 2.0 mg L^{-1}, were found to be effective, the *Chukrasia* plantlets starting to root in 10 days. Different combinations of IBA and NAA auxins were also investigated; 0.1 mg L^{-1} IBA and 0.1 mg L^{-1} NAA produced the best results with more than 85% rooting.

In conclusion, *Chukrasia* is amenable to micropropagation but proof of success of this technique will come when the performance of micro-propagated plants is tested in field trials.

Results from the vegetative propagation experiments described above indicate that clonal propagation is feasible for *Chukrasia* and will offer an alternative to seed-based propagation. As a valuable timber tree with a long generation time, a clonal propagation strategy may be desirable but appropriate vegetative propagation technology will have to be developed for different target users.

Assessment of Genetic Variation

Genetic variation present within wild populations of forest species should be characterised during the domestication process. The variation can be assessed by selecting and testing seedlots, collected from geographically distinct populations within the natural and sometimes naturalised distribution, in provenance and progeny tests.

Species with a wide distribution like *Chukrasia* are expected to show considerable geographic variation in growth traits and possible resistance to pests and diseases. Characterisation of such variation will reveal outstanding provenances and individuals within provenances. Thus, provenance testing has been given high priority at the outset of the *Chukrasia* domestication program. An important aspect of provenance selection programs is the need to include material from a wide range of origins. The range-wide *Chukrasia* seed collection as outlined earlier has made this work possible.

In addition to field provenance trials, glasshouse experiments in which a range of seed sources from natural populations is grown under common environmental conditions are often used in forestry to assess intraspecific variation. Characteristics of seedlings (e.g. leaf size and shape) grown under such conditions have been used successfully as taxonomic characters for assessing geographic variation for *Eucalyptus* and *Acacia* species. A study of intraspecific variation in *Chukrasia* seedlings can help determine, at an early age, the pattern of geographic variation. Populations that are markedly different morphologically are also likely to be physiologically different. Likewise, populations that are morphologically uniform over large areas may display similar performance in field trials.

Geographic variation in seedling morphology

A seedling morphology study has demonstrated that a high degree of genetic variation exists among provenances of *Chukrasia* (Kalinganire *et al.* 2002). Seed from 23 natural provenances and one Australian landrace was used in the study, conducted under temperature-controlled glasshouse conditions. Twenty-four characteristics were measured for each seedling and the resultant data subjected to univariate and multivariate analyses. The multivariate analyses effectively separated the *Chukrasia* genus into three different groups or eco-geographical clusters (Fig. 6). The most important characteristics that separated the groups were bark structure, leaf type and midrib colour. The first group, provenances from China,

Lao PDR, Malaysia, Vietnam and the Australian land race, is characterised by smooth bark, pinnate or intermediate leaves and reddish-green to greenish-red midrib. The second group, provenances from Myanmar and Thailand, is characterised by rough bark, mainly bipinnate leaves and green midrib. The third group, provenances from Sri Lanka, is characterised by rough bark, bipinnate leaves and red midrib. The study clearly shows *Chukrasia* to be a polymorphic genus comprising at least three ecotypes or possibly three species.

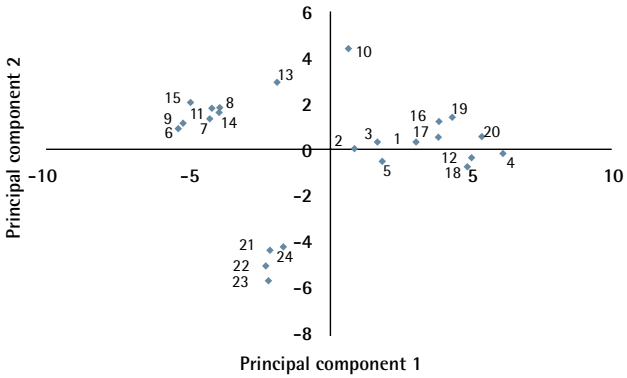


Figure 6. Plot of principal components 1 and 2 from multivariate analysis showing three different groups or eco-geographical clusters of the Chukrasia genus. Bark feature, leaf type and midrib colour are the most seedling characteristics (after Kalinganire and Pinyopusarerk 2002)

Chukrasia provenance trials

Since 1999 more than 15 provenance trials have been established in various countries including Australia, China, Lao PDR, Malaysia, Sri Lanka, Thailand and Vietnam. Most of these trials were established with a common set of 24 seedlots from Australia, China, India, Lao PDR, Malaysia, Myanmar, Sri Lanka, Thailand and Vietnam. Guidelines for the establishment and management of these trials were prepared to ensure standardisation across many participating collaborators (Pinyopusarerk *et al.* 1999). The guidelines emphasised standard procedures for trial establishment, maintenance, assessment and data processing.

Experimental designs

The preferred trial design consisted of 4 replicates of 25-tree (5 x 5) plots of each of the 24 seedlots, laid out in a latinized row-column design with each replicate having 3 rows and 8 columns (Williams *et al.* 2002). The spacing between trees was 3 m x 3 m. The latinized feature of the design required that the four replicates be laid out contiguously giving latinized long columns of 12 plots and overall dimensions of 186 m x 126 m, including a single external perimeter row around the replicates. Where a uniform planting site of these dimensions was not available, the trial was laid out as non-contiguous replicates with incomplete row-column blocking of 3 rows and 8 columns in each replicate. The latinized row-column and non-latinized row-column designs were generated using the computer softare package CycDesigN (Whitaker *et al.* 2002). In some trials where computer-generated designs could not be supplied at the time of planting, randomised complete block designs were used.

Assessment procedure

The assessment procedure adopted for *Chukrasia* provenance trials involves a set of characteristics, which as a whole, summarises the quality of individual trees. Growth characteristics such as height and diameter can be measured directly. Others such as damage from *Hypsipyla* shoot borer are assessed by scoring.

The following characteristics are of particular interest.

Survival: The ability to survive the environment of the planting sites is one of the most important prerequisites in determining the potential of the species.

Height and diameter: These are measured periodically to determine the growth rate. During the first three years after planting it is advisable to measure these parameters annually.

Bark state: There are two distinctive states of *Chukrasia* bark, i.e. smooth or rough, as earlier observed in trees in Thailand and Vietnam and in the seedling morphology study. Variation in bark characteristics of adult trees may be useful in determining the pattern of geographic variation as shown by seedling morphology.

Damage by Hypsipyla shoot borer: Evidence of *Hypsipyla* damage in the provenance trials has been recorded from the very beginning.

Results of provenance trials

Preliminary results from *Chukrasia* provenance trials revealed a high degree of genetic variation in the growth and damage by *Hypsipyla* shoot borers. Tables 4 and 5 show mean values for height growth and damage by shoot borers in selected trials in Thailand and Vietnam respectively.

Survival

Survival was generally higher than 90% after 24 months in most trials, with no evidence of provenance variation. However, severe drought caused high mortality in one trial in northern Thailand (Lampang); the survival at this site decreased from 93% at 12 months to 59% at 24 months.

In the trials planted in Vietnam, the trees of provenances from Myanmar and Thailand suffered leaf desiccation during the winter months while those of provenances from other countries maintained a healthy appearance throughout. This suggests that provenances from Myanmar and Thailand are sensitive to cold.

Height growth

In the trials established in Thailand, most of the provenances from Myanmar and Thailand and the Australian land race grew quickly. However, a few provenances from Thailand did grow slowly, indicating considerable variation among the Thai populations. Provenances from China and India grew slowly while those from Lao PDR, Malaysia and Vietnam were mostly close to the trial average.

There were clear differences in the growth rate between different trial sites, which are established on soils of differing fertility and physical structure. The trial mean height varied from 0.7 m to 2.5 m after 12 months, and 1.3 m to 4.5 m after 24 months. The best growth was obtained at Kanchanaburi which has good deep soil. This site formerly carried a plantation of *Acacia crassicarpa*, a nitrogen-fixing tree which could have improved the soil fertility.

Provenance ranking for height in the Vietnam trials is different from that in Thailand. Apart from the Australian land race which performed well across all four sites in Vietnam, provenances from Myanmar and Thailand were no longer superior to others; in fact many of these provenances were slower-growing (Table 5). Provenances from Lao PDR, Malaysia and Vietnam all performed relatively well.

■ **Table 4. Provenance means for height (m) and damage by Hypsipyla shoot borers (in % of trees attacked) at 12 and 24 months after planting in selected Chukrasia provenance trials in Thailand**

CSIRO Seedlot	Country	Ratchaburi				Kanchanaburi				Uttaradit				Lampang				Prachuap Khiri Khan			
		Ht12	Hyp12	Ht24	Hyp24	Ht12	Hyp	Ht24	Hyp	Ht12	Hyp	Ht24	Hyp	Ht12	Hyp	Ht24	Hyp	Ht12	Hyp	Ht24	Hyp
20186	Australia	1.5	5	2.6	33	2.5	11	4.1	100	1.8	63	2.7	70	0.9	3	1.4	22	1.5	43	2.5	29
20030	China	1.0	1	2.2	24	1.8	15	3.4	100	1.3	40.	1.9	46	0.6	0	1.1	7	1.3	32	2.3	49
20031	China	1.0	1	2.1	23.	2.0	5	3.7	100	1.3	34	2.0	44	0.7	4	1.2	25	1.2	23	2.2	43
20071	India	1.0	0.	2.0	29	1.8	1	3.6	100	-	-	-	-	0.7	8	1.5	23	0.9	365	2.3	39
20105	Lao PDR	1.2	1	2.7	23	2.4	0	4.4	100	1.5	33	2.2	37	0.7	4	1.2	25	1.4	30	2.7	52
20204	Lao PDR	1.3	1	2.7	26	2.4	2	4.0	99	1.6	25	2.2	32	0.8	5	1.3	8	1.4	33	2.2	50
20124	Malaysia	1.2	6	2.3	41	2.6	3	4.2	100	1.5	35	2.1	43	0.7	3	1.1	11	1.4	49	2.3	60
20099	Myanmar	1.0	2	2.0	33	3.0	5	5.3	100	1.7	44	2.7	49	0.8	7	1.6	31	1.1	33	1.9	37
20100	Myanmar	1.1	2.	1.9	21	3.1	2	5.0	100	1.7	56	2.9	69	0.8	1	1.4	37	1.3	37	2.1	38
20101	Myanmar	1.1	8	1.6	68	3.0	4	5.3	99	1.7	36	2.3	48	0.9	20	1.6	61	1.1	45	1.9	56
20102	Myanmar	1.4	0.	2.2	13	3.4	1	5.9	100	1.6	39	2.6	50	0.9	1	1.6	48	1.6	62	2.8	77
20170	Sri Lanka	-	-	-	-	-	-	-	-	-	-	-	-	0.6	6	1.1	49	1.4	43	2.2	68
20117	Thailand	0.7	1	1.5	56	2.6	4	4.4	100	1.2	18	2.2	25	0.6	0	1.4	13	0.9	21	1.9	51
20118	Thailand	1.3	2	2.6	52	3.0	4	5.2	100	1.6	37	2.7	46	0.7	6	1.6	32	1.2	18	2.3	34
20119	Thailand	1.0	3	2.2	46	2.9	6	4.9	100	1.7	40	3.1	51	0.7	3	1.4	30	1.3	10	2.6	39
20120	Thailand	1.3	3	2.3	16	2.7	9	4.9	100	1.8	39	3.4	41	0.8	11	1.7	30	1.4	63	2.8	74
20121	Thailand	1.4	2	2.5	40	2.1	8	3.8	100	1.3	22	1.8	26	0.6	4	1.0	12	1.4	44	2.4	59
20122	Thailand	0.9	3	1.9	15	2.5	2	4.5	100	1.4	19	2.6	25	0.6	0	1.2	15	1.2	13	2.3	38
20194	Thailand	1.0	1	2.1	10	2.8	3	4.8	99	1.6	40	2.7	48	0.8	4	1.5	47	1.2	30	2.3	35
20032	Vietnam	1.1	1	2.4	10	2.2	5	4.1	100	1.5	48	2.3	59	0.6	5	0.9	6	1.3	44	2.3	37
20033	Vietnam	1.1	1	2.4	12	2.0	1	4.0	100	1.4	33	2.3	33	0.7	0	1.2	23	1.4	20	2.7	23
20034	Vietnam	1.1	2	2.3	7	2.2	3	4.3	100	1.4	29	2.4	31	0.6	3	1.0	8	1.4	29	2.3	37
20035	Vietnam	1.1	4	2.5	10	2.3	5	4.5	100	1.6	24	2.6	29	0.6	1	1.1	29	1.5	10	3.0	21
20036	Vietnam	1.1	2	2.4	18	2.1	2	4.2	98	1.5	19	2.4	22	0.6	5	1.2	15	1.2	37	2.2	64
Site mean		1.1	2	2.2	27	2.5	5	4.5	100	1.5	35	2.5	42	0.7	4	1.3	25	1.3	34	2.3	46

■ Table 5. Provenance means for height (m) at 24 months and damage by Hypsipyla shoot borers (in % of trees attacked) at 12 months after planting in four Chukrasia provenance trials in Vietnam

CSIRO Seedlot	Provenance name	Country	Bavi		Hoa Binh		Phu Tho		Gia Lai	
			Ht	Hyp	Ht	Hyp	Ht	Hyp	Ht	Hyp
20186	Atherton	Australia	1.9	86	2.1	59	1.4	23	0.7	10
20030	Shanya Hainam Island	China	1.4	65	1.8	36	0.9	26	0.3	0
20031	Jianfengling, Hainan Island	China	1.7	70	1.6	31	1.0	42	0.7	3
20071	Dehra Dun	India	1.4	38	1.7	41	-	-	-	-
20105	Pak Baeng, Oudomxay	Lao PDR	2.0	69	1.9	41	1.2	13	0.9	8
20204	Nam Bak, Luang Prabang	Lao PDR	2.0	74	1.3	36	1.3	34	-	-
20124	Ulu Tranan Forest	Malaysia	1.6	61	2.0	48	1.4	38	1.1	16
20099	Moeswe Pyinmana	Myanmar	1.6	62	1.0	19	0.7	9	0.6	6
20100	Ledagyi Leway	Myanmar	1.6	56	1.1	18	0.7	10	0.8	1
20101	Popa Kyaukpadaung	Myanmar	1.4	82	1.3	17	0.7	19	0.4	0
20102	Khin Aye Pale	Myanmar	1.7	-	1.8	-	1.0	24	0.4	1
20170	Higurukaduwa	Sri Lanka	1.6	85	1.7	39	-	-	0.5	0
20117	Khao Bin, Ratchaburi	Thailand	1.3	47	0.8	2	0.6	23	0.8	20
20118	Mae Phrik, Lampang	Thailand	1.3	48	0.9	6	0.7	20	0.4	0
20119	Kamphaengphet	Thailand	1.7	82	0.9	16	0.7	15	0.4	0
20120	Obluang, Chiang Mai	Thailand	1.5	44	0.9	8	0.7	32	0.5	2
20121	Kuiburi, Prachuap Khiri Khan	Thailand	1.6	55	1.4	21	1.0	26	0.9	16
20122	Phu Wiang, Khon Kaen	Thailand	1.5	58	0.9	9	0.6	26	0.5	5
20194	Uttaradit	Thailand	1.8	79	1.2	5	0.8	23	0.4	1
20032	Gia Lai	Vietnam	1.6	71	1.7	49	1.0	26	0.8	12
20033	Hoa Binh	Vietnam	1.7	52	1.6	25	0.9	24	0.8	2
20034	Son La	Vietnam	1.8	67	1.2	23	1.1	25	0.8	2
20035	Thanh Hoa	Vietnam	1.8	79	1.6	46	0.9	39	0.8	12
20036	Tuyen Quang	Vietnam	1.8	66	1.4	30	1.1	43	0.7	1
Site mean			1.6	65	1.4	27	0.9	25	0.6	5

The impact of site quality was obvious in Vietnam. Overall, *Chukrasia* trees in provenance trials in Vietnam grew much slower than in those in Thailand, with height after 24 months varying from 0.6 m at Gia Lai (central region) to 1.6 m at Bavi (northern region). This is not unexpected as the lands available for forestry use are generally degraded and very infertile, unsuitable forrice and other agricultural crops.

Figure 7. Poor growth of Chukrasia trees in a trial planting at Bavi, Vietnam

Fugure 8. Chukrasia trees in farmer's garden at Bavi, Vietnam grow rapidly

The growth results indicate that *Chukrasia* is sensitive to soil fertility and structure; growth is clearly poorer on degraded sites. In Vietnam *Chukrasia* trees (from the same provenances) growing on farmers' gardens all show impressive growth, exceeding even that at Kanchanaburi in Thailand. It appears that the best opportunity for growing *Chukrasia* may be in agroforestry systems rather than in plantations on the indifferent sites available for large-scale forestry.

Bark characteristics

Though data are not shown here, the trees from Myanmar, Sri Lanka and all but one Thailand source (i.e. seedlot 20121) have developed rough bark in the nursery, and this feature has become more pronounced with age after being planted out in the field. In contrast, plants from all other countries have developed smooth bark. Such differences in bark had been previously observed in the seedling morphology study. This morphological characteristic may have important implications for taxonomic classification within the genus.

Figure 9. Some individual Chukrasia trees show strong apical dominance, producing replacement leader after attack by Hypsipyla shoot borer

Damage by shoot borers

Attack by *Hypsipyla* shoot borers is widespread in all provenance trials and appears to be more serious once the trees attained 1.5-2 m in height. In Thailand, shoot borer attack in the trials averaged 2%-35% of trees at 12 months, increasing to 25%-100% at 24 months. The trial at Kanchanaburi, which had the best height growth, was practically 100% attacked after 24 months. Although provenances varied in the degree of attack, there was no clear evidence to suggest a geographic pattern of variation.

In Vietnam, the percentage of trees attacked by shoot borers was recorded at 12 months (Table 5) varied between provenances, with a tendency for more extensive attack in the provenances from Australia and Vietnam and less attack in those from Myanmar and Thailand.

Some individual trees were found either not attacked by *Hypsipyla* borers or able to establish a single replacement leading shoot with almost undetectable loss of stem straightness, rather than a fork or cluster of heavy branches (Fig. 9). Although the genetic basis of this characteristic has not been examined, these results suggest that selection for *Hypsipyla* resistance may be feasible in *Chukrasia*.

Overall, preliminary results from provenance trials reveal some genetic variation in growth rate and, to a lesser extent, insect resistance. After a few more years it will be possible to analyse in detail across sites provenance performance in growth, stem form and insect resistance, and subsequently to select superior provenances for particular sites.

Reproductive Biology of *Chukrasia*

Knowledge of the reproductive biology of a tree species is essential for the establishment of seed orchards and operational seed production, and for managing breeding programs. Reproductive biology of *Chukrasia* has been studied in Vietnam, focusing on the flower structure and development,

■ Figure 10. Chukrasia inflorescence

the breeding systems and pollination mechanisms of the species. The study was conducted in a natural stand at Moc Chau (Son La province) and in 6-year-old planted trees in Hanoi. At Moc Chau, flowering and fruiting phenology, the breeding systems and the pollination ecology aspects were studied. In Hanoi, only the flowering and fruiting phenology was investigated.

Flower structure and flowering phenology

There were marked differences between trees in flower, pistil and stamen length, as well as sepal and petal colour (Table 6). The total number of individual flowers per inflorescence varied from 131 to 399, and flowers are 13.9 mm to 19.5 mm long (Fig. 10). The study showed flower characteristics of *Chukrasia* trees in Vietnam conform with other information as summarised by Kalinganire and Pinyopusarerk (2000). The study on petal colour, pistil and stamen characteristics demonstrates that the flowers with violet petals have stamens longer than the pistil. In contrast, those with yellow petals have stamens shorter than the pistil. In the investigated population, the incidence of trees having violet petals is only 5%. Although the effect of floral colour on pollinator behaviour needs further investigation, trees with brighter colour may attract more visitors such as birds.

■ Table 6. Inflorescence and flower characteristics of Chukrasia trees in Moc Chau, Vietnam

Tree No.	Total number of flowers	Flower length(mm)			Pistil length(mm)			Stamen length(mm)			Sepal colour	Petals colour
		Min	Mean	Max	Min	Mean	Max	Min	Mean	Max		
1	131	16.4	17.6	19.2	11.8	13.0	13.7	12.8	13.3	14.1	Green	Violet
2	189	14.0	14.8	15.6	11.5	12.5	13.8	10.1	11.3	12.3	Green	Yellow
3	399	13.9	15.4	19.5	11.7	12.2	13.3	10.6	11.3	12.3	Green	Yellow

■ **Table 7. Time of flowering, fruit ripening and seed dispersal of Chukrasia trees growing in Vietnam**

Location	Start of flowering	End of flowering	Fruit ripening	Seed dispersal
Moc Chau	7 – 13 April 2000	28 April – 6 May 2000	10 Dec. 2000	5 – 20 Jan. 2001
Ha Noi	18 – 28 April 2000	24 – 30 May 2000	25 Dec. 2000	12 – 25 Jan. 2001

For the flowering phenology study, *Chukrasia* trees were randomly selected for observing bud set, duration of flowering periods and flower characteristics. Data presented in Table 7 show differences in flowering, fruiting and seeding times between the two study sites. These observations conform to information summarised by Kalinganire and Pinyopusarerk (2000) in that the flowering pattern of *Chukrasia* is very irregular, and varies (March to August) with localities. Trees at Moc Chau started flowering earlier than those in Hanoi, but in Hanoi flowering duration was longer. Nguyen (1996) observed that trees in a warm climate (Kon Ha Nung, central Vietnam) start flowering earlier than those in a colder climate (Son La, northern Vietnam), which indicates that the onset of flowering of *Chukrasia* is influenced *inter alia* by temperature.

The pollen viability of *Chukrasia* was examined by testing the germination of pollen from each flower separately. At Moc Chau, a low pollen germination percentage (15% to 20%) was obtained. The viability of fresh pollen for most species of the Meliaceae family is expected to be quite high, e.g. 97% for *Toona ciliata* (S. O'Brien, CSIRO Plant Industry 2002 pers. comm.). Low pollen viability has implications for the quantity of pollen needed for controlled pollinations in breeding programs. Further testing of *Chukrasia* pollen is planned.

Pollination, pollen vectors and breeding systems

Chukrasia flowers, like those of all Meliaceae species, show features associated with entomophily. The study in Vietnam found insect visitors to *Chukrasia* flowers included seven butterfly and two bee species. No birds, bats and other mammals were observed visiting the flowers. The results agree with earlier reports that bees and moths were the main pollen vectors of the Meliaceae (Styles and Khosla 1976).

All species of Swietenioideae are reported to be monoecious with single-sex male and female flowers in the same inflorescence (Styles and Khosla 1976). However, Pennington and Styles (1975) reported the presence of well-developed vestiges of the opposite sex within male and female *Chukrasia* flowers. The study in Vietnam showed *Chukrasia* to have hermaphrodite (or bisexual) flowers. These results match those from a naturalised population in the Atherton Tablelands, Queensland, Australia (Hyland and Whiffin 1993), and an earlier study in Vietnam (Nguyen *et al.* 1996).

The selfing ability of *Chukrasia* was investigated in Vietnam as part of the domestication program. Pollination bags were used to isolate inflorescences before flowering. No bagged flowers set fruit. Autogamy or spontaneous self-pollination was the only test used in the study for *Chukrasia.* Other tests such as geitonogamy or artificial self-pollination may provide a better understanding of the breeding system of the species. Open-pollinated flowers had a fruit set of about 14%; the reason for this low set is not known. For *Toona ciliata* the fruit set from open pollination is well above 50% (S. O'Brien, CSIRO Plant Industry 2002 pers. comm.).

Overall these results are useful for potential breeding of *Chukrasia* but more research on reproductive biology and seed ontogeny is needed, especially on a regional scale to cover other locations and perhaps all ecotypes or species. Until more information is available on the pollination and the incompatibility mechanisms of *Chukrasia*, large-scale breeding programs should proceed with care. The results from self- and open-pollinated flowers show the need for pollinators for effective seed production and suggest that future seed production stands should be established where flowering is prolific and insect pollinators are abundant.

III. Recommended Provisional Domestication Strategy for *Chukrasia*

Development of domestication strategy for *Chukrasia* is progressing and a number of knowledge gaps in the biology of the genus, especially genetic variation at the provenance and perhaps species level, have been filled. Although many related research activities are still in progress and final results will not be known for several years, information now available suggests that a multi-faceted strategy is required for effective domestication of *Chukrasia* for a range of beneficiaries. The following key elements need to be addressed:

Selection for *Hypsipyla* Resistance

As attack by *Hypsipyla* shoot borers is a major constraint to growing *Chukrasia*, selection for resistance as one component of a genetic improvement program should be a key aim of the strategy. In field provenance trials some individual trees were either not attacked or were able to establish a single replacement leading shoot with almost undetectable loss of stem straightness, rather than a fork or cluster of heavy branches. Phenotypic selection for resistance and tolerance should be carried out in existing provenance trials, followed by vegetative propagation of selected individuals and clonal trials to determine whether the resistance and tolerance are of genetic origin. This will hopefully lead to the production of trees with much improved stem form.

Genetic variation in apical dominance has been found by decapitation tests on nursery seedlings, e.g. in *Cedrela odorata* (Newton *et al.* 1995) and *Triplochiton scleroxylon* (Leakey and Longman 1986). The process involves removal of the apex of the shoot of a young seedling and the subsequent release of axillary buds. Less vigorous bud activity is associated with relatively strong apical dominance. Thus, decapitation tests on *Chukrasia* seedlings could be used to identify a broadly-based group of tolerant phenotypes for subsequent vegetative propagation and cloning, and give information about the genetic structure of these populations from the point of view of branching architecture and likely responses to shoot borer attack.

Silvicultural Systems

Appropriate silvicultural systems should be identified for *Chukrasia* to realise its full domestication potential. The choice is determined partly by physiological responses of individual species (Fasehun and Grace 1994). The photosynthetic responses of Meliaceae species such as *Swietenia macrophylla*, *Cedrela odorata* and *Khaya ivorensis* are well understood (Kwesiga and Grace 1986). These species are highly light demanding and this factor is taken into account to ensure successful in cultivation. There is evidence of failures in cultivation of *S. macrophylla* due to excessive shading (Newton *et al.* 1994). Although no reports are available, the apparent light-demanding behaviour of *Chukrasia* species suggests that excessive shading could limit growth.

Chukrasia has been found to be very sensitive to soil fertility and structure; growth is very poor on degraded sites as observed in provenance trials. The far better growth of trees on good agricultural soils compared with available forestry sites in Vietnam suggests agroforestry systems as the primary target of domestication. A focus on agroforestry implies that farmers should be the agents of domestication and their participation will be the key to success. Clonal trials should be established on farmland. Subsequently, an effective method to diffuse improved germplasm to this target group will be needed.

A wide range of silvicultural approaches has been applied to planting other Meliaceae species such as mahoganies with the aim of controlling *Hypsipyla* attack. Although the success rate is generally low there are examples of trials in Puerto Rico where damage by the shoot borer has been reduced by line enrichment planting (Vega 1976; Weaver 1987). The low density of mahogany trees in line plantings may prevent the build-up of the *Hypsipyla* populations. *Chukrasia* trees in natural habitats are generally of good stem form with a clear bole more than half of total height. This growth characteristic suggests that a large number of trees in natural forest may have escaped attack by *Hypsipyla* shoot borers. The greater agrodiversity and biodiversity usually present in farmer's fields is likely to be beneficial from the point of view of reducing attack. This suggests the importance of viewing silvicultural systems as one aspect of an integrated domestication strategy. Incorporating shoot-borer resistant genotypes into silvicultural systems to optimise control of damage has also been recommended for pest management in Meliaceae (Newton *et al.* 1994).

Capture of Genetic Variation

Selected genotypes of *Chukrasia* can be captured for use in cultivation by seed and vegetative propagation techniques.

The range-wide seed collection undertaken during the initial stage of domestication will provide interim resources for an expanded program in the course of which superior provenances can be identified. The ability of *Chukrasia* seed to maintain high germination capacity for a long period of storage will ensure the availability of these seed resources. However, new collections from larger numbers of parent trees of identified superior provenances are required to enable establishment of breeding populations for major breeding programs.

Practical protocols developed for vegetative propagation by rooting of leafy cuttings offer an alternative to seed-based propagation. However, more research is required to fully understand the factors affecting rooting potential. Studies to develop a good understanding of stockplant management are also needed.

The level of technology is another issue for consideration. If farmers are to be the main agent for *Chukrasia* domestication, low-technology options, e.g. non-mist propagators (Leakey *et al.* 1990), are very desirable. Techniques will have to be developed that are effective and meet the needs of tree improvement projects in different regions.

Genetic Conservation

Many natural forests where *Chukrasia* occurs continue to be lost through deforestation, a process which will deplete the genetic resources and limit the potential for sustainable production of *Chukrasia*. Therefore, apart from increasing efforts at genetic improvement of *Chukrasia*, attention should be given to genetic conservation. *In-situ* conservation will be complicated by many factors such as national policy and social issues (e.g. land use in natural forest) in different countries. *Ex-situ* conservation through planted stands of selected genotypes is simpler and more practical.

Research results describing the pattern of variation within and between *Chukrasia* populations have important implications for the conservation and sustainable use of the species. *Chukrasia* clearly comprises at least three ecotypes or species. The different populations need to be adequately conserved if the full breadth of genetic variation across the species is to be maintained. Increased knowledge of the species' biology and of the spatial distribution of its genetic diversity will help ensure future availability of highly productive seed sources for reforestation.

Proposed Improvement Strategy for *Chukrasia*

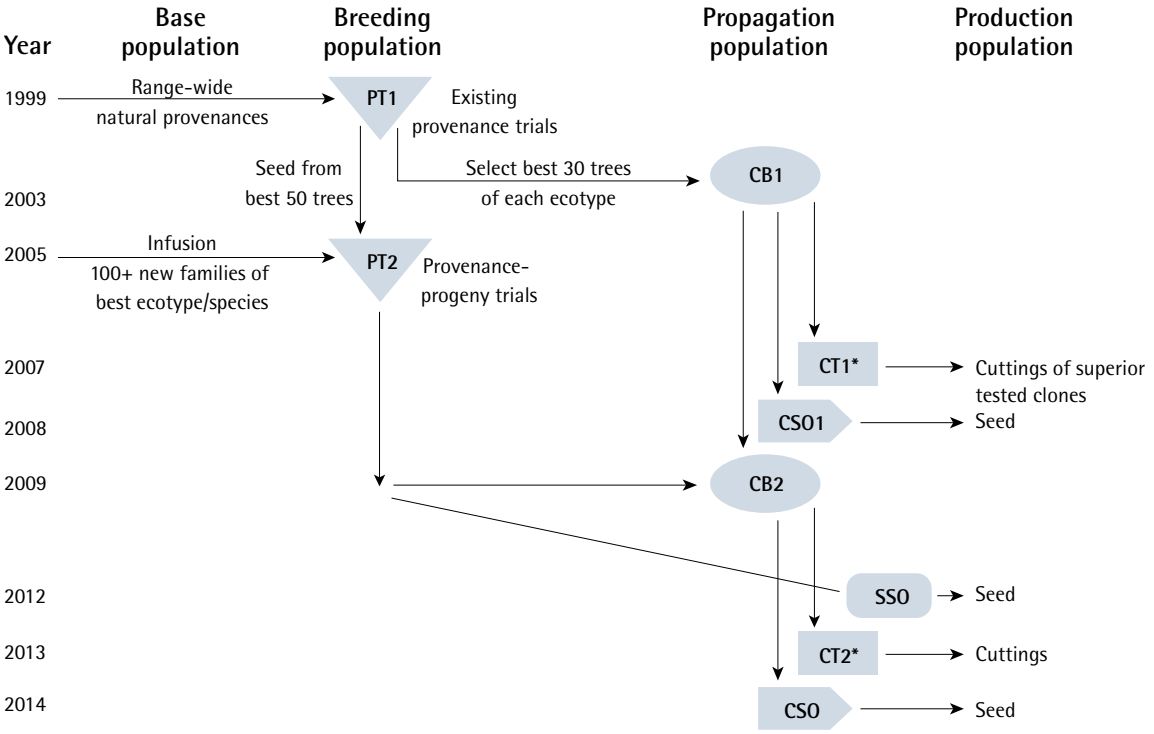
The main product of *Chukrasia* is its high quality timber and therefore the improvement objective is to maximize the production of timber from planted stands, on a per-year and per-hectare basis (or per-tree basis for trees not planted in conventional block plantings). A slightly different way of expressing this objective is to say that we wish to genetically improve *Chukrasia* so as to minimize the cost of producing timber from planted stands.

Selection criteria in the first generation of improvement are resistance to *Hypsipyla* shoot borers, increased wood volume production, and improved stem form. Improvement in wood properties affecting the quality and price of timber could be considered in second and subsequent generations of breeding.

Two options for *Chukrasia* improvement are suggested. The first strategy, shown diagrammatically in Figure 11, is for countries such as Thailand and Vietnam, where the replicated provenance trials established in 1999 will serve as founding populations for selection. The second strategy, shown in Figure 12, is for countries without existing provenance trials which have the capacity to set up pedigreed breeding populations for genetic improvement programs.

Figures 11 and 12 show the main activities to be undertaken and the approximate time scales. Improved planting materials produced from seed-orchard seed and cuttings of field-tested, superior clones will be the outcomes of each breeding cycle.

Detailed breeding plans describing the operations step by step, and their timing, would have to be prepared for countries implementing genetic improvement programs. Such plans will vary between countries depending on factors such as the skills of available local staff, available financial resources and the range of target environments for which breeding is being conducted.



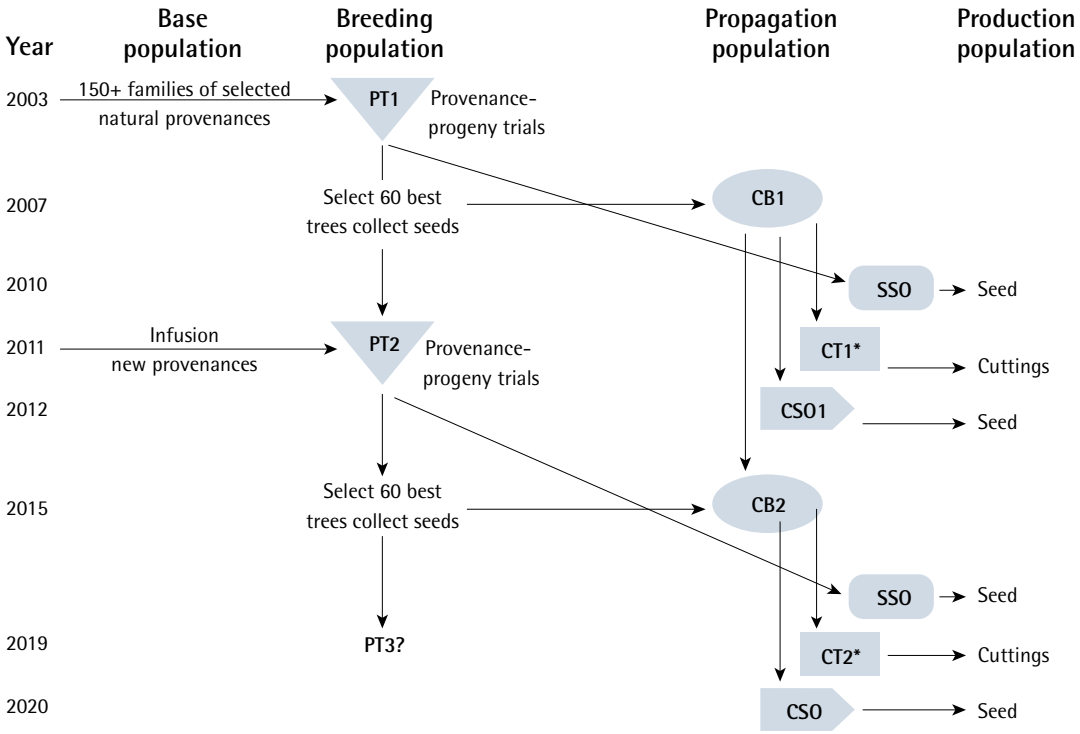
■ Figure 11. Option 1: Basic improvement strategy for countries with existing provenance trials

In Option 1, the provenance trials which were established in 1999 will provide a basis for selection of superior individuals (*Hypsipyla* resistance, fast growth and good stem form).

At around four years of age in 2003, the best 30 trees of each ecotype are selected for grafting into a clone bank. The clones are propagated for clonal testing on at least two sites in the target planting environments. Some clonal genotypes that are susceptible to *Hypsipyla* attack, and unselected seedlings, are included as controls in the clonal trials. As selection for *Hypsipyla* resistance is a major objective, the clone trials should be planted in areas where *Hypsipyla* shoot borers are known to be prevalent.

The best 10-15 clones could be selected for deployment in year 2007-08 provided their performance is clearly superior to that of the controls.

Selected trees assembled in the clone bank are also used to establish one or more clonal seed orchards. Following assessment of growth and insect damage and based on results from the clone trials, the clonal seed orchard(s) are rogued to remove inferior clones. The first seed collection is expected in year 2008 or 2009.



■ Figure 12. Option 2: Improvement strategy for countries without existing provenance trials

In Option 2, for countries that have not yet established provenance trials, the individual family collections from range-wide provenance collections available at the Australian Tree Seed Centre will be used to set up breeding populations. Approximately 150 families from superior provenances (based on information from existing provenance trials in adjacent countries) would be planted in provenance-progeny trials on at least 2-3 sites.

At least one trial would be converted to a seedling seed orchard by heavy, selective thinning. It would deliver somewhat improved seed within 6-8 years.

At about 4 years of age, the best 60 trees would be identified for grafting into a clone bank and subsequent clonal tests. As with Option 1, genotypes known to be susceptible to *Hypsipyla* shoot borers should be included in the clone trials. The best 10-15 field-tested clones are selected for deployment.

If feasible, superior field-tested clones from countries such as Thailand and Vietnam may be imported and included in the provenance-progeny trials and clone bank. Clonal seed orchards can be established from the material assembled in the clone bank.

For both Options 1 and 2, the details of the second cycle of breeding will be determined after a review of the information obtained from the first cycle.

An important question that must be resolved is whether there is sufficient genotype-by-environment interaction to justify separate breeding populations for different target planting zones in the second generation.

Another fundamental question is whether the three ecotypes should be maintained together in a single breeding population, or whether they should be kept separate. Hybridisation between the ecotypes may produce superior individuals that could be propagated clonally. Alternatively, hybridization may be undesirable. It is prudent to assume at this stage that hybridisation might not occur in open-pollinated breeding populations and seed orchards, and this must be taken into account when determining the genetic base; for example clonal seed orchards should include at least 20 individuals of a desired ecotype. Research to establish the genetic relationships between the ecotypes, whether or not they hybridise and consequences of any hybridisation is a high priority.

Conclusions

Efficient domestication requires two important ingredients. Firstly, willing national and international collaboration so that essential resources can be brought to the task; this ranges from sharing the genetic resources of natural population and the capacity to establish and manage field trials, to facilities for coordination. Secondly, strategic plans are needed to guide the overall effort; these identify priorities and provide the framework for collaborative activity, and must be dynamic, evolving as the project progresses.

Much progress has been made in the development of a domestication strategy for *Chukrasia* with strong collaboration and support among the implementing research agencies in Lao PDR, Malaysia, Thailand, Vietnam and Australia. It is important that the momentum developed during the past three years be maintained. There is a good prospect that a successful domestication strategy for *Chukrasia* will overcome the problems which have limited the cultivation of the genus, and stimulate a wider interest in regenerating the depleting resource. The lessons to be learned from the domestication of *Chukrasia* will have significant implications for other commercially-important indigenous tree species in South-east Asia.

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