Integrating cloned and seedling progeny for rapid improvement of teak (*Tectona grandis*)

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Abstract
The rapid and accurate identification of superior clones for commercial deployment has recently become an important goal of many teak (*Tectona grandis*) improvement programs. This paper presents a comparison of three strategies for identifying superior clones from within an initial collection of 200 teak families while initiating the subsequent generation. Due to increased accuracy of predictions of genetic merit, cloned progeny testing is expected to enable a more rapid and accurate selection of clones for pilot-scale deployment and the development of block-plots for commercial-scale testing compared with a strategy that commences with seedling progeny testing. However, the best results are expected from an integration of both approaches into a unified strategy. The integrated cloned and seedling progeny strategy involves taking a proportion of germinants from each family into a second year of propagation to develop them into cloned progeny. The remaining germinants are tested as seedling progeny. Benefits of this strategy include: 1) early clone deployment at 8 years after initiation, 2) refinements to the clonal deployment population at 12 years based on wood property evaluations, 3) the use of results from cloned progeny to improve breeding value estimates and genetic gain in the second generation, and 3) the integration of genetic information across generations and propagule types.

Keywords cloned progeny test, genetic gain, genetic values, tree improvement strategy,

Introduction

Progeny tests underpin the breeding cycle of forest tree improvement programs, providing for the assessment of parent breeding values and selection of elite individuals to be captured into deployment populations (White et al. 2007). Tree improvement strategies often accelerate the genetic gain of deployment populations by assigning greater effort to the most valuable material (e.g. Cotterill et al. 1988; Lstiburek et al. 2004; White et al. 1999). Other key requirements of tree improvement strategies are the maintenance of genetic diversity, accounting for species-specific reproductive biology, and ensuring compatibility with modes of operational deployment.

Although genetic gains in productivity have been achieved from progeny selections in small plots using single-trees or multiple-tree line plots, trials with large block-plots are often required to confirm that selections are genetically superior when competing against others of the same or similar pedigree (Callister et al. 2012). Block-plot testing is probably especially important for improvement of stand volume amongst clones where intra-tree competition is uniform (e.g. Sharma et al. 2008; Stanger et al. 2011).

In recent decades, improvement and deployment strategies for teak (*Tectona grandis*) have been increasingly geared towards clonal deployment (Goh & Monteuuis 2005; Kaosa-ard 1998; Monteuuis & Goh 1999; Monteuuis & Maitre 2007; Murillo & Badilla 2004). Mass production of clones by rooted cuttings or by tissue culture of teak trees of any age is now routine (Goh & Monteuuis 1997). Although controlled-pollination of teak is possible (Kaosa-ard 1998), it is fraught with technical difficulties that are often avoided by the use of open-pollinated seed for progeny testing. On the other hand, a progeny testing innovation with greater potential to transform teak improvement strategies is the cloning of half-sib progeny prior to testing (Callister & Collins 2008).

Teak improvement programs around the world have generally advanced from the early provenance tests (Keiding et al. 1986; Kjaer et al. 1995) to progeny tests of plus-trees and selections (Callister & Collins 2008; Danarto & Hardiyanto 2000; Haque 2000; Murillo et al. 2004; Sharma et al. 2000). The goal of this paper is to describe and compare strategies for advanced-generation improvement of teak with a focus on achieving the most rapid identification of superior clones for operational deployment while maintaining genetic diversity in future generations.

Comparison of tree improvement options

It is assumed that the tree breeder has on hand a collection of 200 half-sib families with sufficient fruit to produce 100 germinants of each family. While starting with the correct provenance will lead to a certain level of genetic gain, the origins of these families are not a consideration of this paper, suffice to say that superior clones may be derived from each and any of the families on hand. The mode of deployment is assumed to be vegetatively propagated clones into a region with unknown patterns of genotype by environment interaction and no requirement for sub-regional deployment populations. It is also assumed that accurate growth and form assessment can be conducted after four years of growth, that seed harvest can be conducted after six years of growth, and that wood properties can be assessed after ten years of growth (Callister 2010).

Option 1. Progeny test of seedlings followed by clone evaluation

The simplest and most conventional approach would be to establish a number of seedling progeny trials from which superior individuals can be identified and cloned (Figure 1). Seedling progeny trials are established after one year of propagation. They are assessed for growth and form at age 4 years followed by wood properties and extensive flowering at age 10 years. Although clone selection can be undertaken at age 4 years, a relatively large number of clones would be captured for evaluation because selection is on the basis of estimated breeding values (EBVs) rather than total genetic values (TGVs). Clone identification is therefore not very accurate at this stage.

I have suggested in Figure 1 that all selected clones from the seedling trials should be entered into block-plot tests (with two years allowed for propagation of sufficient numbers) to avoid further delays in confirming clone values in a deployment context. Another option that reduces the area required for testing could be to establish block-plot clone tests with a subset of superior clones after the clone trials have been evaluated (10 years from commencement).

The first clonal deployment from a program that commences with seedling progeny trials cannot be expected until 12 years after initiation, when clone trials have been evaluated for growth and form at four years and allowing two years for multiplication of tissue culture plantlets (Figure 1). The selection of clones in deployment can be further refined at about age 13 years using measurements of wood properties in the original seedling trials. These measurements will be based on only one tree per clone (i.e. EBV). Stand volume at six years in the block-plot trials will also help to refine the clones included in the deployment population. The final constitution of clones in the deployment population is determined 16 years after commencement, when the wood properties and flowering of the clone trials are measured and TGVs can be assigned for these traits at the clone level (Figure 1).

Option 2. Cloned progeny tests

Cloned progeny tests are planted a year later than seedling progeny tests due to the longer time required for propagation. However, early clone deployment is possible soon after the assessment of growth and form at age four years (Figure 2). Early deployment is four years sooner than for the seedling progeny option, refinement of the deployment population based on TGVs for wood properties and flowering also occurs four years earlier, and the inclusion of stand volume information from block-plot clone tests occurs one year later (compare Figures 1 and 2).

Option 3. Integrated cloned and seedling progeny

The third tree improvement strategy (outlined in Figure 3) is an integration of the previous two testing and evaluation strategies. A proportion of germinants from each family is cloned to produce a cloned progeny population which allows for early clone selection on TGVs. The establishment of block-plot clone trials and the deployment population follow the same timelines presented for the cloned progeny trial option (compare Figures 2 and 3). The remaining

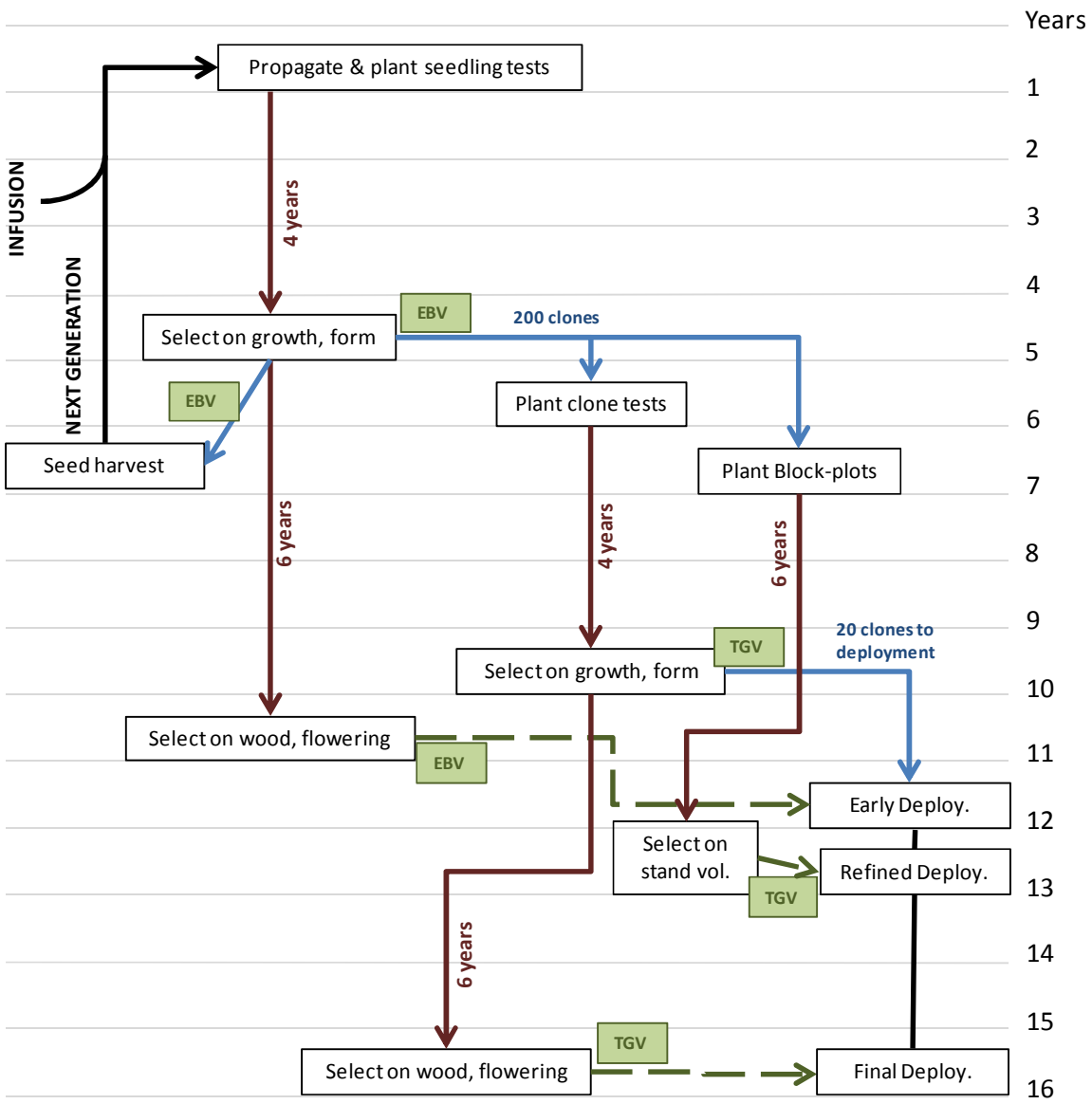

Figure 1. Flow diagram of Option 1. Seedling progeny testing followed by clone evaluation. Brown arrows represent growth of a trial series; blue arrows represent transfer of genetic material; green dashed arrows represent information flow based on estimated breeding values (EBV) or total genetic values (TGV).

germinants are entered into seedling progeny tests, preferably on the same sites. The proceeding generation can be established with seed collected from both the seedling and cloned progeny tests and Figure 3 shows the start of the testing program for this new generation.

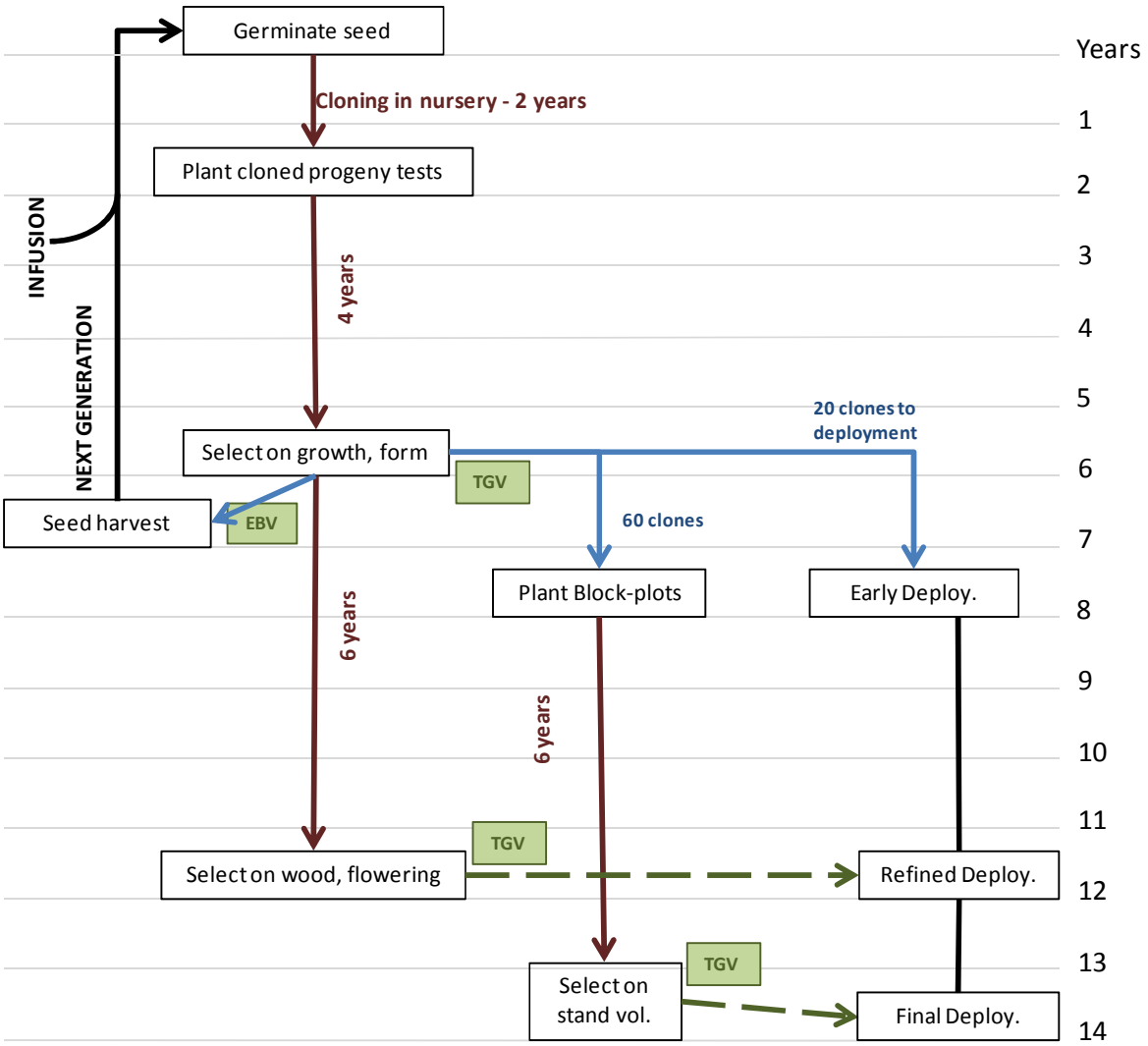

Figure 2. Flow diagram of Option 2. Cloned progeny testing. Brown arrows represent growth of a trial series; blue arrows represent transfer of genetic material; green dashed arrows represent information flow based on estimated breeding values (EBV) or total genetic values (TGV).

While it is difficult to compare the costs involved with the three strategies presented above, models may be developed using relative costs for key variables such as nursery propagation, vegetative propagation, land values, and labour, which vary dramatically around the world. Some of the key variables that influence the amount of land required are explored in Table 1.

The land area required for the seedling progeny strategy is strongly dependent on the number of clones selected for block-plot tests (Table 1). If land availability or cost was a constraint then staging these tests later with a subset of good clones would be a sensible variation. Between 72 and 137 ha would be required for the cloned progeny strategy options presented in Table 1, which would evaluate between 4000 and 8000 clones. Sixteen ramets per clone would be

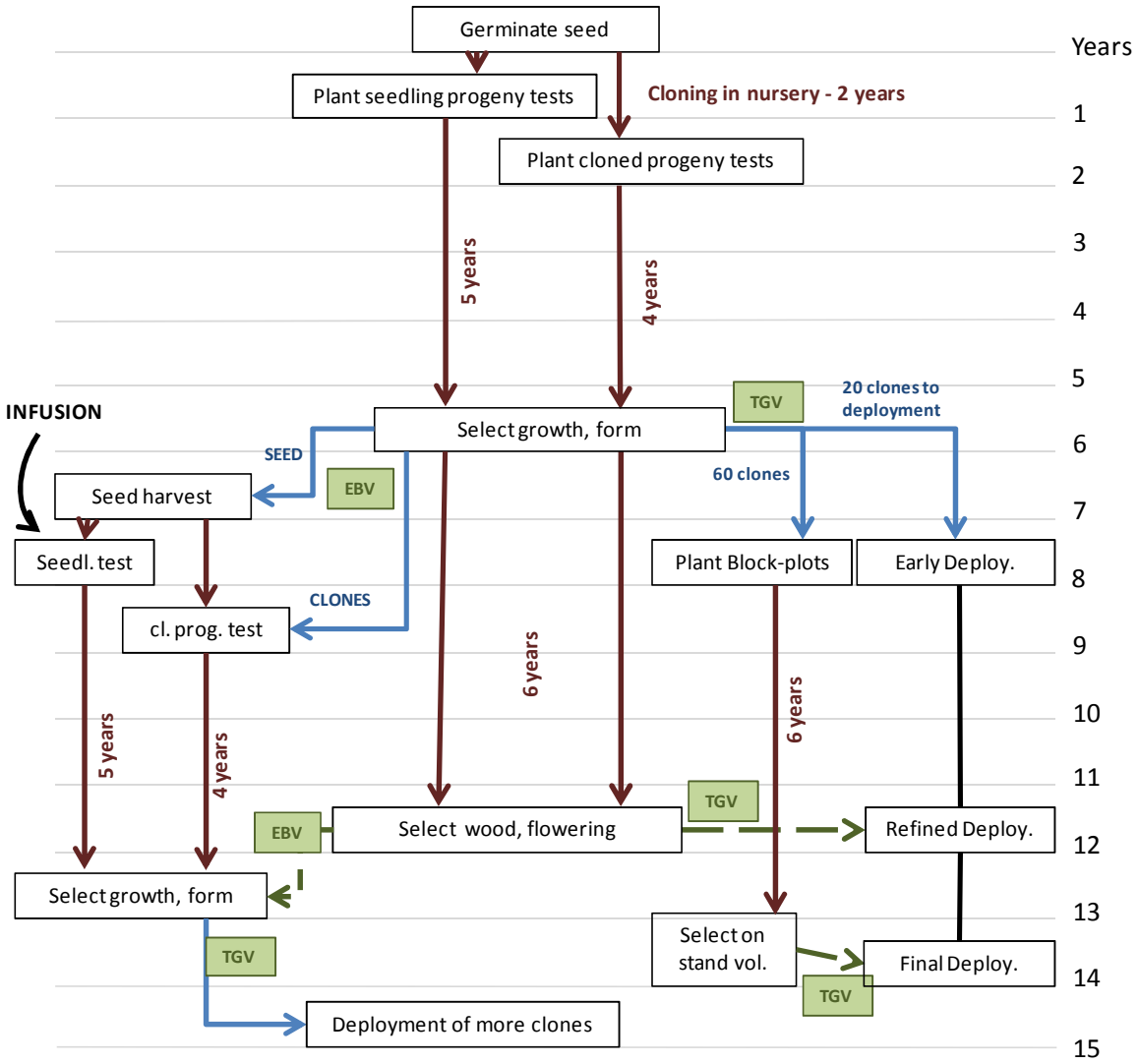

Figure 3. Flow diagram of Option 3. Integrated cloned and seedling progeny testing. Brown arrows represent growth of a trial series; blue arrows represent transfer of genetic material; green dashed arrows represent information flow based on estimated breeding values (EBV) or total genetic values (TGV).

preferable so that each clone could be represented by four ramets in single-tree plots on each of four sites and a rudimentary estimate of clone × site interaction obtained. The integrated cloned and seedling progeny options evaluated in Table 1 did not require larger investments in land for evaluation of up to 30 clones per family (6000 clones in total). The number of clones selected for block-plot tests was an important variable in the land area requirement of the cloned progeny and integrated cloned and seedling progeny strategies.

Table 1. Key variables and resultant land requirements in six scenarios for each of the tree improvement strategies (seedling progeny, cloned progeny, and integrated cloned and seedling progeny). It is assumed that 200 families are represented equally in each scenario, that block-plot clone tests are established with 400 ramets of each clone (four block-plots of 5×5 trees on four sites), and that stocking is 1111 stems per hectare (3×3 m).

Seedling progeny						
Scenario	S-1	S-2	S-3	S-4	S-5	S-6
Seedlings per family	100	100	100	100	100	100
Area of seedling progeny (ha)	18	18	18	18	18	18
Clones selected for line-plot tests	100	100	200	200	400	400
Ramets/clone for line-plot tests	16	80	16	80	16	80
Area of clone tests (ha)	1	7	3	14	6	29
Clones selected for block-plot tests	100	100	200	200	400	400
Area of block-plot clone tests (ha)	36	36	72	72	144	144
Total area required	**55**	**61**	**93**	**104**	**168**	**191**
Cloned progeny						
Scenario	C-1	C-2	C-3	C-4	C-5	C-6
Clones per family	20	20	30	30	40	40
Ramets/clone for line-plot tests	8	16	8	16	8	16
Area of cloned progeny tests (ha)	29	58	43	86	58	115
Clones selected for block-plot tests	120	60	120	60	120	60
Area of block-plot clone tests (ha)	43	22	43	22	43	22
Total area required	**72**	**79**	**86**	**108**	**101**	**137**
Integrated cloned and seedling progeny						
Scenario	CS-1	CS-2	CS-3	CS-4	CS-5	CS-6
Clones per family	10	10	20	20	30	30
Ramets/clone for line-plot tests	8	16	8	16	8	16
Area of cloned progeny tests (ha)	14	29	29	58	43	86
Seedlings per family	90	90	80	80	70	70
Area of seedling progeny (ha)	16	16	14	14	13	13
Clones selected for block-plot tests	120	60	120	60	120	60
Area of block-plot clone tests (ha)	43	22	43	22	43	22
Total area required	**74**	**67**	**86**	**94**	**99**	**121**

Discussion

The integrated cloned and seedling progeny strategy capitalises on the strengths of both single stream systems – the more rapid identification of clones for deployment from the cloned progeny strategy and the greater within-family selection from the seedling progeny strategy. The time reduction for clone identification comes at the cost of greater trial area requirements in the initial testing phase, greater propagating costs and nursery labeling requirements. Therefore, the costs and benefits of each strategy will vary between organisations and the specific balance between numbers of cloned progeny (if any) and seedling progeny must suit organisational budgets, priorities and technological capacities. Nevertheless, the costs associated with cloning will most likely be smaller when using seedling ortets in the nursery than mature ortets in progeny tests. This is one of the weaknesses of the traditional seedling progeny strategy – that such a large

number of clones from the progeny generation must be captured into tissue culture prior to subsequent evaluation.

A strength of the integrated cloned and seedling approach is that the cloned progeny are expected to increase the heritabilities expressed by the population and thereby increase the efficiency of selecting parent trees for the second generation (e.g. Isik et al. 2005). This approach also provides integration across the generations, as superior individuals from the first generation who were represented as seedlings can be cloned and entered into the cloned progeny test with the second generation, and EBVs for wood properties and flowering in the first generation can be used to improve the early selection of deployed clones from the second generation (Figure 3). An additional advantage is that mixtures or small plots of these pre-deployment clones selected from the stage-one trials may be deployed prior to the completion of block-plot trials, assuming this material will provide some level of genetic gain relative to the base population. The strategy demands relatively advanced genetic analyses to combine data across different types of trial designs (e.g. Baltunis et al. 2009).

Further results from empirical studies and modeling would be valuable to assist breeders in determining the optimal number of ramets per clone per site and optimal number of cloned progeny per family.

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