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A Breeding Seed Orchard (BSO) of Dalbergia sissoo in Nepal. Phot. Erik Kjær 1998.

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Authors
L.P. Dhakal, H.L. Aryal, E.D. Kjær and J.P.B. Lillesø

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DK-2970 Hørsholm
Tel. +45 3528 1500
E-mail: sl@kvl.dk

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Content

CFC-TIS

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Please address comments and enquiries to:

Component Support Unit - CFC
Tree Improvement and Silviculture (TIS)
PO Box 6055, Hattisar
Kathmandu, Nepal

Tel: +977 1 4434504, 4437784
Fax: +977 1 4434546
Email: tisc@mos.com.np

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1. Introduction

The present tree improvement programme for *Dalbergia sissoo* in Nepal was initiated in 1994 following a strategy agreed upon by government forestry institutions in 1993. The strategy was outlined by Gibson (1994) based on the so-called BSO concept (see e.g. Barnes 1995 for details). An introduction to BSOs in the context of tree improvement in Nepal is presented in Thomson (1994). The main advantages of BSOs are: faster delivery of improved seed, less resource demanding, and easy management. The major disadvantages are less genetic gain and utilisation limited to similar sites.

According to the planned long-term breeding programme, the Nepali *D. sissoo* BSO programme shall develop into a so-called multiple population breeding strategy (MPBS, Namkoong *et al.* 1980). The idea is to divide the breeding population into sub-populations that are kept separate throughout future generations of tree improvement in order to produce propagation material adapted to different environments. At the time of BSO establishment only limited information on climatic variation on Nepal was available and one BSO was therefore established in each of four geographical zones using major watersheds for delineation. Lillesø *et al.* (2001) provides a more comprehensive interpretation of climatic variation in lowland Nepal. The currently available information indicates that the potential growing area for *D. sissoo* may be divided into two dry zones and two wet zones (fig. 1) that do not overlap with the previous geographic zones used for the 1. generation BSOs. This will be taken into consideration when planning future work, as will be discussed below.

The 1994-1998 progress of the tree improvement has been summarised by Graudal (1996) and Lillesø (1998). Genetic parameters and potential gain from different selection regimes have been estimated by Aryal et al. (2001), and the feasibility of continued breeding of *D. sissoo* has been discussed by Hansen et al (2004). A detailed description of the breeding activities can be found in the above references.

In recent years *D. sissoo* has been severely affected by the die-back disease, a disease known to kill the tree from the top. The causes of this disease are not well understood. The disease was not serious during the start of the BSO programme and was not given high priority during selection. It is now commonly found throughout *D. sissoo*'s natural distribution area in South Asia and is causing widespread death of *D. sissoo*. There are indications in Nepal that fungi of the genus *Fusiform* and some insect species of *Lepidoptera* are involved in this disease syndrome, mostly in plantations. However, the exact mechanisms and triggering factors remain unknown.

The occurrence of disease resistant families in the breeding population of the Nepali BSOs may be of immense value. The challenge now is to improve and maintain the genetic gains of the selected families in the first generation BSOs and enhance the disease resistance qualities in the future generation populations. Below we outline options and suggest specific activities for the Nepali *D.*

sissoo breeding programme in this new context. More specifically, the present paper provides information on the coming generation turnover of *D. sissoo* in the Nepali BSOs. The role of infusion populations is discussed. Design, testing and infusion of these selected populations into the breeding programme are outlined.

The questions that are addressed include:

- When and where can the 2. generation superior trees be selected ?
- How many trees should be selected and from how many families?
- How is inbreeding depression in future generations avoided?
- Should the sub-population structure be maintained?
- Can the number of breeding zones be reduced based on the new information from the BSO?
- Can the new information on climate variation in Nepal be used for estimating breeding zones?
- Should new progenies be included in the breeding population?
- Why are infusion populations necessary for the breeding programme?
- Where and how to select the die-back disease resistant families?
- Where to place the infusion population trials?
- How does the cost associated with establishment of the 2. generation BSO compare to the benefits it will generate?

2. Composition of the 1. generation breeding population

The present 1. generation breeding population consists of progenies from two major rounds of selection:

- **'Super Sissoo Collection (SSC)':** In 1994 seed was collected from 94 out of 103 identified phenotypically superior trees, mainly from natural forests in the western part of Nepal (Table 1).
- **'35 families for progeny test (TKP)'**: Seed for a progeny trial was collected in 1993 from 35 superior trees in 5 populations (all from the Far Western Region, Table 1).

Table 1. Base breeding populations for BSOs of D. sissoo

Development Region[1]	# of candidate trees
'Super Sissoo Collection' (SSC)	
Eastern Region	3
Central Region	9
Western Region	11
Mid Western Region	11
Far Western Region	69
Progenies in the Teel Kane progeny trial (TKP)	
Far Western Region, Tunigori	3
Far Western Region, Lathiya	8
Far Western Region, Barchariya	10
Far Western Region, Kalyogu	5
Far Western Region, Godawari	9

Source: Thomson (1994). Note: not all candidate trees were eventually used, in particular none from the Eastern Region and only a few from the Central Region

BSOs have been established from the 'Super Sissoo Collection' (SSC) in all four regions - although not balanced. The number of progenies varies from 84 in Chitwan (Central Region) to 48 in Kapil Bastu (Western Region), see Table 2. This is mainly a result of decreasing availability of seed from the 1994 collection, as the initial plan was to establish all 1. generation BSOs with the full set of families.

The eastern and central parts of Nepal are poorly represented in the present breeding population, because most of the first generation plus trees were selected in the Western and Far Western Regions. The reason for favouring collection from the western part of Nepal has been an expectation that Western Kailali River provenance was the best in terms of form and growth (White 1994). However, available evidence has since indicated that this is an oversimplification, and

[1] There are five administratively delineated regions in Nepal, which cut across the country from east to west. These administrative regions will in the following be denoted by beginning with capital letters, e.g. Eastern Region; while geographical descriptions will be denoted by beginning with small letters, eastern part of Nepal.

that a more complex situation with local adaptation of *D. sissoo* may exist. The provenance variation and interaction of *D. sissoo* in Nepal is not well enough understood to be decisive on the matter due to lack of testing, but the recent results do indicate that western provenances may well be best in the west and the eastern provenances best in the east (Joshi and Thapa, 1997, Hansen *et al* 2004).

Therefore, the initial strategy to base the breeding exclusively on Western populations needs to be revised and additional progenies selected in the Eastern and Central Region and included in this sub-breeding population. This requires establishment of so-called infusion populations that will serve the purpose of feeding new genotypes from the central and eastern gene pools into the breeding programmes. The infusion populations will also allow a change in focus, putting much more emphasis on selection for disease resistance traits.

'35 families for progeny test' (TKP) has only been used for the Teel Kane Progeny Trial, and has at present not been included in a production population (unless the trial is converted to a seed orchard). They are all of Far Western origin.

Table 2. The present 1. generation BSOs

Region	District	Place	Year	# of families	Eastern families	Selection	Net Size (ha)	# of blocks (replications)
Central	Chitwan	Sauraha	1994	84	0	94 SSC	2,30	7
Western	Kapil Bastu	Hattiausha	1995 1996	48 45	0 0	94 SSC 94 SSC	0,69 0,65	4 4
Eastern	Sunsari	Dharan	1996	70	0	94 SSC	1,50	6
Mid West	Banke	Kohalpur	1998	49	0	94 SSC	1,17	6
Central	Chitwan	Teel Kane	1993	35	0	35 TKP	3,00	96

Sources: Rimal *et al.* (1995); Aryal *et al.* (1994); Jha *et al.* (1996); Aryal *et al.* (1998); Thomson (1993)
SSC: Super sissoo collections; TKP: Teel Kane Progeny Trial

The BSOs are established in a randomised block design where each family is represented in a block by a plot of 16 seedlings. Planting density is 4.444 plants per hectare. The number of families in some BSOs varies, but at least 45 progenies of 'Super *Sissoo* Collection (SSC)' are tested on all four sites.

2.1 Status of the 1. generation breeding population – when can selection for 2. generation take place?

Family breeding values can be estimated in the BSOs and used for selection between families for the next generation. Selection within families is done through selective thinning in the family plots until only one tree is left per plot. Generation turnover can then take place when

- the BSO trees start to produce seed, and

- the trees have reached a stage where it is anticipated that the long term performance has been revealed, i.e. good juvenile-mature correlations are expected (see Lambeth, 1980 for discussion on importance of juvenile-mature correlations in relation to gains from selection).

The juvenile-mature correlation remains unknown at this stage and breeding values used between family selection should probably not be estimated on trees younger than 5-7 years. Based on the high heritabilites found in the Teel Kane trial (Aryal *et al*, 2001), it is assumed that effective selection for growth and stem form can be carried out at this age. However, potential genetic variation in long term health - 'Sissoo die-back' - is not likely to be revealed until a later stage, although initial incidences are likely to be revealed in some cases. Breeding values for health can probably not be estimated until the trees are approximately 8 years old.

The BSO in Kapil Bastu (testing 48 SSC progenies) is the most successful of the BSOs in terms of survival and uniformity. The BSO was established over two years (4 blocks each year), but the full trial can be evaluated for growth and stem form already and juvenile breeding values calculated. Final breeding values for vigour and stem form may be estimated after 5-7 growing seasons. At that time there will be only 4 trees left per plot, but data from previous assessment may be included as additional information. Flowering is expected to have been initiated by that time.

Good survival has also been obtained in the BSO in Chitwan (testing 84 SSC progenies), but this BSO is less uniform, probably due to water logging in parts of the area. The water logging does not follow block limits and this parameter will therefore be included as a co-variate in the future analysis. Re-blocking will also be considered. Additional efforts are made to improve the drainage. The trees are of different height, but between family selection is probably also possible by now.

The BSO in Sunsari (testing 70 of the SSC progenies) has suffered from heavy insect attack, and the future of this BSO is uncertain. It is unlikely that it will be possible to estimate breeding values from this site but data on survival and health will be collected and analysed. The surviving families, which resist the high disease incidences, could be utilised as disease resistant families for seed production and for inclusion into the next generation breeding programme.

The BSO in Banke was established in 1999 and 2. generation selection may not be initiated until 2006.

The progeny trial at Teel Kane was established as a single tree plot with 35 families, 96 replications, and a planting density of 1,600 plants per hectare. This layout is well suited for estimation of family breeding values due to small blocks and many replications. The establishment has been quite successful with good and uniform growth. Family breeding values on stem form and growth rate for the 35 progenies (TKP) have been established (Aryal *et al.*, 2001). Selection for a new generation could take place now or within a few years. Light selective thinning, not eliminating any of the families, was employed in the year 2000.

As discussed above, not all the established 1. generation BSOs are old enough to allow estimation of conclusive breeding values (BVs) for growth and stem form. However, such BVs can probably be estimated for Sauraha and Kapil Bastu BSOs (see table 2) and 2. generation breeding can be practically initiated in the Central and Western Regions within 2-3 years. At that time a new assessment of the progeny test from Far-Western Nepal, Teel Kane trial, can also shed more light on the age-age correlation. Selection should be postponed a few years if the results at that time indicate decreasing age-age correlations.

Breeding values in the Eastern and Mid West Regions sub-populations will at the best be available by 2006. Also, the size of genotype by environment interactions cannot be quantified until this stage as this requires good estimates at all sites.

Breeding values for long term health cannot be estimated for the next 6-8 years because decreasing health is not likely to be fully revealed until then.
The timing of the generation turnover will depend on five important management decisions:

1. Should the present substructure into 4 breeding populations be maintained?

- *Yes*: This would mean that the next generation of BSOs should be selected from within each of the four zones – with no mixing of material between zones. This alternative would provide a faster generation turnover in the oldest BSOs and thus provide quicker gains. However, as the eastern BSO may have been lost due to severe damage of the Eastern Region BSO (see above), new infusion in the eastern breeding population will probably be required in any case. Also, the few families in the Midwestern and Western Region BSOs call for inclusion of more families. It therefore seems that the present substructure can only be maintained for the Central Region BSO, as the remaining three BSOs all need inclusion of the progenies for technical reasons.
- *No*: This would allow selection of material for the next generation across the breeding zones (using material from several BSOs). This alternative would mean that establishment of all 2. generation BSOs should probably wait until selection has taken place in all BSOs which will be around 2006. New genetic material for the eastern breeding zone may then, fully or partly, be selected in the Western and Central Region BSOs. Also, the Midwestern and Western Region BSOs can become genetically diversified with selections in the Central BSO (superior individuals within the families that are only present in this BSO).

2. Should the breeding zones be modified and new progenies infused?

The breeding zones can prior to the generation turnover be modified according to new information that has become available since the initiation of the programme. New information has especially become available on: climatic information, genotype x environment interaction[2] (GxE) suggesting the need to include central and eastern populations, the possible failure of the BSO in the Eastern Region, increased need for selection for health and focus on using well adapted seed sources. More decisive results will become available when the performance of the same set of families in the Central, Midwestern and Western Region BSOs can be compared, probably around 2008. New genetic material for the eastern breeding zone may then (fully or partly) be selected in the Western and Central Region BSOs as discussed above. However, new 1. generation selections of material from the wet breeding zone in the east seems highly required due to the lack of representation of this gene pool in the initial 'Super Sissoo Collection', see fig. 1.

3. When should generation turnover be done?
In principle, generation turnover can be done as soon as the first BSO starts to flower (and the number of trees in family plots has been reduced to one). It is possible to have a breeding programme with a 'rolling generation turnover' (see e.g. Borralho, 1998), where the second generation selection is done gradually, and the progenies in the second generation therefore become of uneven age. However, this is difficult to combine with the simple BSO approach that is the backbone in the Nepali *D. sissoo* breeding programme. Therefore, if it is decided to partly pool the 1. generation breeding populations (a discussed above), this will probably mean that the 2. generation BSOs need to await flowering in all the 1. generation BSO. Selections for the new BSOs in the Central and Western Regions would then be delayed with 3-5 years. A combination, where the 2. generation BSOs in the Central and Western Regions are based on selections only within these two BSOs, but selection for the remaining two BSOs, drawing on all four BSOs, would gain a few years for the Central and Western breeding zones.

It can be argued that the decision on future sub-structure should not be taken before genotype by environment interaction has been tested (around 2008).

The timing of the generation turnover should also be considered within the programme of TISC. The Nepali tree domestication strategy is to work with many species of importance to smallholders and it may therefore be decided to slow down the progress on *D. sissoo* in order to be able to include additional species in the programme.

Prolonged breeding generations will reduce gain in the long run (Figure 1), but in this case it is only a question of prolonging the first generation. Future breeding generations can be shorter once the genetic structure is better understood.

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[2] Genotype by environment interaction (in short GxE) is an expression of how seed sources change rank when compared in different environments. What is best in a given test environment may be inferior at another, and seed sources therefore have to be selected specifically depending on the environmental features of the planting site (see Lillesø *et al.*, 2001).

4. Should long term health be included as an important breeding objective?
The past selection regime has mainly focused on stem form and vigour. However, the decreasing health of the *D. sissoo* plantings in recent years should be taken into consideration before planning and implementing 2. generation activities. There are indications that the disease problem has a genetic background in the sense that improved health can be obtained through selection and breeding (Dhakal *et al.* 2004 in prep) and the TISC breeding programme may be able to contribute significantly to continued planting of *D. sissoo*.

Inclusion of long term health as an important breeding objective calls for a revision of the ways selections are made in the breeding programme, and it is also needed to consider how and whether to include new material into the existing populations.

- One option is to start a whole new set of BSOs selected for disease resistance among natural populations and plantations. However, such a choice would forfeit the gain that will be achieved with the existing BSOs. This lost gain will not only refer to vigour and stem form, but also to health. The SSC selections focused on healthy trees and the existing BSOs will be an effective way to screen these for 1. generation progenies for families and individuals with superior resistance as expressed on the test sites.
- An alternative is to establish a number of new infusion populations based on seed collection from old, healthy trees. Such infusion populations should then be designed to allow strong selection for disease resistance within the progenies. The 2. generation BSOs could then be based on a mixture of improved material from the present BSOs and improved infusion material. If the infusion populations are established in 2002, flowering can be expected to begin around 2010 and the next generation of BSOs then established around 2012.

The infusion populations would allow to correct the strong geographic bias in 'SSC' progenies by including more families from the central and eastern populations that are under-represented in the present BSOs.

5. Should provenance trials be established in order to better understand the GxE in adaptation?
The likely failure of the Eastern Region BSO (Sunsari), that mainly contains Western families, has increased the need to understand the adaptability of the Central provenances when planted in different ecological regions. If there are significant differences between provenances in their adaptability, then future generation *D. sissoo* breeding should obviously consider this.

This suggests that a set of provenance trials should be established. Field trials that test provenances from the entire natural distributional range on planting sites representing all the ecological variations. However, results from traditional provenance test may be difficult to apply in future breeding since the results will not be available for considerable time. One therefore has to consider solutions that do not prevent the continuous progress in health and quality through domestication.

Recommendations

The above reasoning points towards the following management decisions:

(i) the present structure should not be maintained, rather the next generation BSOs be based on new modified breeding zones that better reflect the ecological variation and GxE once this information becomes available;

(ii) the turnover should be somewhat delayed to ensure that GxE is better understood and to allow better testing for long term health;

(iii) new infusion populations should be established based on selections from eastern and central populations, and progeny from these populations (strongly selected for die-back resistance) should be included in the next generation BSOs;

(iv) the central and eastern provenances should be collected and tested along with the infusion tests for assessment of general adaptability of western provenances when grown on sites in the Central and Eastern Regions. These provenance collections (Eastern and Central Regions) will probably not be included in future generation breeding, but shed light on the GxE and adaptational patterns.

3. Selection for 2. generation BSOs

The established *D. sissoo* BSOs will serve as important seed sources for many years, and should be genetically thinned and followed with regular assessments. Tree breeding is a continuous process, where new generations of breeding populations are formed by selecting and crossing between the best individuals in each breeding generation once they have been tested and have started flowering (Figure 1). Continued progress will be obtained if this recurrent[3] selection continues over time and important progress can be achieved with *D. sissoo* due to high heritability[4] of important traits and relatively early flowering (Figure 2).

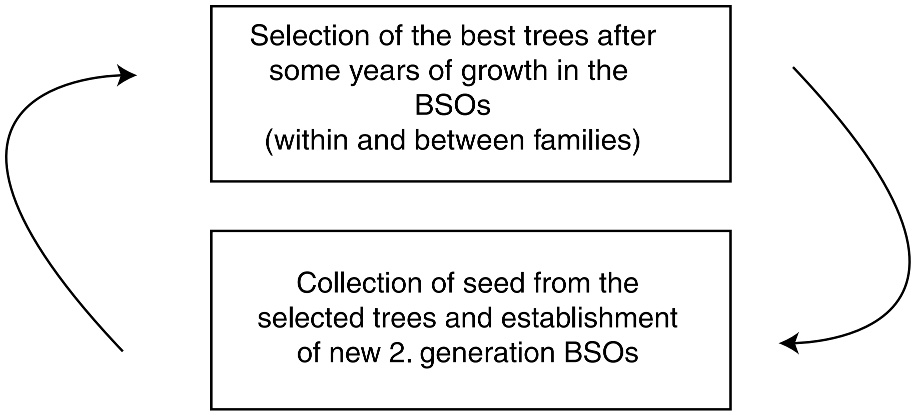

Figure 1. Recurrent selection in BSOs

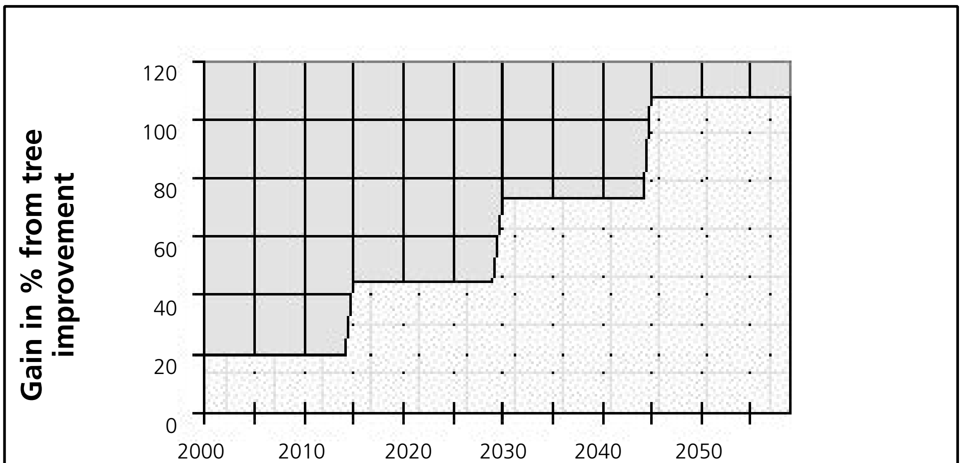

Figure 2. The continued gain from domestication of D. sissoo to be expected over successive breeding generations in Nepal. The steps represent gain obtained in each generation compared to the selected trees, here assumed to be 20% 'height of the steps'. The timing (length of the steps) will depend of the length of the breeding generation.

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[3] Recurrent selections means that selections are made 'again and again' after each cycle of progeny tests.

[4] Heritablity is the relative importance of heredity in determining phenotypic values. Heritability in the narrow sense is the extent to which phenotypes are determined by the genes transmitted from the parents (Falconer, 1989).

The generation turnover in BSO programmes is made in the seed orchard. It is therefore important to consider future generation turnover at a fairly early stage, because thinning of the BSO will influence the options for future breeding. It is especially important that strong between family selection should not take place until trees for the next generation breeding population have been selected. Otherwise, the long term breeding population may become too genetically narrow and lead to inbreeding in future generations.

A relatively large number of families should be selected for the next BSO generation. However, after collection of seed for establishment of the next generation BSOs, a BSO can be converted into a seed production orchard in which only the very best families are retained. The seed from such a production orchard will produce high quality trees for planting, but the seeds should not be used for breeding, as there will be small representation of families.

3.1 Number and origin of progenies to be selected for the 2. generation BSOs including infusions

It is assumed that progenies from the BSOs to some extent themselves will serve as future seed sources (see Aryal *et al.* 2001 for detailed discussion), and the seed producing BSOs include a fairly large number of progenies. This means that there will not be any substantial conflict between the breeding and seed production aspects in the BSOs. For both purposes it is recommended not to reduce the number of families in the BSO to below 40. This means, for example, that only few between family selections will take place in Banke and Kapil Bastu.

The number of trees selected for 2. generation breeding should allow between family selection in the following generation.

For the purpose of the 2. generation BSOs the following selections will constitute the base population:

a) best performing 40 families within the sub-structure of breeding population (each of the 4 existing BSOs). The selection criteria will emphasise high BV's for health (disease resistance), but also vigour and stem form;
b) 10 best progenies from Teel Kane trial with high BV's for health, vigour and stem form;
c) 80 best performing trees from the infusion trials of Eastern and Western Regions, respectively (80 families in total, described below);

It is recommended that the new BSOs be established around year 2010, with a total of 130 progenies in each sub-structure. A typical 2. generation BSO in each zone will constitute 40 selected families from the sub-structure breeding population + 10 families from Teel Kane + 80 families from infusion population.

It is most likely that the eastern BSO will not be able to provide 40 selected families from the sub-structure breeding population and it is recommended that these families are selected in the central BSO in Sauhara, an area which is relatively wet compared to the western BSOs.

3.2 Infusion population

Infusion populations are populations selected for injection of genes of desired traits (resistance to die-back disease) in the breeding programme. The importance of infusions is also to broaden the genetic base of the 2. generation BSO. In principle, it would be appropriate to collect many individuals in all breeding zones for the infusion population tests, meaning the entire Tarai (lowlands) of southern Nepal. The criteria for selection of disease resistance should be selection of the healthiest individuals from the area of high disease incidence sites where *D. sissoo* is growing; the details are described in table 3.

Table 3. Selection criteria for families in central and eastern infusion populations

Character	Economic weight	Score range	Score of the best tree	Weightage %
Health (resistance to die-back disease)	14	0-5	70	70
Age	4	0-2	8	8
Cylindrical bole	4	0-2	8	8
No ramicorn branching	3	0-2	6	6
A tendency to self prune	2	0-2	4	4
Seed production	4	0-1	4	4

N.B. Only trees scoring total of more than 80 could be included in the infusion tests

As described above, the current breeding population of *D. sissoo* mainly represents families from western Nepal. Hence, considering the work load and the availability of the resources it is advisable that infusion population candidate families, 40 families from each of two areas, be selected from the Central and Eastern zone only, since these zones are very poorly represented in the original breeding population. During the selection of infusion population it is important to consider that the seed used in plantations during the 1980's, in the Central and Eastern Regions is presumed to originate mainly from very few seed sources (mainly Koshi Tappu, Sunsari) of eastern Tarai.

3.3 Provenance test

It would be appropriate to collect nine populations from the natural *D. sissoo* range, three from each region, Eastern, Central and Western. Provenances could be delineated along the main river courses falling in each region. The probable collection areas for the eastern provenance could be east from Koshi river and east from Bara to Siraha District in the Central and Eastern Regions and for the western provenance from Mahakali river up to Rapti River in the Western Region. These sites have to be finally decided.

Each of the infusion collections should consider: a) seeds from many trees, in equal proportions; b) only healthy trees with no incidences of disease and well growing individuals; and c) proper documentation of the sources.

3.4 Economics of the 2. generation BSOs

The total gain from using improved *D. sissoo* seed in the Tarai will depend on the planting scale. The demand of *D. sissoo* seed is still more than 2,000 kg per year according to a fairly recent estimate. This corresponds to about 12,000 ha *D. sissoo* plantation per year.

One hectare BSO with final spacing of 8.5 m will have about 140 seed trees and will produce about 500 kg of seed. The current demand therefore corresponds to production from about 4 ha of BSOs.

Table 4 compares the average annual gains from planting 1. generation seed of *D. sissoo* with the annual costs of the improvement programme. Interest rates are taken at 3 and 5% per annum (Aryal *et al*, 2001).

Table 4. Annual gain and cost (millions of rupees) from 1. generation seeds of D. sissoo

Plantings per year with improved planting stock (ha)	Annual gain, i=0.00 NRs in mill	Annual gain (NPV), i=0.03 NRs in mill	Annual gain (NPV), i=0.05 NRs in mill	Yearly costs for tree impr. NRs in mill
250	238	11	5,5	1
500	656	22	11	1
1,000	1,313	45	21	1
2,000	2,625	89	43	1
4,000	5,250	179	86	1

Source: Aryal et al. (2001)

Table 4 shows that the more seedlings used in plantings, the bigger the total gain. Cost and gain can therefore be compared based on different scenarios on the planting scale. The break-even point for gain versus yearly costs can be found as low as 50 ha at 5% pa. This indicates that the cost associated with the 2. generation BSO establishment is negligible compared to the benefits it will generate (Aryal *et al.*, 2001).

4. Location of 2. generation BSOs

The current information on the climatic variation in Tarai (lowland tropical Nepal) indicates that the easternmost part should be considered separate from the remainder of the Tropical Ecological Zones, representing a semi-evergreen tropical rain forest climate. Dry and Wet Monsoon climate appear to be a patchwork across lowland tropical Nepal, perhaps with a tendency for the Siwaliks in the Upper Tropical Ecological Zone to be more humid than the Lower Tropical Ecological Zone. There are not enough stations in the tropical valleys to evaluate how humid they are; we therefore suggest (judging from the surrounding vegetation) tentatively to separate the tropical valley systems from the Tarai (see Lillesø *et al.* 2001 for a detailed discussion).

At present there is one BSO in each of the four administrative regions of Tarai. Two of the BSOs are situated in the western expanse of the dry monsoon forest planting zone, while the easternmost BSO is situated in at the border towards semi-evergreen forest planting zone. One BSO and the progeny trial are situated in the relatively wet monsoon forest planting zone.

The basic idea behind the multiple population strategy is to develop populations that are adaptable to different types of environment and the improved seed should be utilised in those environments. The present sub-structure of the breeding populations (the four "multiple populations") covers the types of environments in lowland tropical Nepal (although the BSO in the semi-evergreen forest planting zone is on the border and moreover has problems related to health).

The present demand is most probably depressed because of concern for die-back disease syndrome. Should it be possible to breed for increased resistance to the disease, and at the same time provide extension advice on proper silviculture and not plant *D. sissoo* on heavy clay soils, it is most likely that the demand for seed will increase substantially.

The sites of the two existing BSO sites in the western expanse of the dry monsoon forest planting zone can be justified also for the next generation BSO provided that they also produce seed for the eastern expanse of the dry monsoon forest. However, it could also be suggested to establish one 2. generation BSO in each expanse of dry monsoon forest. The BSO site in the wet monsoon forest planting zone can be used for the next BSO generation, although the potential planting area is small. The present BSO site in the semi-evergreen forest planting zone is probably not suitable for *D. sissoo* and a new site should be found further to the east. To conclude, the potential sites could be: Sunsari District (but new riverine site) for Eastern Region, Bara or Rautahat (along Bagmati riverside) and Sauraha, Chitwan (but new riverine site) for Central Region and Banke (new riverine site) for Western Region.

4.1 Layout and location of the infusion population trial

In the 1. generation BSOs the central and eastern populations of *D. sissoo* were under-represented (refer above). The populations appear to occur in three different types of climates, wet monsoon forest, dry monsoon forest and semi-evergreen forest.

Ideally, infusion trials should be established in all three types of climates. However, to limit costs, infusion trials will only be established in the two largest climatic areas.

The infusion trials will be in a randomised block design in which Eastern population should constitute 30 families (semi-evergreen forest). Central population should constitute 40 families from eastern dry monsoon forest and 10 families from wet monsoon forest. Each family will constitute of 100-tree block plots with 4 replications at the spacing of 1.5x1.5 m.

The ideotypes[5] used for selection purpose will be: a) health (die-back disease resistance) and vigour; b) age (selection emphasising toward the old aged trees); c) straight, cylindrical bole; c) no ramicorn branching habit; and d) a tendency to self prune.

Each of the populations with 30 and 50 families (40+10), from East and Central respectively, will be put in trials near the existing BSO of the Central and Eastern Regions, or other appropriate site of the respective regions. Each of the sites should set aside approximately one ha. for the infusion trial plantation.

Table 5. Selection and planting of infusion populations

	Families planted	
Families selected	Dry (dry monsoon forest) - Central	Wet (semi-evergreen forest) - East
Dry (dry monsoon forest)	40	
Wet (semi-evergreen forest)		30
Wet (wet-monsoon forest)	10	
Total	50	30

These trials would be analysed primarily for disease resistance during year 3, 5 and 8. Only one out of each 100 trees will be retained in each family after the final genetic thinning. This means that one best tree per family will be retained in each trial at the end of the tests. These 80 selected disease resistant trees will be further used for the next generation BSOs as infusions as soon as they start to flower and produce seed. It is expected that at age 8, these families will be producing seed ready for the next generation.

[5] Ideotype: Donald (1968) suggested the importance of having a model (ideotype) as a goal for breeding programs. This concept has had an impact on breeding programs and ideal plant types of many crops have been described (in Zaheer *et al.*, 1999).

Infusion trials could be placed in the Koshi river sites in Sunsari district in the east and in the Bagmati river sites in the Bara or Rautahat district.

4.2 Layout and location of provenance trial

The layout of the provenance trial should be a randomised block design. Three populations, one each from new breeding zones (covering Central, Eastern and Western Regions) will be placed in 8 tree square plots with 10 replications at a spacing of 2x2 m. Relatively wide spacing is needed in order to limit the thinnings. The thinning could be done mechanically at later stages of development.

Provenance trials should be planted with the infusion trials for Central and Eastern Regions but the Western Region Provenance trials could be planted with the existing Banke or Kapil Bastu BSO sites.

4.3 Layout of 2. generation BSO

Layout of the 2. generation BSO can almost follow the design in 1. generation BSOs, i.e. 12 tree row plots with 6 replications. The plots are planned with 12 rather than 16 trees because the number of families is planned to 130 in the 2. generation BSO.

The plots should be randomised within the blocks. However, breeding between related trees should be reduced by avoiding that neighbouring plots have progenies from trees of the same 1. generation progeny. This can be done by application of computerised algorithms or simply by checking the layout manually.

It is very important to maintain the information on family structure in order to be able to develop a pedigree in the following generations. Each selected 2. generation tree should therefore be assigned an identity code and its origin (1. generation family number) should be recorded. 2. generation progenies can then be assigned a progeny code that includes the first generation identity. A simple written file will be used to store the information allowing the breeders to be able to keep track of the pedigree at all times.

The inbreeding coefficient will gradually increase over the generations. However, as n = 130 families are maintained at generation turnover, this increase will be slow and counteracted by the selection against recessive semi-lethal genes during the within-family selection. Still, caution should be taken to keep track of pedigree and to choose a layout in the BSO that isolates related trees by distance.

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