Appendix 8

Report to OFI on funding to the International Centre for Research in Agroforestry (ICRAF) under the LEUCNET initiative

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Introduction

The successful adoption of agroforestry technologies depends to a large extent on the availability of high quality tree germplasm, both at the right time and in appropriate quantities. Natural stands of many important provenances are small and unable to meet user's demand for seed, which is often high. In such situations, the establishment of specific seed production stands, managed to optimise the production of high quality seed, is needed to fulfil seed requirements.

In the genus *Leucaena*, a large number of field trials have been undertaken to identify those species and provenances which are superior for particular products in specific geographical regions. However, less attention has been placed on how best to meet the seed demands of the end users, farmers, for the key provenances identified in screening trials. A common cry of development projects is the absence of sufficient high quality tree seed to meet the demands of their users. As a result, two areas of research at ICRAF are to understand the processes involved in tree seed production and how germplasm is delivered to users. Research involves technical aspects of tree management to optimise quality and quantity of seed production, and surveys establishing the pathways of distribution, dissemination and diffusion of germplasm through different players in the supply chain.

The primary focus of the research described here, undertaken by ICRAF with the Oxford Forestry Institute (OFI), was to meet gaps in knowledge on the technical aspects of tree seed production in the genus *Leucaena*. Family-based seed production trials of four provenances from the genus were established in Kenya in May 1996 (Were *et al*. 1998). Provenances were chosen on the basis of preliminary results on growth performance, pysllid resistance/tolerance and the availability of family-structured collections. Provenances were: Corral Falso (*Leucaena diversifolia* [Schltdl.] Benth. Hooker), Los Guates (*Leucaena trichandra* [Zucc.] Urban), San Pedro Chapulco (*Leucaena pallida* Britton & Rose) and San Martin Pachívia (*Leucaena esculenta* [Sessé & Moc. Ex DC] Benth). All were established at two sites (Muguga and Machakos), except for San Martin Pachívia, which was established at one site only (Machakos).

The following were the specific objectives of the research: (i) to evaluate the genetic characteristics of seed production, including heritability, relationship to tree growth characteristics and the influence of different thinning strategies on genetic constitution, (b) to determine the influence of different management strategies (such as coppicing) on seed production (c) to evaluate the optimum ecological conditions for seed production; and (d) to produce seed of key provenances which can then be made available to users for planting.

In addition to the above, a small part of the grant allocated to ICRAF was used to establish an experiment to study hybrid seed production between *L. leucocephala* and *L. trichandra* at Machakos field station, in November 1998. The primary objective was to assess the production of triploid seed with *L. trichandra* as the maternal parent, as a function of different planting ratios of the two species. Triploid progeny are expected to be sterile and demonstrate positive heterosis for growth characteristics. The former characteristic is of ecological importance when weediness concerns preclude the distribution of *Leucaena* seed. Weediness is a major issue for certain *Leucaena* provenances and sterile seed production is therefore an important area of research (Hughes 1998). Only the methodology of this experiment is described here, as no substantial results have yet been obtained.

Finally, a small portion of the grant was used to establish a *Leucaena* species trial at Machakos in June 1996, to study resistance/tolerance to, and management of, the *Leucaena* psyllid, *Heteropsylla cubana* (work primarily undertaken by CABI). The results of this trial are not reported here, but are described by CABI elsewhere (Day 1999).

Family-based and hybrid seed production trials currently remain on-going under separate funding from DfID. Measurements will be terminated in 2000 and 2001, respectively.

Materials and methods

Family-based seed production trials

Table 1 describes the OFI seed lots used for establishment of seed production trials. Three provenances (Corral Falso, San Pedro Chapulco and Los Guates) were obtained as individual tree collections from 20 trees, while one provenance (San Martin Pachívia) consisted of 24 tree families. Progeny within tree families are likely to demonstrate a half-sib relationship to each other (i.e. different paternal parents), although this may not always be the case. In particular, L. diversifolia is self-compatible (Hughes 1998) and a degree of selfing may occur in the Corral Falso provenance. Seedlings were raised in polythene pots in forest soil at ICRAF-Machakos field station before field planting at Machakos and Muguga in May 1996. Table 2 describes the conditions at the sites where trials were established.

The planting sites had been cropped in the previous two years with corn and beans. After the last crop of corn, the land was ploughed twice and harrowed once before it was marked for planting.

Each of the provenances was established at both Machakos and Muguga (except San Martin Pachívia at Machakos only), using a similar design. Twenty families (24 for San Martin Pachívia) were planted in 4-tree line plots replicated 10 or 20 times (see Table 1). Therefore, either 40 or 80 trees represented each family in a stand. Within rows, tree spacing was 1 m, with 4 m between rows. This design allows thinning by up to a factor of 4 within family plots, to an average spacing of 4 m within and between rows. Table 1 also indicates any treatments subsequently applied to individual stands to study the effects of different management strategies (type of thinning and coppicing) on seed production. In some cases, no treatments have been applied to date because of limited early seed production or limited competition between trees (for San Martin Pachívia and San Pedro Chapulco).

Stands were separated from each other by a minimum of 100 m in order to minimise pollen contamination between stands. This was particularly important for *L. pallida* and *L. diversifolia*, which are both tetraploid and are expected to have a high crossability index and produce highly fertile progeny (Hughes 1998).

Trees were assessed at 6-monthly intervals for length of longest stem and total number of stems. The diameter of all stems larger than 1 cm diameter was measured at 30 cm from the ground to provide estimates of cross sectional area. Seed was collected by handpicking pods twice monthly from individual trees. Seed was extracted, cleaned, dried and weighed on an individual tree basis. For the purpose of the present report, seed yield per tree was averaged on a family basis

Leucaena *hybrid trial*

Raising of seedlings and land preparation was carried out in a similar manner to the family-based seed production trials.

Bulk seed collections of Los Guates (*Leucaena trichandra*) and K636 (one of the highest performing provenances of *L. leucocephala* currently available), both obtained from OFI, were planted in rows at a ratio of 1:4, 1:6 and 1:8 (*L. trichandra*: *L. leucocephala*). For example, at a ratio of 1:4, one *L. trichandra* was followed within a row by four *L. leucocephala*. Spacing between trees within rows was 2 m. Each row formed a replicate and contained all three treatments. Nine replicates in total were planted in three blocks, with 10 m between rows within a block.

Trees were assessed at 6-monthly intervals for length of longest stem and total number of stems. The diameter of all stems larger than 1 cm diameter was measured at 30 cm from the ground. Seed will be collected from *L. trichandra* trees by handpicking pods twice monthly from individuals and will be extracted, cleaned, dried and weighed on an individual basis. A proportion of this seed is expected to be triploid (*L. leucocephala* as the paternal parent). The proportion and performance of triploid seed will be evaluated by field planting (the triploid has a distinctive phenotype) and using molecular techniques (specific genetic markers are available for each species [Stephen Harris, OFI, personal communication], allowing hybrid individuals to be readily identified.

Table 1 *Provenances, species and OFI references of seed used for the establishment of family-based seed production trials at Machakos and Muguga, Kenya. Also indicated are details of experimental treatments (selective or systematic thinning, coppicing, spacing) and culminative seed processed for storage*

Provenance (country)	**Species**	**OFI ID**	**No. of Families**	**Site**	**No. of reps.**	**Treatments[1]**	**Date of treatment**	**Seed processed, in Kg (June 1999)**
Corral Falso (Mexico)	*Leucaena diversifolia*	45/87/1 to 20	20	Machakos	20	1. Systematic thinning of 10 reps. (2 blocks of 5 reps.) to 4 m within rows 2. Selective thinning of 10 reps. (2 blocks of 5 reps.) to (average) within row spacing of 4 m	9/6/98	238
				Muguga	20	1. Selective thinning of 5 reps. to (average) within row spacing of 2 m 2. Selective thinning of 5 reps. to (average) within row spacing of 2 m and coppicing at 50 cm 3. Selective thinning of 5 reps. to (average) within row spacing of 4 m 4. Selective thinning of 5 reps. to (average) within row spacing of 4 m and coppicing at 50 cm	15/6/98	5
San Martin Pachívia (Mexico)	*Leucaena esculenta*	47/87/1 to 24	24	Machakos	10	No treatment applied to date	-	94
San Pedro Chapulco (Mexico)[2]	*Leucaena pallida*	52/87/1 to 20	20	Machakos	10	No treatment applied to date	-	150
				Muguga	20	No treatment applied to date	-	33
Los Guates (Guatemala)	*Leucaena trichandra*	53/88/1 to 20	20	Machakos	10	1. Selective thinning of 5 reps. (1 block of 5 reps.) to (average) within row spacing of 4 m 2. Coppicing of 5 reps. (1 block of 5 reps.) at 50 cm	09/6/98	202
				Muguga	20	1. Selective thinning of 10 reps. (2 blocks of 5 reps.) to (average) within row spacing of 4 m 2. Coppicing of 10 reps. (2 blocks of 5 reps.) at 50 cm	15/6/98	127

[1] The primary criterion for selective thinning, when applied, was culminative seed yield of a tree (individual with highest seed yield remained). In the case of Corral Falso at the Muguga site, no seed production data was available at the time of selective thinning and selection was based on cross sectional area (CSA) (individual with highest CSA remained)
[2] According to Hughes (1997), San Pedro Chapulco may represent a hybrid (the parentage of which is unknown)

Table 2 *Site characteristics of Machakos and Muguga field stations*

Station	Altitude (m)	Latitude	Longitude	Mean Annual Rainfall (mm)	Mean Annual Temperature (^{0}C)	Soil type (FAO classification)
Machakos	1660	1^0 33' S	37^0 08' E	740	24-26	Haplic Lixisol
Muguga[3]	2150	1^0 14' S	36^0 38' E	970	18-21	Rhodic Nitisol

Results

Family-based seed production trials – seed production

Seed production data on a per tree basis are summarised in Table 3 and Figure 1. In the case of Corral Falso and Los Guates, data shown precedes management applied to trees in June 1999. To date, data collected from these stands subsequent to management indicate no obvious trends in the influence of different treatments (copping and selective/systematic thinning; see Table 1 for treatments) on seed production. In the case of coppicing, very little seed has so far been produced from coppiced trees, making accurate comparison on a family basis difficult. Effects of treatments require longer-term observations that are currently on-going – preliminary results of treatments are therefore not shown here.

Based on seed production figures without management interventions, for all four provenances it is clear that considerable variation exists among families in average seed production per tree at a site (Fig. 1). This is particularly evident for Corral Falso (24 MAP) and San Pedro Chapulco (37 MAP), less so for Los Guates (24 MAP) and San Martin Pachívia (30 MAP). In addition to family variation, seed production varies greatly between Machakos and Muguga sites, with overall average seed yield per tree being 2- and 6-fold greater at Machakos for Los Guates (24 MAP) and San Pedro Chapulco (37 MAP), respectively. Twenty-four MAP, Corral Falso had produced considerable seed at Machakos but none at Muguga. Comparing the two sites further, it is evident that, in the case of Los Guates (24 MAP), seed production shows a similar trend between families across the two locations (see Fig 3. for correlation of families across sites). In the case of San Pedro Chapulco (37 MAP), the highest producing family is also the same across locations.

Family-based seed production trials – height data

Height data on a per family basis are summarised in Figure 2. Data are 17 MAP except San Martin Pachívia (23 MAP).

For all four provenances, variation exists among families in average tree height at a site, although this variation is less than for seed production data (see Fig. 1). Variation also exists between Machakos and Muguga sites, although this is considerably less than for seed production data. In contrast to seed production, in the three cases where comparisons are possible, provenances perform slightly better at the Muguga site. Similar to seed production data, a correlation between family performance across sites exists (see Fig 3. for Los Guates – a similar correspondence is also observed for Corral Falso and San Pedro Chapulco). For Los

[3] The Muguga site is subject to frequent morning mist in the months of July and August

Guates, although height variation among families is less than that for seed production across families, the correlation across sites for family height is greater than for seed production (see R values in Fig 3).

Comparing family seed production and family height data, no obvious correlation was observed for any provenance at either site.

Table 3 *Summary of seed production data for four Leucaena provenances at Machakos and Muguga field stations, Kenya*

	Cumulative average seed yield per tree (g)[4]						
	Corral Falso *L. diversifolia* 45/87[5]		Los Guates *L. trichandra* 53/88[6]		San Pedro Chapulco *L. pallida* 52/87[7]		San Martin Pachívia *L. esculenta* 47/87[8]
Family no.	Machakos (14 MAP)	Muguga	Machakos (13 MAP)	Muguga (17 MAP)	Machakos (15 MAP)	Muguga (21 MAP)	Machakos (18 MAP)
1	175.5	0.0	111.9	41.1	11.7	1.5	23.8
2	11.6	0.0	106.8	59.3	13.7	0.2	31.0
3	10.1	0.0	82.7	29.4	14.8	1.3	28.8
4	119.7	0.0	166.4	102.5	73.4	2.3	70.7
5	56.8	0.0	214.8	89.4	29.9	8.2	47.0
6	159.7	0.0	133.9	76.1	43.6	1.4	46.9
7	8.1	0.0	132.8	43.9	14.2	1.7	26.0
8	60.1	0.0	88.5	41.9	9.0	4.8	2.1
9	81.8	0.0	165.4	69.2	37.6	0.9	11.0
10	80.6	0.0	101.1	103.1	64.9	1.3	8.3
11	210.0	0.0	94.7	14.3	4.5	0.0	31.8
12	45.1	0.0	112.8	41.1	23.6	0.1	25.7
13	7.1	0.0	104.1	37.9	20.4	0.3	21.4
14	33.3	0.0	81.8	59.3	7.2	2.6	33.0
15	38.2	0.0	139.7	97.1	2.8	1.3	51.0
16	32.8	0.0	70.9	29.0	21.8	0.0	35.0
17	14.9	0.0	73.8	56.9	18.6	0.6	20.5
18	104.8	0.0	162.6	100.0	276.7	69.4	65.3
19	236.4	0.0	132.3	99.7	26.0	24.4	63.8
20	240.3	0.0	70.1	47.8	4.6	0.0	96.9
21	-	-	-	-	-	-	24.7
22	-	-	-	-	-	-	48.6
23	-	-	-	-	-	-	64.5
24	-	-	-	-	-	-	37.4
Average	86.3	0.0	117.4	62.0	36.0	6.1	38.1

4 Value in brackets indicates months after planting (MAP) when seed could first be collected from each stand
5 Production figures 24 MAP
6 Production figures 24 MAP
7 Production figures 37 MAP
8 Production figures 30 MAP

Discussion

Our results allow a number of important practical conclusions to be drawn from data.

First, the high variation observed among families in seed production, particularly for Corral Falso (*L. diversifolia*) and San Pedro Chapulco (*L. pallida*), suggests strong genetic control of this character. Data provide an indication of what can be expected when bulking seed from production stands in which family structure is not accounted for. It is evident that a considerable reduction in the genetic base, and a large shift in the genetic constitution of seed, will occur. Collected seed may consist primarily of a small number of high-producing families, with a considerably narrower genetic base than the original population. This narrowing will likely lead to inbreeding depression and loss in subsequent performance. This is of particular concern in understanding the performance of material in subsequent generations which has been distributed to farmers. The similar trend between families in seed yield across the Machakos and Muguga sites, for Los Guates (*L. trichandra*), is additional evidence of the genetic control of seed production. In the case of San Pedro Chapulco, a similar trend may also exist across sites among families, but the overall low level of seed production at the Muguga site precludes firm conclusions from being drawn at present.

Second, the differences in seed production observed between Machakos and Muguga sites provide an indication of the ecological range and preferences of the different provenances for producing seed. At Machakos, with a lower altitude and rainfall, higher temperatures and haplic lixisol soil, seed production of all three provenances in common is higher than at Muguga, for the first two years (three years for San Pedro Chapulco) after stand establishment. For all three provenances, seed production began earlier at the Machakos site. Since early seed production is a desirable characteristic of seed stands, it is apparent that Machakos is the preferred site for the three provenances tested at both sites. Most interesting is the relative difference between Corral Falso and Los Guates stands at the two sites. While the average seed production at Muguga of Los Guates is half that at Machakos (24 MAP), Corral Falso had produced a similar quantity of seed as Los Guates at Machakos but no seed at all at Muguga (24 MAP). This difference in relative production is not surprising when the different altitudes of the two populations in their native ranges (Corral Falso = 800 m, Los Guates = 1450 m) (Hughes 1998) are compared with the altitudes of the Machakos and Muguga sites (1660 m and 2150 m, respectively). Corral Falso is clearly outside its ecological limits for good seed production at the Muguga site, possibly relating to the low mean annual temperature there, although small quantities of seed have been produced subsequent to 24 MAP (see Table 1).

Third, the difference between families in seed production is not directly related to height data. Seed production is much greater at the Machakos site, but height data are relatively similar across sites. Furthermore, no correlation between seed production and tree height was observed among families at a particular site. Therefore, seed production does not relate directly to the size of the tree. Between sites, it may relate to variable ecological conditions that influence flowering, pollination or seed set. At a particular site, variation among families is currently unaccounted for. It is possible that seed production may relate to the form of the tree (measured by number of stems and cross-sectional area), and this will be the subject of further investigation. Although it is therefore too early to say from the present analysis if a genetic shift toward high seed producing families (as discussed above) will directly influence the overall performance of trees in terms of biomass production, it appears that height will be unaffected. It is important to clarify the effect of such a genetic shift on performance, because it is possible that high seed production may be negatively correlated with growth characteristics such as leaf and woody biomass. In this situation, sampling of seed from production stands in which family structure is not maintained may lead to a direct loss of performance in subsequent generations.

Seed distribution

As well as providing important research results on seed stand management issues, trials have produced large quantities of seed of key *Leucaena* provenances for distribution to users. Overall figures for seed processed for storage were indicated in Table 1. Table 4 indicates quantities of seed distributed from family-based seed production trials to various partners.

***Table 4** Seed distribution from Leucaena family-based provenance seed production trials (distributed as provenance bulks unless otherwise stated)*

Quantity (Kg)	Organisation	Purpose of distribution
Ex. Corral Falso (*L. diversifolia* 45/87)		
150	World Vision Zambia	Integrated Agroforestry Project
11[9]	Oxford Forestry Institute	For distribution through LEUCNET
3	Forestry Research Institute Malawi	Distribution/establishment of seed production stands
1	ICRAF (Kenya)	On-farm research/on farm seed production stands
Ex. Los Guates (*L. trichandra* 53/88)		
150	World Vision Zambia	Integrated Agroforestry Project
29[10]	Oxford Forestry Institute	For distribution through LEUCNET
3	Forestry Research Institute Malawi	Distribution/establishment of seed production stands
1	ICRAF (Kenya)	On-farm research/on farm seed production stands

Of particular interest is the World Vision Integrated Agroforestry Project, based in Chipata, Zambia. A major component of this project is to work directly with 15,000 farmers in the Chipata region to increase food security through soil fertility replenishment by planting agroforestry trees. *Leucaena diversifolia* and *L. trichandra* are two of the five species chosen for planting in this project because of their coppicing ability and will be distributed to several thousand farm-families for this purpose. Apart from playing a role in all aspects of seed supply in the World Vision project, ICRAF is also involved in monitoring the processes involved in seed distribution and dissemination. The project provides a unique opportunity to assess such processes, and, as such, it is intended that the subsequent distribution pathways of the two *Leucaena* provenances will be followed in the region, as well as genetic monitoring of any interactions between the two species. This will provide a valuable insight into the interventions that can me made to increase the sustainability of agroforestry systems in the tropics.

[9] Bulk and single tree collections
[10] Bulk and single tree collections

Conclusion

In conclusion, our results indicate the role of family structure and site in determining seed production in *Leucaena* provenances representing four different species. In addition, comparison of seed production data with height data provides a preliminary insight into the relationship between seed production and growth characteristics.

More detailed analysis needs to be undertaken, in which a formal analysis of variance is conducted on individual trees, considering the self-compatibility or incompatibility of the different species. Further, the relative pollen contribution of different trees must be considered. In combination with on-going work evaluating the relationship between seed production and tree growth characteristics other than tree height, the influence of selective and systematic thinning and the effect of coppicing on seed production, further light will be shed on the factors influencing seed production in the genus *Leucaena*. Together, data will enable optimum strategies for the sustainable supply of good quality seed of *Leucaena* species, in the quantities required by users, to be devised.

Finally, seed produced from stands has already been distributed to a number of partners. Of particular note is the World Vision Integrated Agroforestry Project in Zambia, where seed is being distributed to several thousand farmers to increase crop production through soil fertility improvement in the Chipata region.

References

Hughes CE (1998) Leucaena: *A genetic resources handbook*. Tropical Forestry Paper 37, Oxford Forestry Institute, Oxford, UK.

Day RK (1999) *Integrated control of Leucaena psyllid*. Final technical report R6524 presented to DfID.

Were J, Dawson I, Mbora A, Pottinger A, Simons T (1998) Seed production in *Leucaena* species: initial results on family and site variation from Machakos and Muguga, Kenya. *LEUCNET News*, **5**, 16-21.

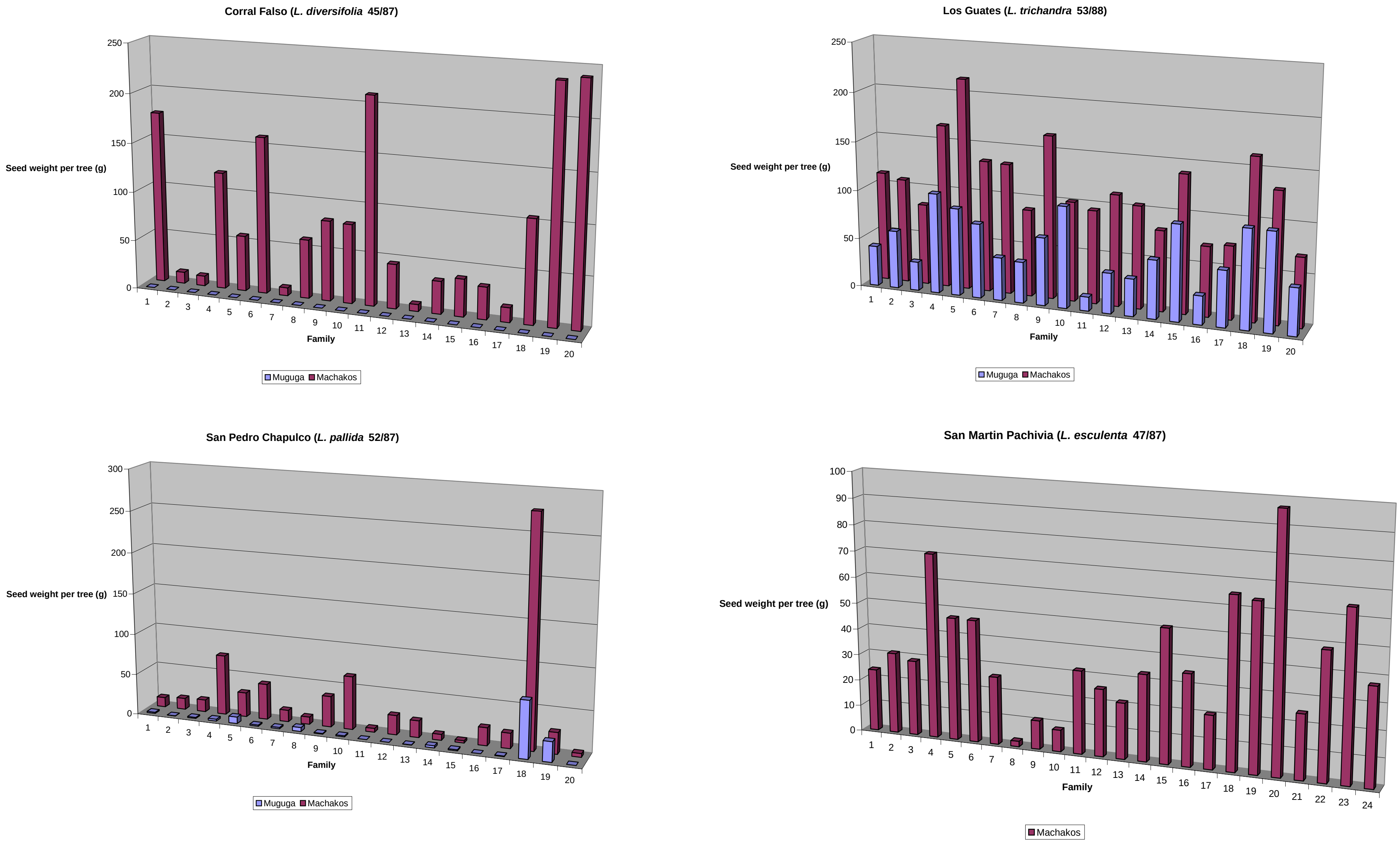

Figure 1 Summary of seed production data for four *Leucaena* provenances at Machakos and Muguga field stations, Kenya. For further details, see Table 3

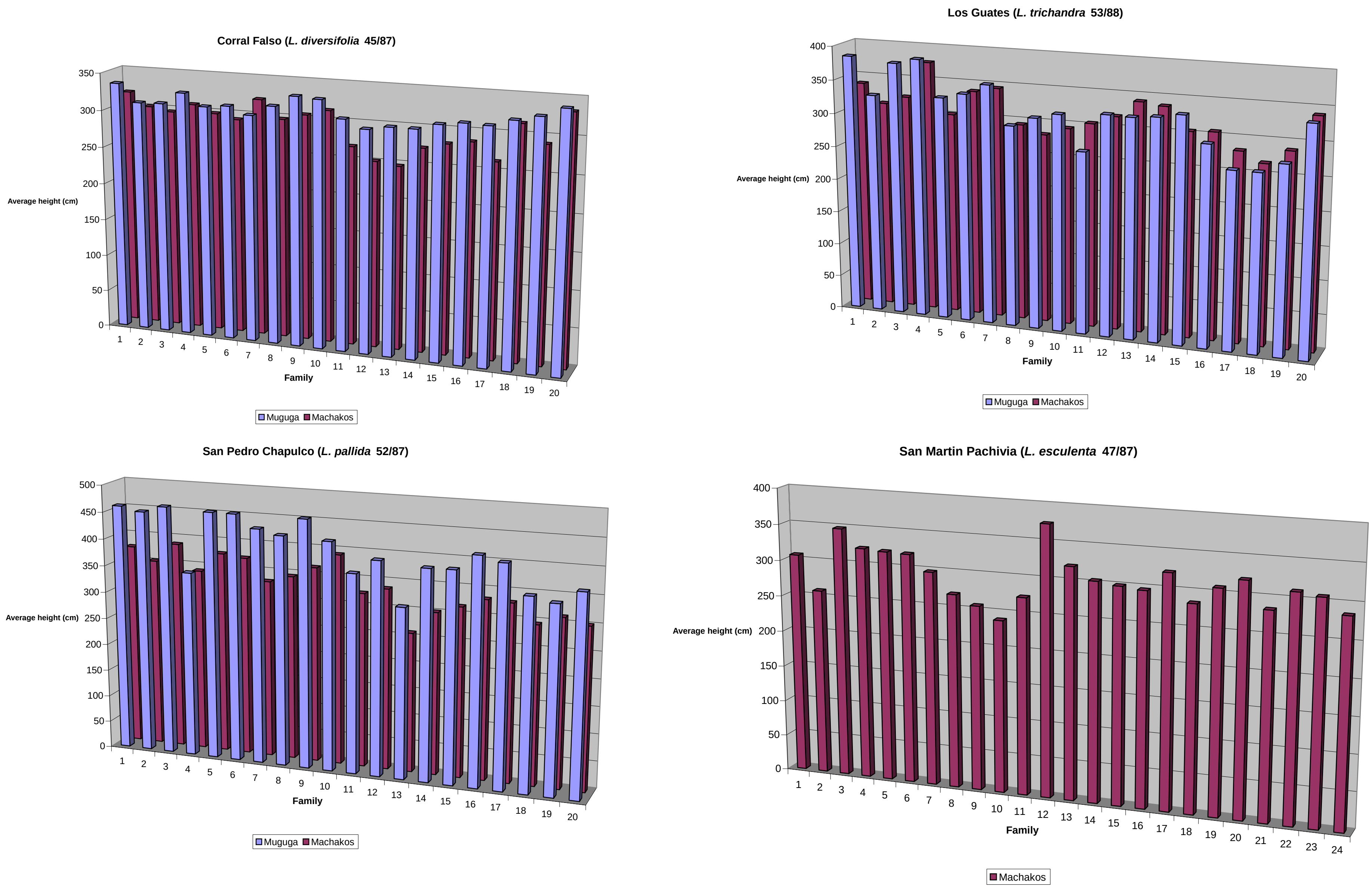

Figure 2 Summary of height data for four *Leucaena* provenances at Machakos and Muguga field stations, Kenya. All measurements 17 MAP except San Martin Pachivia (23 MAP)

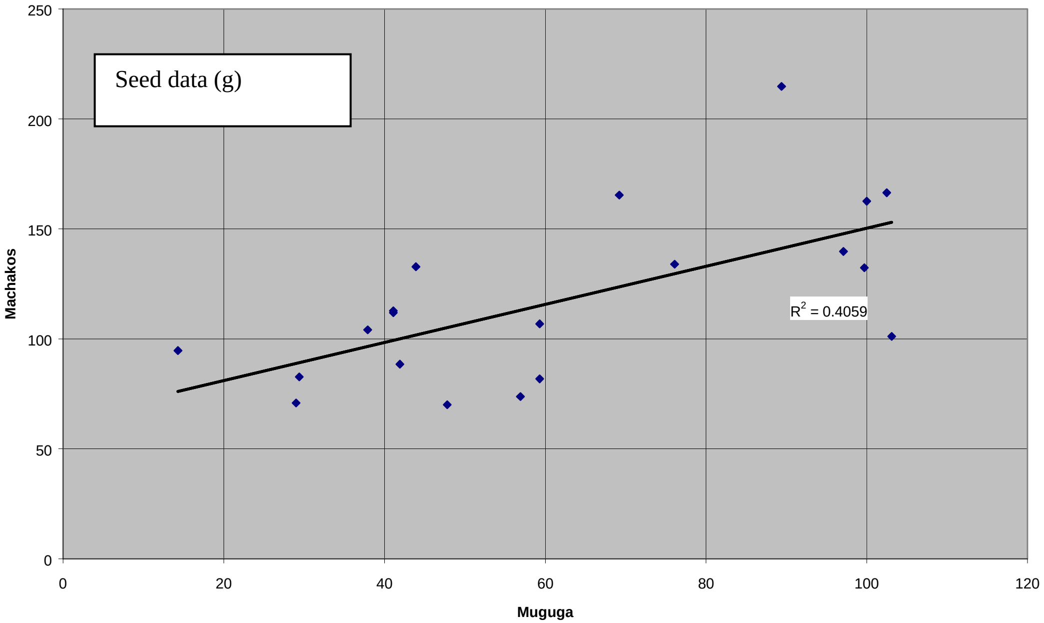

Figure 3 Comparison of seed and height data for Machakos and Muguga sites for Los Guates provenance (*L. trichandra* 53/88). Seed Table 1 and Figs. 1 and 2 for further information

Height data (cm)