Genetic Diversity of Three Size Classes of Seeds of *Eucalyptus globulus* ssp. *globulus*

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Abstract

Variation in seed size is often observed in samples of eucalypt seeds and this leads to heterogeneous populations of plants, principally through variation in the early stages of plant development. It follows that samples of seeds more uniform in size could produce more uniform populations of plants. In studies of *Eucalyptus globulus* ssp. *globulus* it was of interest to determine whether or not the genetic diversity within a population, through the use of isozyme markers, was altered in the subpopulations developed from seeds of different size classes. A commercial sample of seed was separated by seed size into three subpopulations and the percentage germination and mean fresh weight of the seedlings were determined. Proteins extracted from leaves of the seedlings were separated by electrophoresis and tested for activity of eight different enzymes. These eight enzymes showed activity at 20 loci and mean genetic diversity and fixation index were determined using 13 of these loci. The subpopulation of the smallest seeds contained a greater proportion of abnormal seeds and had a lower percentage germination and plant weight compared to the other subpopulations. No significant differences were found in the number of alleles per locus, percentage of polymorphic loci, mean heterozygosity. The major part of the endogamy, indicated by F statistic, was found within the subpopulations: $F_{(IS)}$ = 0.518; $F_{(ST)}$ = 0.010 and $F_{(IT)}$ = 0.523. We conclude that the use of seeds of uniform size will lead to more uniform germination and plant growth without alteration in overall genetic diversity.

Key words: seeds, isozyme, genetic diversity, outcrossing, *Eucalyptus globulus*.

FDC: 181.525; 232.312; 232.318; 165.5; 176.1 *Eucalyptus globulus*: (81).

Introduction

Variations in the size of seeds, commonly observed in eucalypt seed lots, cause differences in the growth of the seedlings in the nursery, even when those seeds are only from improved genetic sources are used. The production of different sizes of seedlings causes low utilization of plants for field plantations. There is a direct relation between the size of the eucalypt seeds, the percentage of germination and the initial development of the seedlings (MENDES *et al.*, 1978; GOZZO, 1963). According to BALLONI *et al.* (1978), larger seeds germinate faster, produce larger seedlings for up to 60 days of age and show a higher seedling survival rate. However the relation between this variation and the seed genetic nature is not known.

The reproductive system of *Eucalyptus* species offers a great opportunity for self-fertilization. Having hermaphrodite flowers they present a rate of self-fertilization of up to 40% (MORAN and BELL, 1983), provided that protandry does not eliminate the possibility of self-fertilization (ELDRIDGE, 1978). There is no conclusive evidence for self-incompability reactions in *Eucalyptus* species (GRIFFIN *et al.*, 1987), although they have been inferred from self-fertility observations (PRYOR, 1976). Often mixed pollination occurs as the eucalypt stigmas can get pollen from the same plant and/or from other plants through crossing. So it is possible that seeds of both parental types can coexist inside the capsules (GRIFFIN *et al.*, 1987). The specie *E. globulus* ssp. *globulus* is of great economical importance for the paper and cellulose companies for producing light wood free of lignin, besides being of excellent productivity in countries such as Portugal, Spain and Australia (ELDRIDGE *et al.*, 1993). But in Brazil its performance is not satisfactory after 3 years of age. The present study has the objective of to analyse if different seed size influence the genetic structure of a population of *E. globulus* ssp. *globulus*.

Material and Methods

Sampling and preparation of the material

The population of *E. globulus* is from a Seed Collection Area (SCA) located in Camanducaia, state of São Paulo, Brazil. The seed lot was composed of seeds collected from at least 30 trees at SCA, at 10 years-old.

Seeds were separated according to size, through a set of GRANUTEST sieves. The denomination of the subpopulations was given by the mesh size: subpopulation I (seeds between 0.84 mm and 1.19 mm), subpopulation II (seeds of 1.4 mm) and subpopulation III (seeds between 1.68 mm and 2.0 mm).

The seeds were submitted to 3 analysis in phases, from of the same seedlot. Plants of different ages were used for the electrophoresis analysis: (i) at 14 days from seeds recently collected – phase 1, (ii) at 60 days – phase 2 and (iii) at 14 days from seeds that had been stored at cold temperature without humidity for about 12 months – phase 3.

The seeds were germinated in Gerbox plastic boxes on filter paper and incubated in a seed germinator at 26 °C. After germination the normal plants and the abnormal plants were counted and weighed. For the variation analysis there was a transformation of the germination data through the $\sqrt{x}$ formula.

In phase II the seedlings were produced following regular nursery procedures. Under these conditions plants grew in half-shade, being submitted to daily irrigation and weekly soil fertilization with nitrogen compounds. At about 30 days after sowing the seedlings were thinned and only one plant per vase was kept.

Preparation of extracts

For each sample, cotyledonary leaves (phases 1 and 3) or parts of the foliar tissue (phase 2) were ground in the extraction solution described by NAMKOONG (s/d), and cited by ALFENAS *et al.* (1991). Fragments of WHATMAN paper of 3 mm were moistened with the extracts and frozen in small Eppendorf tubes in a freezer at −80 °C, for approximately 15 days.

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Table 1. – Enzymes, gel buffer systems and references for staining procedures.

Enzyme	EC number	Gel buffer	Reference
Aspartate aminotransferase (AAT)	EC2.6.1.1	LB	Cheliak and Pitel (1984)
Acid phosphatase (ACP)	EC3.1.3.2	TC	Cheliak and Pitel (1984)
Catalase (CAT)	EC1.11.1.6	TC	Cheliak and Pitel (1984)
α-Esterase (α-EST)	EC3.1.1.1	TC	Stuber *et al.* (1983)
Isocitrate dehydrogenase (IDH)	EC1.1.1.42	MC	Soltis *et al.*(1983)
Leucine aminopeptidase (LAP)	EC3.4.11.1	MC	Soltis *et al.*(1983)
Malate dehydrogenase (MDH)	EC1.1.1.37	MC	Soltis *et al.*(1983)
Shikimate dehydrogenase (SKDH)	EC1.1.1.25	TC	Soltis *et al.*(1983)

Gel buffer systems used were: MC – gel, 1 in 20 diluition of tray buffer, pH 6.1; tray, 0.04M citric acid, ajusted to pH 6.1 with N-(3-aminopropyl) morpholine (CLAYTON and TRETIAK, 1972); TC – gel, 1 in 35 dilution of tray buffer, pH 7.5; tray, 0.223 M tris, 0.086 M citric acid, adjusted to pH 7.5 with 10 N tris (SOLTIS *et al.*, 1983); LB – gel, 90% 0.05 M tris and 0.007 M citric acid, pH 8.3 and 10% tray buffer; tray, 0.19 M boric acid, 0.04 M lithium hydroxide, pH 8.3 (JARRET and LITZ, 1986).

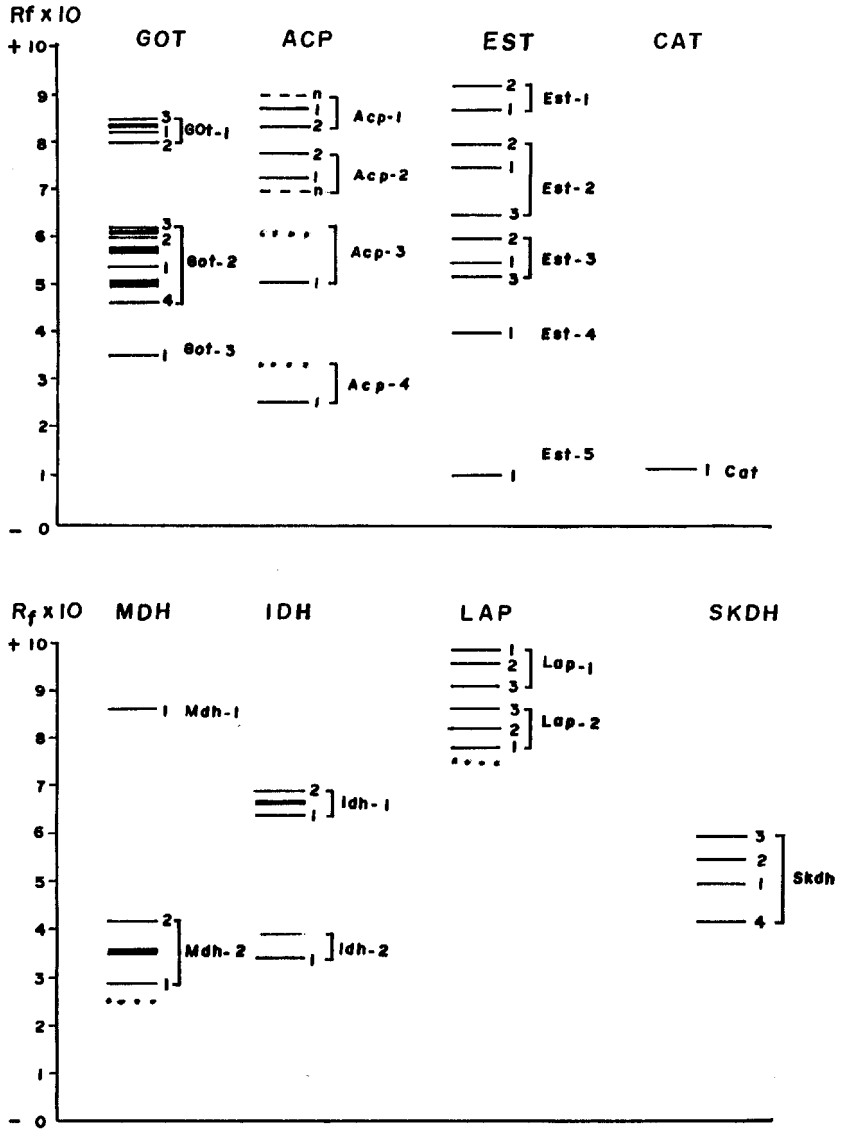

Figure 1. – Diagram of the isozyme variation of the *Eucalyptus globulus*. The isozyme ACP, EST, LAP and SKDH are monomeric, and isozyme MDH, GOT and IDH are dimeric.

Table 2. – Allelic frequencies, observed heterozygosity and sample size of three subpopulation (I, II and III) of *E. globulus* in different phases.

		Subpopulation								
		------- Phase 1 --------			--------- Phase 2 --------			--------- Phase 3 --------		
Locus	Allele	I	II	III	I	II	III	I	II	III
Acp-1	1	0.621	0.722	0.408				0.780	0.683	0.708
	2	0.097	0.111	0.338				0.122	0.190	0.185
	'n'	0.282	0.167	0.254				0.098	0.127	0.107
	(Ho)	0.274	0.127	0.231				0.146	0.206	0.185
	(N)	62	63	65	54	58	34	82	63	65
Acp-2	1	0.680	0.578	0.477				0.487	0.598	0.609
	2	0.221	0.367	0.377				0.382	0.311	0.328
	'n'	0.098	0.055	0.146				0.132	0.090	0.063
	(Ho)	0.164	0.188	0.338				0.197	0.098	0.281
	(N)	61	64	65	60	60	60	76	61	64
Acp-3	1	1.000	1.000	1.000				1.000	1.000	1.000
	(N)	64	62	62	61	69	42	84	62	67
Acp-4	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	(N)	64	63	66	61	69	42	84	62	67
Cat	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	(N)	62	62	69	66	71	67	63	84	84
Est-1	1	0.861	0.850	0.915	0.250	0.462	0.538	0.741	0.864	0.877
	2	0.139	0.150	0.085	0.750	0.538	0.462	0.259	0.136	0.123
	(Ho)	0.093	0.033	0	0.328	0.338	0.258	0.232	0.091	0.140
	(N)	54	60	59	58	65	66	56	55	57
Est-2	1	0.875	0.875	0.868	0.992	0.817	0.695	0.975	0.900	0.778
	2	0.092	0.125	0.132	0	0.161	0.258	0.025	0.100	0.222
	3	0.033	0	0	0.008	0.022	0.047	0	0	0
	(Ho)	0.150	0.050	0.118	0.017	0.156	0.328	0.049	0.133	0.095
	(N)	60	60	68	59	90	64	61	60	63
Est-3	1	0.992	0.937	0.963	0.922	0.764	0.806	0.798	0.788	0.857
	2	0.008	0.032	0.022	0.070	0.224	0.119	0.121	0.161	0.095
	3	0	0.032	0.015	0.008	0.011	0.075	0.081	0.051	0.048
	(Ho)	0.016	0	0.015	0.063	0.202	0.119	0.016	0.017	0
	(S)	64	63	67	64	89	67	62	59	63
Est-4	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	(N)	64	63	68	66	71	67	52	64	38
Est-5	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	(N)	41	59	65	66	71	67	40	47	42
Got-1	1	0.692	0.808	0.775				0.672	0.602	0.654
	2	0.051	0.038	0.008						
	3	0.256	0.154	0.217				0.328	0.398	0.346
	(Ho)	0	0.038	0.017				0.069	0.020	0.026
	(N)	39	52	60	35	75	58	58	49	39
Got-2	1	0.310	0.255	0.327	0.157	0.193	0.276	0.381	0.414	0.381
	2	0.371	0.355	0.227	0.443	0.450	0.422	0.056	0.009	0.025
	3	0.121	0.309	0.364	0.371	0.250	0.233	0.397	0.440	0.508
	4	0.198	0.082	0.082	0.029	0.107	0.069	0.167	0.138	0.085
	(Ho)	0.190	0.291	0.255	0.229	0.257	0.310	0.556	0.500	0.458
	(N)	58	55	55	35	70	58	63	58	59
Got-3	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	(N)	60	60	62	66	75	58	62	61	61

(*Table 2* continued)

Locus	Allele	Subpopulation								
		Phase 1			Phase 2			Phase 3		
		I	II	III	I	II	III	I	II	III
Idh-1	1	0.469	0.405	0.180	0.326	0.331	0.346	0.413	0.441	0.465
	2	0.531	0.595	0.820	0.674	0.669	0.674	0.587	0.559	0.535
	(Ho)	0.041	0.016	0.016	0.042	0.017	0	0.127	0.118	0.097
	(N)	49	63	64	72	59	59	63	76	72
Idh-2	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	(N)	63	63	68	83	60	65	63	83	84
Lap-1	1	1.000	1.000	1.000	0.933	0.905	0.960	0.669	0.855	0.805
	2				0.067	0.095	0.032	0.290	0.145	0.169
	3						0.008	0.040		0.025
	(Ho)	0	0	0	0.133	0.190	0.079	0.242	0.194	0.186
	(N)	59	53	68	45	58	63	62	62	59
Lap-2	1	0.462	0.441	0.425	0.929	0.825	0.754	0.452	0.468	0.426
	2	0.019	0.017	0.022	0.018	0.067	0.098	0.008	0.040	0.041
	3	0.519	0.542	0.552	0.054	0.108	0.148	0.540	0.492	0.533
	(Ho)	0.923	0.881	0.896	0.107	0.200	0.246	0.887	0.937	0.852
	(N)	52	59	67	56	60	61	62	63	61
Mdh-1	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	(N)	63	62	69	65	61	65	63	84	84
Mdh-2	1	0.742	0.790	0.819	0.776	0.825	0.831	0.778	0.747	0.839
	2	0.258	0.210	0.181	0.224	0.158	0.169	0.222	0.253	0.161
	(Ho)	0.419	0.290	0.362	0.328	0.333	0.277	0.317	0.289	0.250
	(N)	62	62	69	67	60	65	63	83	84
Skdh	1	0.886	0.764	0.760	0.907	0.855	0.910	0.732	0.635	0.779
	2	0.114	0.236	0.240	0.093	0.100	0.090	0.146	0.311	0.163
	3					0.045		0.098	0.027	0.058
	4						0.024	0.027		
	(Ho)	0.053	0.019	0.058	0.047	0.091	0.020	0.244	0.081	0.058
	(N)	57	53	52	43	55	50	41	37	52

Blank spaces indicate zero values.

Electrophoresis

In each phase about 180 plants (60 per subpopulation) were analyzed, totalling 540 individuals.

In the separation of the isoenzymes the technique of horizontal electrophoresis in corn starch gel (penetrose 13%) was used. Eight enzymatic systems were used: Aspartate aminotransferase (ATT), Acid phosphatase (ACP), Catalase (CAT), α-Esterase (α-EST), Isocitrate dehydrogenase (IDH), Leucine aminopeptidase (LAP), Malate dehydrogenase (MDH), Shikimate dehydrogenase (SKDH). *Table 1* presents a summary of the experimental conditions adopted in this present study.

The genetic control of the isoenzymes was inferred from a comparison of the phenotypes in different phases of the development and through studies of various species of *Eucalyptus* (BROWN *et al.*, 1975; MORAN and BELL, 1983; MARTINS-CORDER *et al.*, 1996; MARTINS-CORDER and LOPES, 1997). For each enzymatic system the most anodic isoenzyme was designated locus 1, the next locus 2 and so on. In the same manner the most rapid band at each locus was denominated allele 1 and in order of decreasing mobility the other bands were called 2, 3, etc.

Data analysis

The analysis was performed with the use of a computer software (BIOSYS-1)[4]) developed by SWOFFORD and SELLANDER (1989). It was made for each of the three phases separately, for

[4]) From the zymograms the allelic frequencies were calculated for each locus to estimate the heterozygosity per locus ($h_1 = 1-\sum x_i^2$; where x_i^2 is the allele frequency) and the average heterozygosity [$H = (\sum h_1/r$; where h_1 is the heterozygosity per locus, and r is the number of locus analysed)], fixation index [$F = 1-(Ho/He)$; where $Ho = \sum h_{ij}$ and $He = \sum 2p_ip_j$], F statistic {$F_{IT} = F_{ST} + (1-F_{ST})$. F_{IS}}; and the genetic distance ($D = -n\ I$; $I = J_{xy}/\sqrt{J_x J_y}$, in that J_{xy}, J_x and J_y are arithmetic means of alleles j_x , j_y and j_{xy}). The values of genetic distance were used to generate dendrograms using the unweighted pair-group method with arithmetic averaging (UPGMA) as described by SNEATH and SOKAL (1973). Other calculations were made to estimate the percentage of polymorphic loci (P = number of polymorphic loci/total number of loci) and the average number of alleles per loci (Ap = total number of alleles per polymorphic loci/number of polymorphic loci).

pairs (phases 1 and 2, phases 1 and 3) and for the three phases together.

The observed allelic frequencies for the enzyme systems were subjected to statistical analysis to compute various within-phase variability measures such as mean number of alleles per locus, percentage of polymorphic loci, observed and expected levels of heterozygosity (BROWN and WEIR, 1983). Fixation index and F statistic (WRIGHT, 1965); and genetic distance (NEI, 1978) were subjected to statistical analysis to compute between-phases analysis.

Results and Discussion

The gel patterns found in the various enzymatic systems are shown in *figures 1*. For most of the systems analyzed the patterns obtained from 14 day old plants were the same as those from 60 day plants. This probably occurred because the tissues analyzed were the same in all 3 phases. However there was a lack of Acp-2, Got-1 and α-Est-5 in the plants of 60 days, which may have been a consequence of differential gene action (SCANDALIOS, 1974) related to the different ages of the tissues. Reproducible profiles of ACP activity were not obtained from phase 2 plants. The cause(s) of this anomaly have not yet been determined.

Isoenzymatic variability

For the estimates of genetic variation of the three subpopulations following the frequencies were used: (i) 44 alleles in 20 loci related to phases 1 and 3, (ii) 31 alleles in 15 loci related to phase 2. *Table 2* shows the allelic frequencies of the three subpopulations.

The genetic variation within the sub-populations is shown in *table 3*. Subpopulations I, II and III in phase 3 (plants from stored seed) showed slight increases in the values of the genetic estimatives when compared with the subpopulations in phase 1. When phases 1 and 2 were compared, using 15 loci, the heterozygosity of subpopulation I of phase 2 (Ho = 0.086) was less than that of the same subpopulation in phase 1 (Ho = 0.126). In the combined analysis – 3 phases together – the subpopulations maintained constant values of genetic diversity (*Table 4*).

For populations in HARDY-WEINBERG equilibrium the fixation index should be zero. Values above zero indicate the occurrence

Table 3. – Means of number of alleles per locus (Ap), percentage of polymorphic loci (P%) (criterion 0.95), mean observed heterozygosity (Ho) and mean expected heterozygosity (He) in subpopulations (I, II and III) of *E. globulus* in different phases.

Subpopulation		N	Ap	P%	Ho	He
	I	57.9 (1.7)	1.9 (0.2)	50.0	0.116 (0.05)	0.214 (0.06)
Phase 1	II	60.0 (0.9)	1.9 (0.2)	55.0	0.097 (0.05)	0.216 (0.05)
	III	64.4 (1.1)	2.0 (0.2)	50.0	0.117 (0.05)	0.216 (0.06)
	NL_1*	20				
	I	64.6 (2.3)	2.0 (0.2)	55.0	0.154 (0.05)	0.256 (0.06)
Phase 3	II	63.7 (2.9)	2.0 (0.2)	60.0	0.134 (0.05)	0.254 (0.05)
	III	63.3 (3.1)	2.0 (0.2)	60.0	0.131 (0.05)	0.243 (0.05)
	NL_3	20				
	I	59.4 (1.2)	1.8 (0.2)	46.7	0.126 (0.06)	0.188 (0.06)
Phase 1	II	60.1 (0.9)	1.8 (0.2)	53.3	0.105 (0.06)	0.201 (0.06)
	III	64.8 (1.4)	1.8 (0.2)	46.7	0.115 (0.06)	0.177 (0.06)
	NL_1**	15				
	I	58.3 (3.5)	1.9 (0.2)	53.3	0.086 (0.03)	0.161 (0.05)
Phase 2	II	67.5 (2.8)	2.0 (0.3)	60.0	0.119 (0.03)	0.233 (0.06)
	III	61.7 (1.8)	2.0 (0.3)	53.3	0.109 (0.03)	0.225 (0.06)
	NL_2	15				

N is mean number of sample.
NL1, NL2 and NL3 is number of loci analyzed in each phase.
Values in brackets are standard deviations.

Table 4. – Means of number of alleles per locus (Ap), percentage of polymorphic loci (P%, criterion 0.95), mean observed heterozygosity (Ho) and mean expected heterozygosity (He) in three subpopulations (I, II and III) of *E. globulus*.

Subpopulation	(N)	(Ap)	(P%)	(Ho)	(He)
I	170.4 (6.3)	2.2 (0.2)	60.0	0.123 (0.039)	0.241 (0.054)
II	176.1 (9.2)	2.1 (0.2)	60.0	0.112 (0.037)	0.252 (0.053)
III	173.9 (8.4)	2.2 (0.2)	60.0	0.120 (0.039)	0.247 (0.054)

Standard deviations are enclosed in brackets.
Pool analysis of the three phases.

Table 5. – Indicies of fixation (F) in three subpopulations (I, II and III) of *E. globulus* in different phases.

Locus	Subpopulation								
	Phase 1			Phase 2			Phase 3		
	I	II	III	I	II	III	I	II	III
Acp-1	0,478	0,710	0,648	--	--	--	0,601	0.572	0,593
Acp-2	0,657	0,645	0,444	--	--	--	0,671	0,817	0,456
Acp-4	0	0	-0,015	0	0	0	0	0	
Est-1	0,613	0,869	-0,015	0,126	0,319	0,482	0,395	0,614	0,349
Est-2	0,333	0,771	1,000	-0,009	0,493	0,267	-0,025	0,259	0,724
Est-3	-0,008	1,000	0,488	0,569	0,447	0,639	0,953	0,952	1,000
Got-1	1,000	0,881	0,953	--	--	--	0,843	0,957	0,943
Got-2	0,734	0,589	0,638	0,643	0,625	0,548	0,167	0,189	0,222
Idh-1	0,918	0,967	0,947	0,905	0,962	1,000	0,738	0,760	0,805
Lap-1	0	0	0	-0,071	-0,105	-0,034	0,481	0,220	0,422
Lap-2	-0,785	- 0,724	-0,744	0,204	0,340	0,385	-0,760	-0,744	-0,600
Mdh-2	-0,095	0,124	-0,244	0,055	-0,212	0,015	0,082	0,235	0,073
Skdh	0,740	0,948	0,842	0,724	0,647	0,878	0,437	0,837	0,841
Mean	0,417	0,616	0,481	0,350	0,391	0,464	0,382	0,472	0,490
Means of the phases		**0.448**			**0.505**			**0.401**	

Means for subpopulacion	**I**	**0.383**
	II	**0.493**
	III	**0.478**

of endogamy and values below zero represent a selection in favor of heterozygotes (BROWN *et al.*, 1975). Subpopulation I showed lower values of fixation index than subpopulations II and III, and in phase 2 there was a decrease in the rate in all subpopulations (*Table 5*). In general the subpopulations of *E. globulus* presented high rates of self-fertilization among the recently collected seeds, stored seeds and in the seedlings of the nursery. However the subpopulations I presented smaller rates of endogamy. It is probably true that the reduced fixation index of subpopulation I can be explained through the elimination of the self-fertilized individuals shown by the low percentage of germination and by the higher number of abnormal plants in this class. Among the losses individuals are included with high genetic load which originated from the more drastic form of depression of endogamy that is self-fertilization (FALCONER, 1987). The results obtained from the seed germination rate and plant weight of *E. globulus* confirm this and will be discussed later. However there was no evidence for selection in the nursery against self-fertilized individuals had not that been eliminated through natural selection. Eucalypts are autocompatible hermaphrodite plants that show different levels of self-fertilization and of depression by endogamy (ELDRIDGE and GRIFFIN, 1983). Small variations on the endogamy level can drastically change the performance of individuals, families and populations (POTTS *et al.*, 1995). These authors related that the individuals and the population can react differently to endogamy due to the casual variations in the genetic load of the recessive alleles. Positive fixation indexes are frequently observed in the seeds and young plants due to self-fertilization, but rarely in the adult phase (MITTON, 1989). HARDNER and POTTS (1995) noticed that self-pollination reduced seed set severely and the growth of plants of *E. globulus* in the field. These authors noticed that the levels of depression due to endogamy for growth and progeny survival in open pollination, were intermediate between controlled self-pollination and controlled crossing, and that there was a strong tendency for the depressive effects of the endogamy to increase with plant age. ELDRIDGE and GRIFFIN (1983) observed that in *E. regnans* endogamy depresse plant mortality significantly up to 5 years of age. For *E. gunnii*, POTTS *et al.* (1987) related an even lower survival rate in progenies from controlled self-pollination than in those from crossed pollination, in nursery and field conditions one year after the sowing. VOLKER *et al.* (1994) detected high self-pollination levels in a test of progenies from open pollination of *E. globulus*.

Table 6. – Means of the F statistic for the subpopulations of *E. globulus*.

Phase	F_{IS}	F_{ST}	F_{IT}
1	0,485	0,026	0,498
2	0,480	0,027	0,495
3	0,438	0,012	0,444
1 and 2	0,434	0,090	0,485
1 and 3	0,460	0,037	0,479
1, 2 and 3	0,518	0,010	0,523

Fixation index within (F_{IS}), between (F_{ST}) and total (F_{IT}), according WRIGHT (1965).

The results obtained in this study suggest that the elimination of endogamic individuals was more drastic in the initial phases of development, mainly in subpopulation I. However the viable seeds could have originated both from cross-pollination and from self-pollination, probably with different endogamy levels. It is possible that the depressive effects of endogamy are more significant after planting in the field.

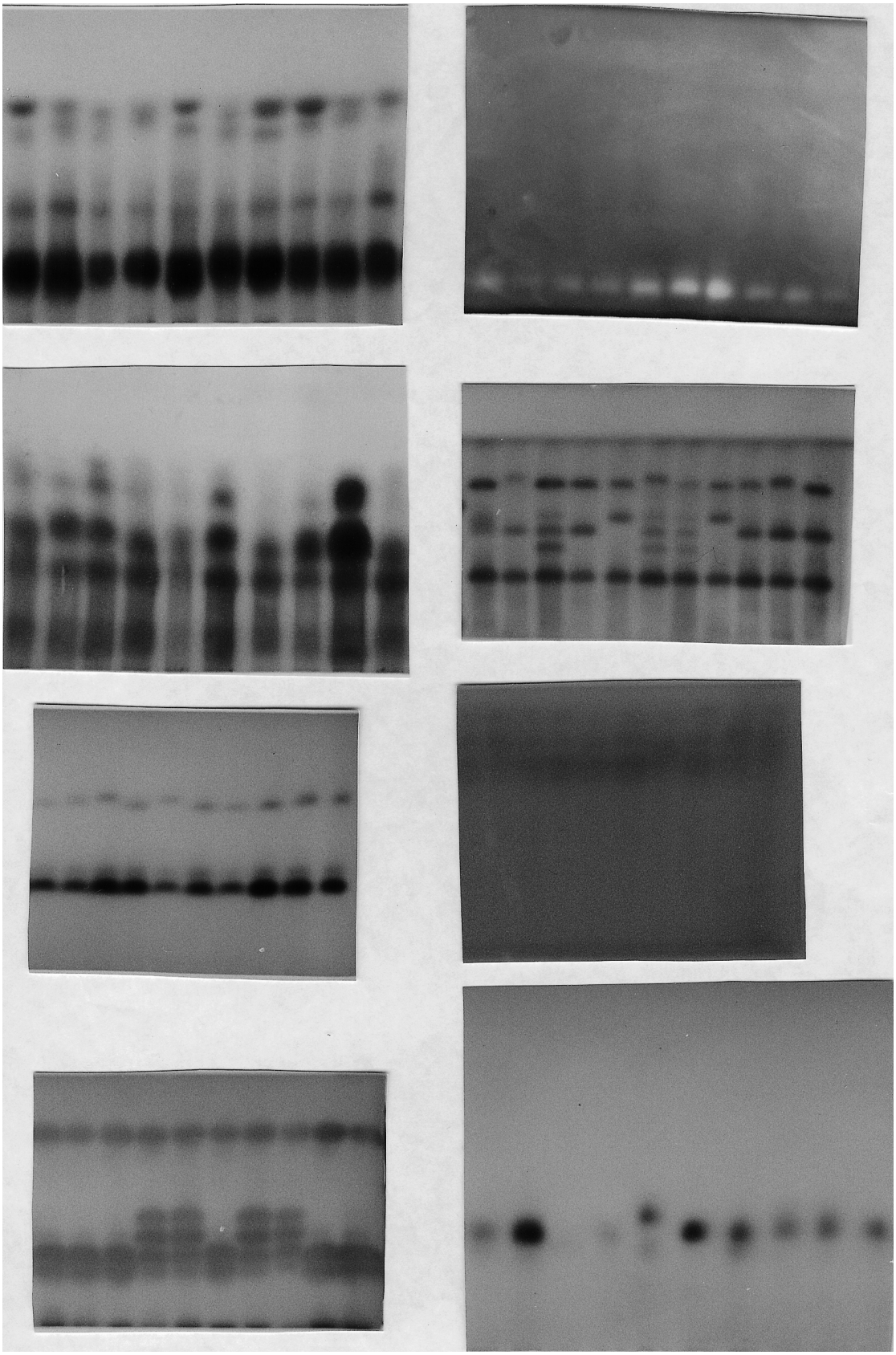

Figure 2. – Dendrogram based no UPGMA clustering of *Eucalyptus globulus* subpopulation using the genetic distances of NEI (1978).

The F statistics (WRIGHT, 1965) presented in *table 6* show that the major part of total endogamy is due to fixation within the subpopulations that presented higher values.

The degree of differentiation among the subpopulations was low, although there were changes in the genetic composition of the populations due to the differences in the allelic frequencies and the presence of a greater number of alleles at specific loci. Cluster analysis using NEI's unbiased genetic distance (*Figure 3*) revealed that the distances between populations were very small. In phase 1, the subpopulations I and II were more related, in phase 2 subpopulations I and II were more related and in phase 3 the genetic distance of the subpopulations was close to zero.

Germination analysis and plant weight

Seed germination, average plant weight and number of abnormal plants of subpopulations I, II and III were shown in *table 7*.

Subpopulation I differs statistically from both populations II and III, in terms of percentage of germination, so forming two distinct groups. For the average weight of the plants all 3 subpopulations were statistically distinct. The number of abnormal plants was greatest in subpopulation I; possibly due to a greater number of self-fertilized seeds in this class. According to HARDNER and POTTS (1995) self-pollination in *E. globulus* appears to result in fewer fertilized ovules and in an increase in the rate of embryo abortion. The effects of self-pollination in eucalypts cause a reduction of seed set (POTTS and SAVVA, 1988; SEDGLEY and SMITH, 1989; SEDGLEY *et al.*, 1989), an decrease in the germination percentage (ELDRIDGE and GRIFFIN, 1983), an increase in the frequency of abnormal individuals (HODGSON, 1976; POTTS *et al.*, 1987), a reduction in the growth and vigour of the plants in field conditions (ELDRIDGE and GRIFFIN, 1983; POTTS et al., 1987; GRIFFIN and COTTERILL, 1988) and a reduction of seedling survival both in nursery and in field conditions (ELDRIDGE and GRIFFIN, 1983; POTTS *et al.*, 1987).

A high positive correlation (r = 0.83) was found between seed germination and the average weight of the plants. The medium and large sized seeds presented a higher percentage of germination than the smaller ones and also produced bigger and stronger seedlings. In nursery conditions the differences were

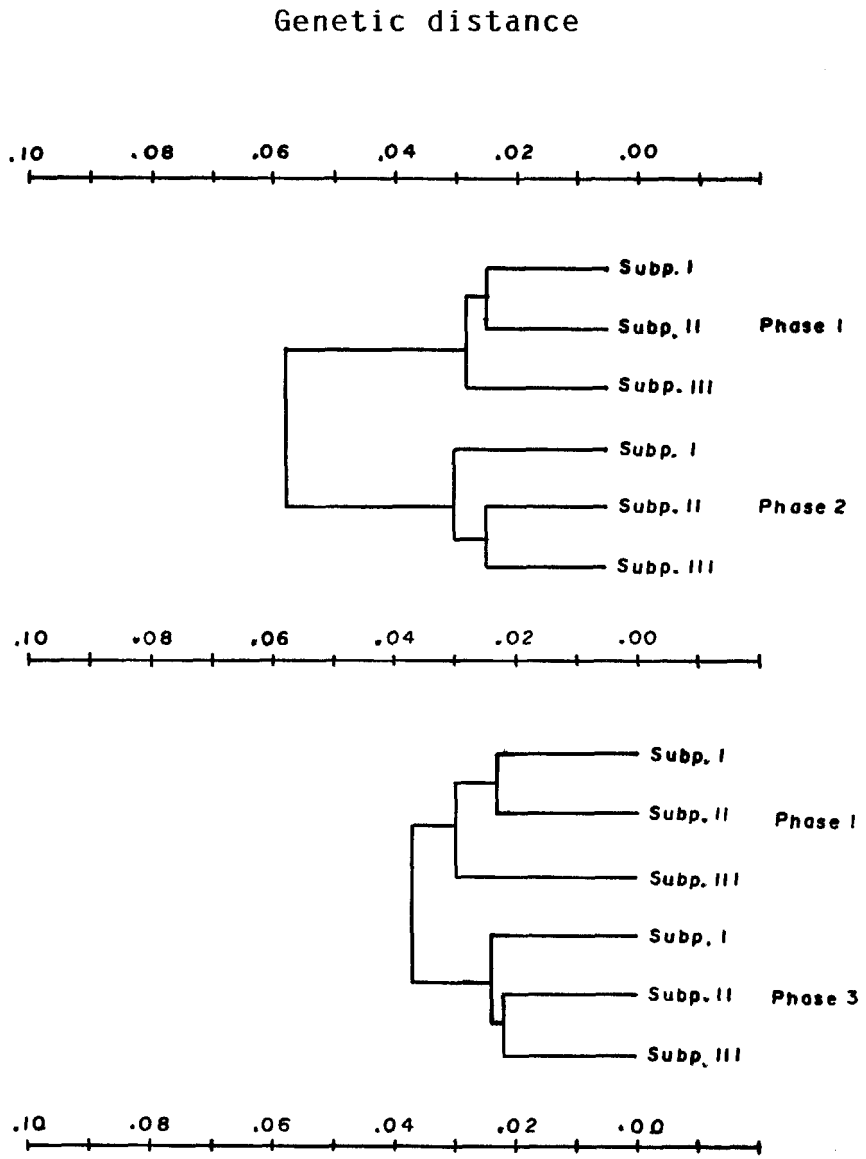

Figure 3. – A dendrogram based on UPGMA clustering of *E. globulus* subpopulations using the genetic distances of NEI (1978).

Table 7. – Test of TUKEY for mean values of seed germination transformed to $\sqrt{x}$, mean weight of seedlings and number of abnormal plants in each of the three subpopulations of *E. globulus*.

Subpopulation	Germination[1)] ($\sqrt{x}$)	Weight[1)] (mg)	Number of abnormal plants
III	0,9136 A	0,02845 A	8
II	0,8624 A	0,02041 B	18
I	0,7236 B	0,01219 C	33
CV (%)	6,7	12,2	

[1]) Determined after 14 days (8 replicates).

notable. Similar results have been reported for other species of *Eucalyptus* by GOZZO (1963), CANDIDO (1970) and MENDES *et al.* (1978).

Conclusions

In practical terms the use in nurseries of seeds that were chosen by size is beneficial, due to the differences in development that the different classes present. Through the use of uniform seeds it is possible to obtain more uniform germination and a lower variation in the plots without changing the genetic diversity. As a consequence the selection of seedlings for field sowing will be more efficient.

In general the population of *E. globulus* studied presented a high rate of endogamy, which probably has accumulated with time. In the field the population presented an unsatisfactory behavior in terms of growth and form. The narrowing of the genetic base in this population could be the factor responsible for the low levels of heterozygozity and for the increase in the levels of self-fertilization.

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