GOT MACROGAMETOPHYTE ISOENZYMES OF VIRGINIA PINE 1/

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Abstract.--Analysis of populations of macrogametophytes from Pinus virginiana L. demonstrated genetic control at two loci for glutamate oxalo-acetate transaminase (GOT) isoenzymes. A comparison among four populations illustrated that natural stands and seed orchard progeny are not significantly different at the GOT locus A either in allelic composition or percentage of individuals heterozygous at the A locus.

The technique of protein electrophoresis permits the separation of multiple molecular forms of enzymes (Markert and Moller, 1959). Since different isoenzymes arise from different genes (Scandalios, 1974; Feret and Bergmann, 1976), isoenzyme analysis may be used to define genetic markers for studies relating to forest tree improvement and genetics.

In the Pinaceae, the genetics of isoenzyme inheritance may be investigated without extensive or intensive breeding programs. The nutritive tissue of pine seeds (female gametophyte tissue) originates from a single megaspore (Ferguson, 1904). The megaspore is the last remaining spore resulting from meiotic division of a megaspore mother-cell, the three other spores presumably disintegrating at random. Thus, analysis of female gametophyte isoenzyme variation among seeds collected from a single tree (genotype) will demonstrate segregation ratios if the tree is heterozygous. By analysis of a series of genotypes the inheritance mechanisms of isoenzyme phenotypes may be elucidated and genetic markers defined.

Reports may be found in the literature describing, for example, the use of isoenzyme genetic markers in studies relating to provenance variation (Bergmann, 1975; Rudin et al., 1974; Tigerstedt, 1973), seed lot and clone identification (Bergmann, 1972; Miyazaki and Sakai, 1969), and developmental genetics (Conkle, 1971; see also Conkle, this proceeding). Published reports relating an applied use of the technique for the solution of problems encountered in seed orchards and genetic management of seed orchard gene pools are scarce, if in existence at all. Thus, current studies by personnel of the U. S. Forest Service and State Universities are of extreme interest (Personal communication, R. Wier, North Carolina State Cooperative).

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If inventories of isoenzyme genetic markers can be developed for the major southern pine species, it will be possible to accomplish the following:

1. Determine the amount of heterozygosity in seed orchards, seed orchard progeny, and natural stands.

2. Determine if gamete fertilization in seed orchards is random among clones.

3. Determine the percent of seed orchard progeny arising from selfing.

4. Identify clones or progeny for patent purposes, registration for seed certification, etc.

5. Monitor changes in gene frequencies as breeding programs move toward second and third generation populations to ensure maintenance of a proper "genetic base."

6. Provide quantitative measures of the effectiveness of "pollen management" procedures such as misting, etc.

7. Investigate the relationships between ubiquitous gene markers and economically important tree growth parameters.

Presented here are the results of an analysis of glutamate oxalo-acetate transaminase (GOT) isoenzymes in four populations of Virginia pine (Pinus virginiana L.). The analysis is presented as an example of how genetic markers can be used to compare gene and genotypic frequencies in domesticated and natural populations of Virginia pine.

MATERIALS AND METHODS

Virginia pine seeds were collected from four populations: the Virginia Division of Forestry seed orchard at Appomattox, Virginia, two natural stands, one near Critz, Virginia and the other at Blacksburg, Virginia, and a planted stand near Critz, Virginia, originating from seedlings obtained in 1971 as nursery-run stock from the Virginia Division of Forestry Augusta nursery. Following extraction from cones by air drying, seeds were stored at 4°C until used.

Female gametophyte tissue was extracted in 0.1 M, pH 8.0 Tris-HCl (.10 ml/seed), homogenized with a glass rod and centrifuged at 3500 XG for 10 minutes. Following centrifugation the supernatent was removed with a micropipette and layered into the electrophoretic apparatus.

Electrophoresis followed the methods of Davis (1964). Enzyme electrophoresis separations were made in 7.5% polyacrylamide gel slabs (.75 mm x 100 mm x 140 mm). Electrophoresis was conducted at 50 V and 7.5 ma for 100 minutes followed by 125 V and 12.5 ma for 130 minutes using a 0.09 M tris-glycine pH 8.9 reservoir buffer and a .2 M Tris-HCI pH 8.2 gel buffer. GOT isoenzymes were stained according to the procedures described by Schwartz, et al. (1963).

RESULTS

GOT Genetics

Using the techniques described above, eight GOT isoenzymes could be identified with a high degree of reliability (Fig. 1). Of the eight, those labeled $A3_1$ and $A3_2$ could not be reliably distinguished in some seed populations, thus A31 and $A3_2$ frequencies were combined and given the A3 designation.

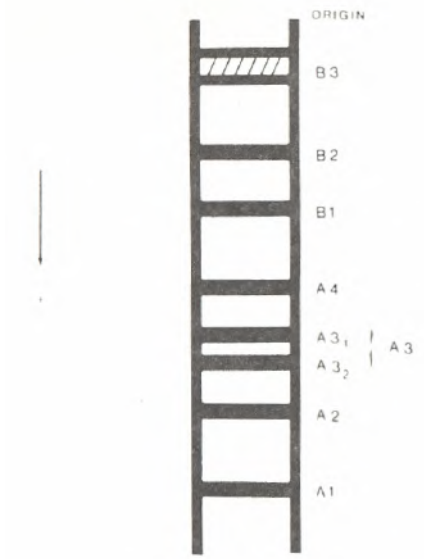

Figure 1.--Composite representation of the GOT isoenzymes in macrogametophyte tissue

Based upon the segregation patterns exhibited by seed from individual trees, there were found to be two loci: A and B. Locus A had four alleles (A1-A4, Fig. 1) and locus B, three alleles (B1-B3, Fig. 1). Chi square analyses of segregation ratios are presented in Table 1. Alleles A2 and A4 occurred in the samples analyzed here in intermediate frequencies, A3 less commonly, while A1 was found in one seed orchard tree. The B locus was essentially fixed for the B1 allele, except for one tree in the planted stand and one tree in the Critz natural stand. These two trees were heterozygous for B1/B2 and B1/B3 respectively.

Two trees in the sampled populations were heterozygous at both locus A and B; hence it was possible to test for linkage. The results of a Chi square test for linkage illustrated that linkage is not present (P = 0.05).

Table 1.--GOT segregation ratios for locus A and B derived from analysis of female gametophyte populations

Postulated seed tree genotype	No. trees analyzed	Sample size	Segregation ratio	χ^2	P
A2/A4	12	344	OBS: 169:175 EXP: 172:172	0.10	0.7-0.8
A2/A3	4	82	OBS: 46:36 EXP: 41:41	1.22	0.2-0.3
A1/A2	1	20	OBS: 10:10 EXP: 10:10	0.00	> 0.9
A3/A4	6	140	OBS: 73:67 EXP: 70:70	0.26	0.5-0.7
B1/B2	1	53	OBS: 25:28 EXP: 26.5:26.5	0.17	0.5-0.7
B1/B3	1	100	OBS: 52:48 EXP: 50:50	0.16	0.5-0.7

Population Comparisons

GOT allelic frequencies are listed in Table 2 by population. With the exception of the Blacksburg natural stand where sample size was a limiting factor, it can be observed that each population possessed a unique and relatively rare allele. A1 was unique to the seed orchard while B2 and B3 were unique to the Critz planted and natural stands, respectively. For the other allelic frequencies the four populations were not significantly different according to the arcsin transformation t test of Sokal and Rohlf (1961). Genotypic frequencies (Table 3) show a similar amount of homogeneity among the four populations analyzed. The only statistically significant differences were the frequency of A4/A4 genotypes in the Virginia Division of Forestry seed orchard compared with the Critz planted stand and the frequency of A2/A4 seed orchard genotypes compared with both Critz stands.

Table 2.--Frequency of the GOT alleles in four populations of Virginia pine.

Population	N[a/]	Locus A				Locus B		
		A1	A2	A3	A4	B1	B2	B3
Critz natural stand	20	0.00	0.50	0.02	0.48	0.98	0.00	0.02
Blacksburg natural stand	7	0.00	0.50	0.14	0.36	1.00	0.00	0.00
Critz planted stand	28	0.00	0.50	0.11	0.39	0.98	0.02	0.00
VDF seed orchard	25	0.02	0.34	0.06	0.58	1.00	0.00	0.00

[a/] Number of trees sampled

Table 3.--GOT genotype frequencies in four populations of Virginia pine

Population	N[a/]	Genotype									
		A2/A2	A3/A3	A4/A4	A2/A4	A2/A3	A3/A2	A1/A2	B1/B1	B1/B2	B1/B3
Critz natural stand	20	.20	.00	.15	.60	.00	.05	.00	.95	.00	.05
Blacksburg natural stand	7	.14	.00	.14	.43	.29	.00	.00	1.00	.00	.00
Critz planted stand	28	.21	.04	.11	.50	.07	.07	.00	.96	.04	.00
VDF seed orchard	25	.20	.00	.40	.24	.00	.12	.04	1.00	.00	.00

[a/] Number of trees sampled

A comparison is given in Table 4 of the four populations for the percentage of individuals found to be heterozygous at locus A. The data demonstrates that for GOT locus A the seed orchard was the most homozygous, heterozygosity occurring in only 40% of the trees. However, assuming panmixia, the levels of heterozygosity can be calculated for progeny of the populations from which seed was collected for this study. This was done using the gene frequencies given for locus A **in** Table 2. It was found that seed orchard progeny would be 54% heterozygous. This value is within the range found for the other three populations (Table 4).

Table 4.--Frequency of individuals heterozygous for GOT locus A in the four Virginia pine populations analyzed and (assuming panmixia) their progeny

Population	Frequency of heterozygous individuals	
	Population analyzed	Progeny of analyzed populations
Critz natural stand	.65	.52
Blacksburg natural stand	.71	.60
Critz planted stand	.63	.58
VDF seed orchard	.40	.54

DISCUSSION

The results of this study indicate that the technique of gel electrophoresis may be effectively utilized to study gene frequencies, genotypic frequencies and heterozygosity in southern pine populations. This can be done without breeding studies. For the GOT locus, allelic variability is approximately the same in the three population types studied here; these being natural stands, a stand derived from "nursery-run" stock and a seed orchard. Study of additional populations with larger sample sizes than those used here, coupled with additional gene loci and enzyme systems will permit application of the technique for the solution to the problem areas outlined above.

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