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The genetics of glutamate oxaloacetate transaminase (GOT) in *Pinus merkusii* Jungh. et de Vriese

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ABSTRACT

Siregar IZ, Yunanto T (2010) The genetics of glutamate oxaloacetate transaminase (GOT) in Pinus merkusii *Jungh. et de Vriese. Biodiversitas 11: 5-8.* Inheritance and linkage analysis of glutamate oxalacetate transaminase (GOT) in *P. merkusii* were performed using megagametophyte tissues of single tree seed-lots. One monomorphic locus (GOT-A) and three polymorphic loci (GOT-B, GOT-C and GOT-D) were identified, based on the allelic 1: 1 segregation of putatively heterozygous trees. Linkage analysis revealed that GOT-C and GOT-D were closely linked loci with an average recombination rate (R) of about 5%. This result which is in contrast to the GOT genetics in other pine species was discussed with regard to the probably occurring gene duplication of one GOT locus.

Key words: *Pinus merkusii*, GOT, isozymes, inheritance and linkage.

INTRODUCTION

The glutamate oxaloacetate transaminase (GOT) which is identical to aspartate amino transferase (AAT, E.C. 2.6.1.1) is one of the enzyme systems often used in isozyme studies on plant and animal genetics. It catalyzes the reversible reaction of glutamate and oxaloacetate to 2-oxoglutarate and aspartate, thus having an important role in nitrogen metabolism by distributing this nutrient originally assimilated into glutamate to other compounds (Ireland and Joy 1985). In surveys of higher plant isozymes, three to four electrophoretic GOT zones have been reported (for review, see Gottlieb 1982). In maize, three different isozymes associated with the mitochondria (mGOT), with the glyoxysomes (gGOT), and with the soluble fraction of the cytosol (sGOT) were described, however, in later studies it was established that GOT occurs also in plastids (Weeden and Gottlieb 1980).

In genetic studies on forest tree species, GOT has frequently been assayed to determine genetic diversity and differentiation (Aguirre-Planter et al 2000; Ledig 2000; Ledig et al. 2001; Shea et al. 2002), since its controlling gene loci were found to be largely polymorphic. In conifer, GOT is generally encoded by three gene loci, one of which (GOT-C or GOT-3) specifies an isozyme consisting of double- or triple-banded variants near the cathode in zymograms. If the separation buffer in electrophoretic analysis has a pH value of 8.0 or 8.1, one or two bands of GOT-C migrate to the anode and one band migrates to the cathode, but all bands of this isozyme reveal a comigration, so that they were believed to be encoded by the same gene locus. In pine species, GOT has also been found to be encoded by three gene loci, i.e. GOT-A, GOT-B and GOT-C (Mejnartowicz and Bergmann 1985). However, Chung (1981) reported a fourth locus of GOT in *P. sylvestris* and classified it as GOT-D, although this additional isozyme may correspond to one of the above-mentioned subbands comigrating with bands of GOT-C (Mejnartowicz and Bergmann 1985). Similarly, four isozyme zones were found in seed samples of *Pinus merkusii* from Thailand, however, one of these zones near the origin was invariant and was assigned to GOT-C which controls another variable zone (Changtragoon and Finkeldey 1995). However, new data with *P. merkusii* single tree seed-lots from Indonesia revealed the existence of two separate gene loci, i.e. GOT-C and GOT-D, controlling these multiband configurations in GOT zymograms (Siregar and Yunanto 2008). The studies on inheritance and linkage were carried previously with objective to confirm the number of GOT loci in *P. merkusii*. Output of the studies would be of significant importance in future biodiversity assessment, i.e. genetic diversity, of the genus *Pinus*.

MATERIALS AND METHODS

Seeds

Bulk seed-lots of *P. merkusii* from Aceh and Java (Indonesia) were primarily used to optimise the experimental methods for GOT resolution. In the second step haploid megagametophytes (endosperms) of 18 single tree seed-lots were analysed according to the procedure largely used by Changtragoon and Finkeldey (1995). Seeds were immersed overnight in water, dissected and the embryo carefully separated from the megagametophytes. The megagametophytes were ground in two drops of homogenising buffer (0.1 m Tris-HCl pH 7.3, containing 0.03% DTT and 2.5 % PVP).

Electrophoresis

These crude homogenates were then subjected to horizontal starch gel electrophoresis, using the buffer system of Ashton and Bradon, pH 8.6. Further details of electrophoretic and staining procedures were given by Liengsiri et al. (1990).

Segregation analysis

Inheritance of isozyme variants was confirmed through analysis of segregation ratios of haploid megagametophytes from putatively heterozygous mother trees. A test for goodness of fit to the 1: 1 ratio, chi-square test (χ^2), was performed separately for each parent tree. In this analysis, all statistical tests were set up at 5% level of significance.

Linkage analysis

Chi-square tests (χ^2 test) of linkage were performed for each pair of segregating loci (Weir 1990). Two chi-square tests (χ^2) were calculated: the first one tests Mendelian segregation of alleles at each individual locus in the pair, and the last one tests for linkage between loci. A linkage analysis was performed for each individual parent tree when its progeny was found to be heterogenous at a given pair of loci. When linkage between loci was detected, the estimated frequency of recombination (r) was calculated using the formula $r = l/n$, where l is the number of recombinants and n is the total number of megagametophytes.

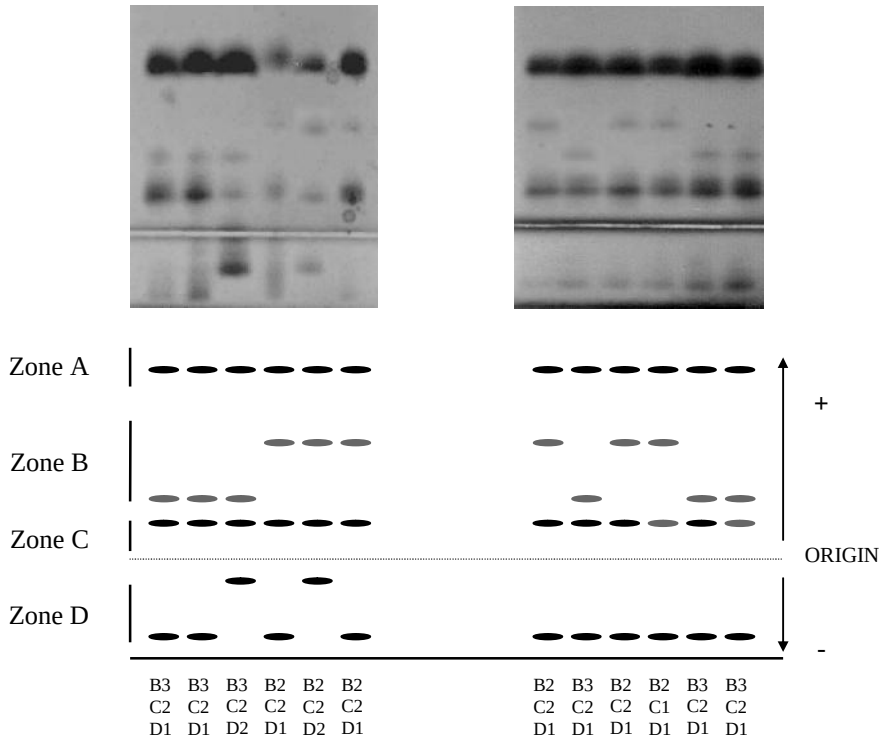

Figure 1. The GOT banding patterns of megagametophytes (*1n*) from two trees. The indication of independent GOT-C and GOT-D zones is shown on the left zymogram.

RESULTS AND DISCUSSION

The phenotypic variation and inheritance of GOT

After electrophoretic separation of extracts from seed megagametophytes, the gels stained for GOT showed four zones where one zone migrated towards the cathode. Figure 1 shows the four GOT zones in zymograms as well as variations in zone B and zone D. Contrary to this study, Zhelev et al. (2002) reported a monomorphic GOT enzyme system on *Pinus peuce*.

Estimates of the statistics used to test the conformity to the Mendelian mode of inheritance for each polymorphic locus of GOT zone are presented in Table 1. The zone GOT-A was invariant in the material used and supposed to be controlled by a single gene locus. Contrary to *P. merkusii* in Thailand, three allozymes were observed in the GOT-B zone and genetic analysis conducted with 10 single tree seed-lots showed that this zone is controlled by one single gene locus (Table 1).

Four allozymes were identified in the GOT-C zone. The same number was also observed by Na'iem and Indrioko (1996). The segregation at GOT-C was regular for several conifers species (Sousa et al. 2001). Nine out of twelve seed lots tested for segregation showed Mendelian 1: 1 ratio, confirming that GOT-C is controlled by a single gene locus with four alleles (Table 1). Two heterozygous trees (J54 and J47) with genotype C_2C_3 and C_2C_4, respectively, indicated a very strong segregation distortion.

The GOT-D zone was highly polymorphic, possessing two allozymes and genetic analysis conducted with 18 single tree seed-lots showed that this zone is controlled by a separate gene locus. Two seed lots showed segregation distortion and the allele D_2 seemed to be in favour.

Linkage relationships

As was shown in Figure 1, the zones GOT-C and GOT-D in zymograms appear to be independent isozymes which are encoded by two separate gene loci, however, these loci may be linked. In the following analysis, linkage among the three variable GOT loci will be assessed. Three pairs of heterozygous GOT loci were tested in different tree samples and the results of linkage analysis and statistical tests are presented in Table 2. According to the observed recombination values for significantly linked loci, only one linkage group can be constructed. GOT-C/GOT-D was closely linked with an average recombination value of 0.046. Other possible linkages were GOT-B/GOT-D (R=0.167) based on only one significant case of linkage, however, the allelic distribution in the other three samples

also tends to some loose linkage. Significant linkage in one but not all trees has also been reported for other conifer species (Fallour et al. 2001; Pastorino and Gallo 2001).

Table 1. Observed segregation of allozymes from mega-gametophytes trees of *P. merkusii* at gene loci of GOT and goodness of fit to the 1: 1 expected ratio.

Tree	Genotype	Sample size (Nij)	Observed segregation (Ni)	(Nj)	¡ ²test
GOT-B					
J04	B_2B_3	12	7	5	0.333
J09	B_2B_3	12	6	6	0.000
J14	B_2B_3	15	10	5	1.667
J29	B_2B_3	22	13	9	0.727
J33	B_2B_3	16	4	12	4.000*
J34	B_2B_3	29	15	14	0.034
J37	B_2B_3	16	9	7	0.250
J54	B_2B_3	43	24	19	0.581
AI40	B_2B_3	18	11	7	0.889
A04	B_2B_3	20	8	12	0.800
Pooled	B_2B_3	203	107	96	0,596
GOT-C					
J01	C_1C_2	27	12	15	0.333
J02	C_1C_2	34	21	13	1.882
J36	C_1C_2	40	25	15	2.500
Pooled	C_1C_2	101	58	43	4.715
J16	C_2C_3	36	16	20	0.444
J26	C_2C_3	62	39	23	4.129*
J54	C_2C_3	43	39	4	28.488***
Pooled	C_2C_3	141	94	47	282***
J27	C_2C_4	57	31	26	0.439
J38	C_2C_4	16	9	7	0.250
J47	C_2C_4	36	26	10	7.111**
J48	C_2C_4	20	12	8	0.800
J52	C_2C_4	43	18	25	1.140
AI41	C_2C_4	25	12	13	0.040
Pooled	C_1C_2	197	108	89	1.980
GOT-D					
J01	D_1D_2	27	16	11	0.926
J02	D_1D_2	34	14	20	1.059
J04	D_1D_2	12	7	5	0.333
J06	D_1D_2	12	6	6	0.000
J09	D_1D_2	12	6	6	0.000
J11	D_1D_2	22	9	13	0.727
J12	D_1D_2	39	6	33	18.692***
J27	D_1D_2	57	48	9	26.684***
J29	D_1D_2	21	10	11	0.048
J31	D_1D_2	22	12	10	0.182
J34	D_1D_2	29	14	15	0.034
J36	D_1D_2	40	14	26	3.600
J47	D_1D_2	36	14	22	1.778
J51	D_1D_2	24	10	14	0.667
J52	D_1D_2	40	20	20	0.000
J54	D_1D_2	43	20	23	0.209
AI41	D_1D_2	25	13	12	0.040
A39	D_1D_2	17	11	6	1.471
Pooled	D_1D_2	512	250	262	0.281

Note: Significant levels are 0.05 (*), 0.01 (**), 0.001 (***)

Table 2. Pairs of loci for which significant levels of linkage were detected in at least one tree.

Tree no.	Observed number by allelic combination				¡ 2 Test (df=1)			Recom-bination Fraction
	A_iB_i	A_iB_j	A_jB_i	A_jB_j	locus A	locus B	Linkage	(R)
GOT-B/GOT-C								
J54	20	4	19	0	0.581	28.488***	0.209	0.535
GOT-B/GOT-D								
J54	14	10	6	13	0.581	0.209	2.814	0.372
J04	6	1	1	4	0.333	0.333	5.333*	0.167
J12	8	7	6	8	0.034	0.034	0.310	0.448
J22	14	10	6	13	0.581	0.209	2.814	0.372
GOT-C/GOT-D								
AI41	2	10	11	2	0.040	0.040	11.560***	0.160
J01	0	13	12	0	0.040	0.040	25.000***	0.000
J02	0	11	7	0	0.889	0.889	18.000***	0.000
J36	0	25	14	1	2.500	3.600	36.100***	0.025

Note: Significant levels are 0.05(*), 0.001(***)

Discussion

The genetic analysis conducted in this study has been based on megagametophytes of seeds from putatively heterozygous trees, since progenies from controlled crosses were not available. In coniferous tree species, isozyme analysis of haploid megagametophytes, which genetically represent the female gametes, allows observation of regular meiotic segregation at individual loci (Bergmann 1973). This method therefore allows for both, the identification of the number of loci controlling an enzyme system and the independent transmission or linkage of alleles among these different loci. The study on the genetic control of observed isozyme phenotypes in *P. merkusii* was mainly carried out to confirm the results of previous works (Changtragoon and Finkeldey 1995; Szmidt et al. 1996). However, in contrast to these studies it was noticed that the enzyme system GOT turned out to be controlled by four gene loci. This result is surprising because it is much in contrast to all other pine species where only three GOT loci were identified. Furthermore, it was established that the allelic segregation at two loci did not occur independently. However, Mejnartowicz and Bergmann (2002) and Lewandowski et al. (2002) reported that four AAT or GOT activity zones were found in *Abies alba* and *Picea abies*, respectively.

The close linkage between the loci GOT-C and GOT-D has not been reported previously because the two loci are often regarded as one locus. In many conifers, the GOT-C and GOT-D are actually two widely separated, always co-migrating bands and one of these bands is often moving towards the cathode. This band configuration has led to the one-locus hypothesis. This interpretation may be correct if we assume a gene duplication which has occurred just very recently. In this case, recombinants between adjacent gene loci can only be detected among thousands of seed megagametophytes, which were not included in the former inheritance studies. Later on, the distance between the duplicated GOT loci may have become larger (e.g. by some insertions) and, hence, recombinants between the two loci can be detected with smaller seed samples, as is the case with *P. merkusii*. The various recombination frequencies between GOT-C and GOT-D observed in four trees, i.e.

from 0% to 16%, might be attributed to the genotype at the GOT-C locus. The highest recombination rate (R=16%) was observed in tree AI41 which is C_2C_4, while the other three trees were C_1C_2. Indication of possible influence of temperature during meiosis has been mentioned by Rudin and Ekberg (1978) to explain the different levels of recombination frequency observed among trees of *P. sylvestris*. The later reason should be excluded from our results because of the absence of such high temperature fluctuation in the tropics.

Additionally, it must be emphasized that the linkage analysis was only based on female gametes. In spite of the general observation that gamete selection is less pronounced on the female than on the male side in flowering plants (Rudin and Ekberg 1978), female gamete selection may have influenced recombination data. In *P. radiata*, it was found that the rate of recombination is 43% greater in male gametes than in female gametes. In addition, significant linkage in one but not in all trees has also been reported for other conifer species. The possible reason for different recombination values among trees is related to environmental effects or modification of chromosome structure, i.e. insertion, translocation, deletion, reversion, etc. A high frequency of events leading to such meiotic irregularities has been observed in conifers (Andersson et al. 1969). Furthermore, the history of colonization of a species might contribute also to slightly different chromosomal arrangement resulting in a deviating linkage pattern as shown in *P. sylvestris* from southern and northern Sweden (Rudin and Ekberg 1978).

The gene duplication followed by mutation is a major mechanism of evolution. Duplication results in two identical copies, each of which can retain original function, becomes functionless (formation of pseudo-gene) or develops a new function. The GOT loci has often been reported to be tightly linked with PGI loci in pines and other coniferous species and this linkage has become an indication of a striking conservatism of chromosomal structure in this species (Ledig 1998). Using *P. merkusii* samples from Thailand and Vietnam, Szmidt et al. (1996) have found polymorphism in PGI and this opens up the possibility for future work to confirm the finding of such conservatism of linkage groups.

CONCLUSION

In *P. merkusii*, GOT was identified to have one monomorphic locus (GOT-A) and three polymorphic loci (GOT-B, GOT-C and GOT-D). Linkage analysis confirmed that GOT-C and GOT-D were closely linked loci with an average recombination rate (R) of about 5%.

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