SHORT COMMUNICATION

GENETIC INFERENCE ON THE EMBRYO OF YEW (*TAXUS BACCATA* L.)

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Received May 5, 1997, revised November 11, 1998, accepted January 20, 1999

ABSTRACT

The enzyme gene locus *Pgm–A* was scored in buds, macrogametophytes and, using a new technique, for the first time in the small embryos of yew (*Taxus baccata* L.). Segregation analysis of zymograms yielded conformity to expectations both in haploid and diploid seed tissue.

Keywords: *Taxus baccata* L., buds, macrogametophyte, embryo, segregation analysis.

INTRODUCTION

In the first paper ever published on the genetic control of isoenzyme systems in yew, LEWANDOWSKI *et al.* (1992) analysed the segregation of zymograms among the haploid macrogametophytes of single seed trees. The results of these studies were largely confirmed later.

THOMA (1992) identified several gene loci by analysing samples of macrogametophytes collected from single trees and genotyped ♀ trees in several relic populations. More recently, HERTEL (1996) analysed enzyme systems in both macrogametophytes and buds of the same seed trees and could thus establish the ontogenetic stability of gene expression. On this basis, HERTEL and KOHLSTOCK (1996) were able to infer the genotype of ♂ trees also.

The previous authors had identified a gene locus *Pgm–A* with two (LEWANDOWSKI *et al.* 1992) or three codominant alleles. LEWANDOWSKI *et al.* (1992) also reported preliminary results on the zymograms of embryos in several enzyme systems. In a more recent investigation, RAJEWSKI and LANGE (1997) studied both the identification of gene loci using extracts of various tissues and certain properties of the reproductive system of yew.This present paper is based on part of the results of RAJEWSKI and LANGE (1997) concerning the expression of this gene locus also in the embryo. The paper deals with the segregation among embryo phenotypes and the consistency of these results with the segregation among macrogametophytes.

Ours is the first report on inference of the genotype of yew embryos. As far as we know, the identification of enzyme gene loci first studied by BARTELS in 1971 has always been consistent in different nuclear phases. However, the technical problems involved in manipulating the small yew embryo are enormous. It is, therefore, necessary to test the segregation also in the embryo.

MATERIAL AND METHODS

Both seeds and buds were collected from presumably heterozygous seed trees showing double bands in zone *Pgm–A*. Methods of extract preparation from buds, macrogametophytes, and embryos have been described in detail by RAJEWSKI and LANGE (1997).

The genotype of the seed trees at the gene locus *Pgm-A* was inferred by two different approaches. One of these employed segregating macrogametophytes according to BARTELS (1971). The other approach was based on segregating embryos according to GILLET and HATTEMER (1989). The latter method was successfully used by PAPAGEORGIOU *et al.* (1993) in *Cupressus sempervirens*, a conifer without haploid segregation among the macrogametophytes.

The embryo of yew is rather small and requires extensive stratification for germination (SUSZKA 1985). LEWANDOWSKI *et al.* (1992) stratified their seed material for six months prior to electrophoretic analysis. This pretreatment could be avoided by excising the embryo out of the fresh seed under a microscope (40×) and subsequent storage in the homogenate buffer at 8 °C for at least 36 hours. The zymogram of the macrogametophytes was unaffected by this treatment. Therefore, it was possible to handle both macrogametophyte and embryo in the same way for simultaneous analysis. Without the described pre-

treatment, only rather faint bands, if any, became visible in our material.

The balanced segregation among the macrogametophytes of a tree with putative genotype A_iA_j was studied by testing the hypothesis of equality of the frequencies N_i and N_j of macrogametophytes carrying the allele A_i and A_j, respectively. A more complex hypothesis was tested among the embryos contained in the seed of a seed tree with this putative genotype. It implies both balanced meiotic segregation and subsequent random fusion of effective gametes (GILLET & HATTEMER 1989). In situation I, equal frequencies N_{ij} of heterozygous and $N_{ii}+N_{jj}$ of homozygous carriers of the tree's own alleles are concerned. The question in situation II is, whether the control of an additional band found in zymograms of seeds but not in those of the seed parent can be attributed to a third allele A_k. Under the above hypothesis, its association with A_i and A_j is to be expected with equal frequency, or $N_{ik} = N_{jk}$. To analyse the data on segregation, the exact two-tailed binomial test was applied.

RESULTS

Figure 1 shows the zymograms of buds and macrogametophytes of a presumably heterozygous tree. In Figure 2, the zymograms of the macrogametophyte and embryo of five seeds are shown. The macrogametophytes (on the left of each pair) carry either bands A_1 or A_3, respectively. In the embryos with their less intensively stained zymograms, they are associated with bands A_3, A_2, A_3, A_3 and A_1. These are presumably expressed by the alleles contributed by the pollen. However, the zymogram of the rightmost embryo is only faintly stained.

Table 1 reveals highly balanced segregation among the phenotypes in zone *Pgm–A* of macrogametopytes produced by all types of putative heterozygotes. Also the frequencies of the embryo phenotypes (see Table 2) were close to their expectations under the assumption of one controlling gene locus with three codominant alleles. No significant deviation was detected.

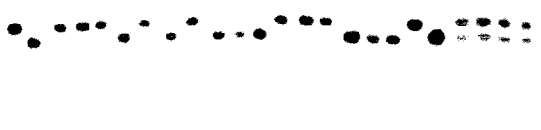

Fig. 1. *PGM* zymograms of ♀ tree 85 with phenotype A_1A_3. Left: four buds; right: 20 macrogametophytes displaying phenotypes A_1 and A_3 in equal proportions.

Fig. 2. *PGM* zymograms of five seeds of ♀ tree 85. Macrogametophytes and embryos are shown side by side: A_3 and A_3A_3, A_3 and A_2A_3 (with putative ordered genotype A_3^{φ} A_2^{σ}), A_3 and A_3A_3, A_1 and A_1A_3 (with putative ordered genotype A_1^{φ} A_3^{σ}), A_3 and A_1A_3 (with putative ordered genotype A_1^{φ} A_3^{σ}). Note that the zymograms of the macrogametophytes are more intensively stained. The zymogram of the rightmost embryo is difficult to be seen even in the original photograph..

Table 1. Segregation of *Pgm–A* phenotypes among the macrogametophytes contained in the seeds of several presumably heterozygous trees.

Tree #	No. of macrogametophytes	Segregation of macrogametophyte phenotypes		*P*
		Phenotype A_1A_2		
27	40	18	22	.64
		Phenotype A_1A_3		
5	83	40	43	.83
30	39	14	25	.11
85	82	41	41	1.00
97	15	4	11	.12
Total	219	99	120	.18
		Phenotype A_2A_3		
2	45	25	20	.55
6	57	22	35	.11
8	20	13	7	.26
26	81	38	43	.66
43	20	10	10	1.00
54	75	38	37	1.00
57	40	15	25	.15
Total	338	161	177	.41

Table 2. Segregation of *Pgm–A* phenotypes among the embryos of several presumably heterozygous trees.

Tree #	Phenotype	No of seeds	Number of progeny phenotypes						P_I	P_{II}
			A_1A_1	A_1A_2	A_1A_3	A_2A_2	A_2A_3	A_3A_3		
27	A_1A_2	34	3	6	10	6	9	–	.61	1.00
5	A_1A_3	83	5	2	37	–	5	34	.91	.45
85		82	8	7	28	–	3	36	.08	.34
2	A_2A_3	41	–	1	2	1	22	15	.42	1.00
26		80	–	8	6	4	31	31	.71	.79
54		60	–	3	3	4	27	23	1.00	1.00

DISCUSSION

The fairly close approximation of the data by the expectations based on balanced segregation and random fusion of gametes prove beyond doubt the eligibility of the controlling gene locus *Pgm–A* as a marker gene locus. The latter test makes no use of the ordered genotypes. It rather makes sense for genetic inference on the diploid genotypes without the lengthy process of raising progeny and on embryos without concomitant assay of the macrogametophyte.

The simultaneous analysis of macrogametophytes and corresponding embryos in yew seeds greatly facilitates the identification of pollen contributors to the subsequent generation of yew populations. This means easier inference and more information on the mating system following the method of MÜLLER[-STARCK] (1976). Otherwise, only the buds of young progeny could be used.

LEWANDOWSKI *et al.* (1992) already reported that besides *Pgm*, banding patterns of macrogametophytes and corresponding embryos indicated that in other enzyme systems such as LAP, PGI, IDH and SOD presumably the same loci were active in both tissues. From the results of RAJEWSKI and LANGE (1997), it is highly likely that genotyping of the embryo will be possible also in PGI, GOT and LAP. A good chance exists also for 6PGDH; in this system, HERTEL (1996) has already been able to demonstrate a controlling gene locus by macrogametophyte segregation. It is to be hoped that, with still more refined electrophoretic methods, more gene loci can reliably be assayed in the embryo. These methods possess importance for the study of gene flow in the context of genetic conservation.

In the beech forests of the Northern hemisphere, yew deserves much interest as the only dioecious species. It may be hoped that the drastically reduced populations of this species will attract more interest among the research workers in forest genetics. More insight into the reproductive system of yew will help in the development of adequate measures of genetic conservation.

ACKNOWLEDGEMENTS

The authors are heavily indebted to Dr H. HERTEL, Waldsieversdorf, for making her unpublished manuscripts available to us. We are also thankful to Dr E. GILLET for help in data analysis by using her computer program for the binomial test, and to her and Dr H.-R. GREGORIUS for making valuable suggestions for data interpretation. Drs F. BERGMANN and M. ZIEHE kindly read the manuscript and pointed out several shortcomings. We are also thankful to the reviewers for making critical comments.

LITERATURE CITED

BARTELS, H. 1971: Genetic control of multiple esterases from needles and macrogametophytes of *Picea abies*. *Planta* **99**: 283–289.

GILLET, E. & HATTEMER, H.H. 1989: Genetic analysis of isoenzyme phenotypes using single tree progenies. *Heredity* **63**:135–141.

HERTEL, H. 1996: Vererbung von Isoenzymmarkern bei Eibe (*Taxus baccata* L.). *Silvae Genetica* **45**: 284–290.

HERTEL, H. & KOHLSTOCK, N. 1996: Genetische Variation und geographische Struktur von Eibenvorkommen (*Taxus baccata* L.) in Mecklenburg-Vorpommern. *Silvae Genetica* **45**: 290–294.

LEWANDOWSKI, A., BURCZYK, J. & MEJNARTOWICZ, L. 1992: Inheritance and linkage of some allozymes in *Taxus baccata* L. *Silvae Genetica* **41**: 342 –347.

MÜLLER [-STARCK], G. 1976: A simple method of estimating rates of self-fertilization by analysing isozymes in tree seeds. *Silvae Genetica* **25**: 15–17.

PAPAGEORGIOU, A.C., BERGMANN, F., GILLET, E. & HATTEMER, H. H. 1993: Genetic analysis of isoenzyme variation in Mediterranean cypress (*Cupressus sempervirens* L.). *Silvae Genetica* **42**: 109 –111.

RAJEWSKI, M. & LANGE, S. 1997: Genetische Strukturen in

verschiedenen ontogenetischen Stadien der Eibe (*Taxus baccata* L.). Forstwiss. Diplomarb. Univ. Göttingen.

SUSZKA, B. 1985: Conditions for after-ripening and germination of seeds and for seedling emergence of English yew (*Taxus baccata* L.) *Arboretum Kórnickie* **30**: 285–338.

THOMA, S. 1992: Genetische Variation an Enzymgenloci in Reliktbeständen der Eibe (*Taxus baccata* L.). Forstwiss. Diplomarb. Univ. Göttingen.