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Short Communication

**THE GENETIC STRUCTURE OF TETRAPLOID *AVENA*:
A COMPARISON OF ISOZYME AND RAPD MARKERS**

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Abstract: Isozymes were the first widely used molecular markers in plant population analysis. They yielded valuable information on the amount and the structure of genetic variability. DNA technology has provided new types of markers based on DNA sequence, which make it possible to study polymorphisms in a much greater proportion of the genome. This is the reason why the use of isozymes is less popular nowadays. This effect would be justified if all markers provided the same type of information on polymorphism and genetic relationships among populations; otherwise, it would be necessary to use different markers to obtain the complete picture of the genetic structure of populations and species.

In this study, we compared data of isozyme and RAPD markers in the populations of two tetraploid species of wild oats: *Avena barbata* populations collected in Argentina, and *Avena murphyi* populations collected in Spain and Morocco. The samples were evaluated for 9 isozymatic systems and 10 primers. The structure of genetic variability was studied using Nei's method, and the relationships between populations were estimated using Hedrick and Jaccard's similarities for isozymes and RAPDs, respectively. As expected, RAPDs were more polymorphic than isozymes, but the information obtained from both markers was weakly correlated. The various reasons for this observation are discussed, but our conclusion is that in order to study the structure of genetic variability, several types of markers should be used.

Key Words: *Avena barbata*, *Avena murphyi*, RAPD, Isozymes, Molecular Markers.

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INTRODUCTION

Avena barbata is a tetraploid grass ($2n = 4x = 28$ chromosomes) derived by polyploidization from the diploid ($2n = 14$ chromosomes) *Avena hirtula*–*Avena wiestii* complex [1]. It is very common throughout the countries of the Mediterranean basin and from the Middle East to Nepal. It thrives under a wide range of ecological conditions encompassing arid sites with shallow soils to wet sites with deep soils. It has also been a highly successful colonizer in the Mediterranean climates of North and South America, South Africa, and Australia.

Avena murphyi ($2n = 4x = 28$) was first described in Tarifa (Spain) in 1971 [2]. In further expeditions across Spain and Morocco, other populations were located in Tangier and Khénifra [3]. It has a morphological similarity with cultivated oat. In both countries, natural populations of *A. murphyi* are gradually diminishing due to human activities.

Characterization of genetic diversity has long been based on isozyme markers; however, the number of loci available is often restricted. Recently, a variety of DNA genetic markers has been proposed to assess genetic variability as a complementary tool in genetic resources management.

MATERIALS AND METHODS

Ten populations of *A. murphyi* from Spain and Morocco and twelve populations of *A. barbata* from Argentina were used in this study. In order to compare the variability estimates using isozyme and RAPD loci, ten plants per population were analyzed.

Isozyme assays for nine enzymatic activities: Leucine aminopeptidase (LAP, EC 3.4.11.1), malic dehydrogenase (MDH, EC 1.1.1.37), 6-phosphogluconate dehydrogenase, (6-PGD, EC 1.1.1.44), peroxidase (PRX, EC 2.6.1.1), glutamic oxaloacetic transaminase (GOT, EC 2.6.1.1), esterase (EST, EC 3.1.1.2), acid phosphatase (ACPH, EC 3.1.3.2), phosphoglucose isomerase (PGI, EC 5.3.1.9), and phosphoglucose mutase, (PGM, EC 2.7.5.1) were performed as described by Hutchinson *et al.* [4]. The genetic interpretation of the band patterns followed that of Allard *et al.* [5].

For RAPD analysis, genomic DNA was extracted following the procedure described by Brook [6]. PCR amplifications, electrophoretic separation of the amplified products and staining were performed as described by Álvarez *et al.* [7]. The primers used were obtained from OPERON Technologies (OPC-04, OPD-02, OPD-04, OPD-07, OPD-16, OPH-05, OPP-15, OPW-04 and OPW-10 for *A. barbata*, and OPC-02, OPC-07, OPG-01, OPG-07, OPH-17, OPP-05 and OPW-07 for *A. murphyi*). For each primer, only bands which could be unambiguously interpreted in all the populations of the same species samples were chosen. The polymorphic RAPD markers were scored as either present (1) or absent (0) in all the individuals from all the populations and for each primer

used. All the plants were considered homozygous since these two species have very high levels of self-fertilization (higher than 95%).

In order to estimate the level and structuration of the genetic variability in the two species, several parameters were used in each population: the number of alleles per locus (A), the percentage of polymorphic loci (P) and Nei's gene diversity value (H). The average values of each species are shown in Table 1. The analysis of genetic diversity within and between populations for each species was performed using Nei's method [8], in which the total diversity in the species (H_T) is the result of the average diversity within the populations (H_S) plus the average gene diversity between populations (D_{ST}). The relationship $D_{ST}/H_T = G_{ST}$ measures the relative magnitude of differentiation between populations.

RESULTS AND DISCUSSION

A total of 14 and 12 isozymatic loci were respectively analyzed in *A. barbata* and *A. murphyi*. For RAPD, 80 and 67 markers were scored in these species. The values of the parameters related to the level of genetic variability and its distribution in and between populations are shown in Table 1.

Tab. 1. Comparison of gene diversity parameters in *A. barbata* and *A. murphyi*.

	<i>A. barbata</i>		<i>A. murphyi</i>	
	Isozymes	RAPDs	Isozymes	RAPDs
Number of loci	14	80	12	67
A	1.60 (0.15)	1.54 (0.38)	1.53 (0.13)	1.51 (0.13)
P	8.50 (12.39)	40.30 (11.64)	34.70 (14.59)	51.40 (13.80)
H_S	0.05 (0.06)	0.14 (0.05)	0.15 (0.06)	0.17 (0.04)
H_T	0.18 (0.23)	0.23 (0.15)	0.29 (0.16)	0.27 (0.15)
D_{ST}	0.13 (0.16)	0.10 (0.10)	0.13 (0.13)	0.10 (0.08)
G_{ST}	0.72 (0.13)	0.41 (0.23)	0.47 (0.22)	0.36 (0.16)

A: number of alleles per locus in populations; P: percentage of polymorphic markers in populations; H_S : average diversity in populations; H_T : diversity in the species; D_{ST} : diversity between populations; G_{ST} : coefficient of genetic differentiation; standard deviation in parenthesis.

For isozymes, several loci and populations were completely monomorphic in both species, while for RAPD markers every plant showed a unique band pattern. The difference between the two types of markers can mainly be attributed to the number of loci available for study, which is much higher for RAPDs than for isozymes, and to polymorphism, which was similar or higher for RAPDs than for isozymes. This concurs with similar studies on other plant species [9].

The coefficient of genetic differentiation (G_{ST}) was high for both species and both types of markers, indicating the important contribution of diversity amongst populations to the global variability. The difference between the G_{ST} values for both markers was studied using the bootstrap method and was statistically significant ($p < 0.01$) for *A. barbata*, but not for *A. murphyi*.

In order to evaluate whether both types of marker yielded similar relationships between populations, we estimated Nei's genetic distance for all the pairs of populations in each species, using the isozyme and RAPD data, and a Pearson correlation (r) was obtained, the significance of which was analyzed by means of Mantel's test. The correlation was significantly positive, although moderate, for *A. barbata* ($r = 0.47$, $p < 0.01$), whilst it was almost zero for *A. murphyi* ($r = 0.09$). We can therefore consider that these markers provide independent estimates of genetic relationships.

Most estimates of the genetic variability in our populations using both markers did not show any significant correlations, pointing out the existence of differences of variability in the different regions of the genome studied, using isozymes and RAPD markers. In *A. barbata*, the differences in the distribution of the variability could be attributed to natural selection, since this process has been repeatedly invoked to explain the variability found with isozymes [10], while the variability detected with RAPDs seems to be neutral.

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