INHERITANCE AND LINKAGE OF ALLOZYMES IN *JUNIPERUS PHOENICEA* L. (CUPRESSACEAE)

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ABSTRACT

Eight enzyme systems coding for 14 loci were assayed in *Juniperus phoenicea*. Mendelian inheritance was confirmed for allozymes at 10 loci (Fle 2, Gdh, Got 1, Idh, Mdh 1, Mdh 2, Men 1, Pgi, Pgm 1, Pgm 2) by testing the fit of band-pattern segregation in macrogametophytes from heterozygous trees to the expected 1:1 ratio. Linkage relationships were examined for 21 pairs of allozyme loci and these pairs do not appear to be linked in our material.

KEY WORDS: *Juniperus phoenicea*, Cupressaceae, allozymes, inheritance, linkage, electrophoresis.

INTRODUCTION

Juniperus phoenicea L. is a small tree 6-8 m tall with scaly leaves, typical for junipers from section *Sabina*. It occurs in southern Europe, south-west Asia and northern Africa, most frequently in the western part of the Mediterranean region. The species occupies coastal dunes, maritime or somewhat inland slopes and enters the mountain regions in the western part of its range, up to altitudes of 2400 m in Morocco and 1800 m in Spain. It grows in various types of Mediterranean forests, sometimes forming also pure stands (Browicz 1982). *J. phoenicea* belongs to variable species, especially in the western part of its range, where two subspecies are recognised (Amaral Franco 1986).

Knowledge of inheritance and confirmation of genetic control of allozymes with simple Mendelian inheritance is the first step facilitating their use as gene markers in population genetics and taxonomic studies. The haploid condition of macrogametophyte tissue in seeds of conifers allows direct observation of Mendelian segregation at heterozygous allozyme loci, without the need for controlled crosses. Few studies of variation of allozymes in Cupressaceae have been published (Copes 1981; Harry 1986; Conkle 1987; Yeh 1988; Perry and Knowles 1989; Millar and Marshall 1991; Xie et al. 1991 and 1992; Papageorgiou et al. 1993 and 1994; Gallo and Geburek 1994; Ferreyra et al. 1996; Lewandowski et al. 1996; Korol et al. 1997; Pastorino and Gallo 1998). However, to our knowledge so far there is not information about inheritance of allozymes in the genus *Juniperus*.

This paper describes the inheritance and linkages of allozymes in *Juniperus phoenicea*.

MATERIALS AND METHODS

Plant material

Material for the present study has been collected on the Iberian Peninsula. Cones of 87 trees from 3 populations

(Cabo de Espihcel from Portugal, Metalascanas and Nueno from Spain) were collected during the autumn of 1999. Seeds were removed from cones by hand and dried at room temperature for five days. They were stored at 4°C until analysis. Prior to electrophoresis, seeds were placed on moistened filter paper in Petri dishes at room temperature for two days. Seeds were prepared for electrophoresis by separating embryos from macrogametophytes. Seven macrogametophytes from each of the studied trees were first sampled to define the genotype. Afterwards, additionally 6 to 46 macrogametophytes per tree were analysed from selected highly heterozygous trees to study linkage and to verify the segregation ratio.

Electrophoretic analysis

Macrogametophyte tissues and embryos were homogenized in TRIS-HCl buffer pH 7.2 with 0.15% 2-mercaptoethanol. Homogenates were subjected to horizontal 12% starch gel electrophoresis by applying the following two buffer systems (electrode buffer / gel buffer): system I, 0.06 M lithium hydroxide – 0.3 M boric acid, pH 8.1 / 0.03 M TRIS – 0.005 M citric acid – 1% electrode buffer, pH 8.5 (Ridgeway et al. 1970). System II, 0.13 M TRIS – 0.043 M citric acid, pH 7.0 / 1:10 dilution of electrode buffer (Siciliano and Shaw 1976). Gel slices were stained for activity of 8 different enzymes using recipes described by Cheliak and Pitel (1984). The following enzymes were investigated in this study (their abbreviations, enzyme commission codes and buffer system upon which they were run are in parenthesis): fluorescent esterase (FLE, EC 3.1.1.1, I), glutamate dehydrogenase (GDH, EC 1.4.1.3, I), glutamate oxaloacetate transaminase (GOT, EC 2.6.1.1, I), isocitrate dehydrogenase (IDH, EC 1.1.1.42, II), malate dehydrogenase (MDH, EC 1.1.1.37, II), menadione reductase (MEN, EC 1.6.99.2, II), phosphoglucose isomerase (PGI, EC 5.3.1.9, I), phosphoglucomutase (PGM, EC 2.7.5.1, I).

Statistical methods

Inheritance of allozyme polymorphism in haploid tissue from heterozygous trees was tested for confirmation with the

expected 1:1 ratio, using the chi-square test. The chi-square test was also used for estimation of homogeneity of results pooled over all trees (Mather 1963). When homogeneity was not rejected, the 1:1 segregation ratio of the pooled data was tested. The statistical evaluation of linkage relationships was done using chi-square tests as described by Mather (1963).

RESULTS AND DISCUSSION

Inheritance of isozyme patterns

For the 8 enzyme system analyzed 14 loci were identified (Fig. 1). Mendelian inheritance was tested for the ten polymorphic allozyme loci. In summary, except locus Fle 2, no significant deviation from the expected 1:1 segregation ratio nor chi-square heterogeneity were observed at any of the loci studied (Table 1), indicating that these allozymes exhibited distinct, co-dominant expression and simple Mendelian segregation in their mode of inheritance. We analyzed seven trees for allelic segregation at Fle 2 and one of them displayed significant excess of allele 3 over allele 2 (Table 1). Segregation distortion at allozyme loci have commonly been observed in

conifers (for references, see Strauss and Conkle 1986). Such phenomena could be caused by many factors, including meiotic drive and natural selection (Strauss and Conkle 1986).

Description of the observed allozyme patterns

Fluorescent esterase (FLE)

Two zones of activity were evident on gels stained for this enzyme. The most anodal zone, designated as Fle 1, stained less intensely and has not been scored in our materials. The lower zone (Fle 2) had three variants. Two zones of FLE activity have been reported in *Thuja orientalis* (Xie et al. 1991) and *Chamaecyparis lawsoniana* (Millar and Marshall 1991). On the other hand only one zone has been observed in *Calocedrus decurrens* (Harry 1986) and *Thuja occidentalis* (Perry and Knowles 1989).

Phenotypes from macrogametophytes and from embryos were compared to deduce subunit associations of enzymes. Monomeric enzymes are characterized by the presence of two bands in heterozygous embryos. Mobilities of the two bands are identical to parental alleles. Dimeric enzymes are characterized by an additional heterodimeric band in heterozygotes,

Fig 1. Enzyme phenotypes found in *Juniperus phoenicea*. The most frequent alleles are printed dark.

TABLE 1. Observed allozyme segregation in macrogametophytes of heterozygous trees and chi-square tests for goodness of fit 1:1 ratio and heterogeneity among employed trees.

Locus	Allele					Deviation χ^2 test (1 df)	Heterogeneity χ^2 test (df)
	1	2	3	4	N		
Fle 2	13	17				0.533	----
		10	17			1.814	----
		17	10			1.814	----
		7	25			10.125	----
		15	16			0.032	----
		17	9			2.462	----
		5	2			1.286	----
		20	16			0.444	----
	joint	91	95			0.086	17.891 (6) **
Gdh	62	65				0.071	3.724 (8)
Got 1	52	59				0.441	2.833 (3)
Idh	85	104				1.910	7.594 (6)
	14				13	0.037	----
Mdh 1	76	75				0.007	0.991 (4)
		42	36			0.462	0.321 (1)
Mdh 2	87	80				0.293	2.614 (5)
	16		17			0.030	0.023 (1)
Men 1	18	28				2.174	0.011 (1)
	20		27			1.043	1.296 (1)
		26	32			0.621	1.660 (4)
			40	38		0.051	1.025 (3)
Pgi	29	41				2.057	1.383 (1)
Pgm 1	28	25				0.170	0.142 (1)
		19	23			0.381	----
Pgm 2		67	60			0.386	6.740 (5)

**significant at p < 0.01

with a mobility intermediate to parental bands. By this criterion, monomeric structure was identified for FLE, concordant to that reported for *C. decurrens* (Harry 1986) and *T. orientalis* (Xie et al. 1991)

Glutamate dehydrogenase (GDH)

One zone, with two variants was evident on gels stained for this enzyme. Similarly a single locus of GDH has been reported for some others species belonging to the Cupressaceae family (Copes 1981; Harry 1986; Conkle 1987; Perry and Knowles 1989; Xie et al. 1991; Papageorgiou et al. 1993; Pastorino and Gallo 1998).

Glutamate oxaloacetate transaminase (GOT)

Three zones of activity were evident on gels stained for GOT. The two lowest zones (Got 2 and Got 3) stained less intensely and probably were invariant in the investigated material. The most cathodal zone (Got 3) was triple-banded, similarly as in some other Cupressaceae species (Gallo and Geburek 1984; Harry 1986; Xie et al. 1991). The most anodal zone (Got 1) was coded by one locus with two alleles. Three zones codified by three different loci have been found in *C. decurrens* (Harry 1986), *T. orientalis* (Xie et al. 1991) and *Austrocedrus chilensis* (Pastorino and Gallo 1998). On the other hand Yeh (1988) and Perry and Knowles (1989) observed only two zones in macrogametophytes of *T. plicata* and *T. occidentalis*, respectively. Heterozygous embryos of *J. phoenicea* displayed three-banded dimeric patterns, similarly as in *C. decurrens* (Harry 1986).

Isocitrate dehydrogenase (IDH)

This enzyme system shows only one variable zone with two alleles. In other members of Cupressaceae two zones of IDH activity have usually been reported (Harry 1986; Perry and

Knowles 1989; Xie et al. 1991; Pastorino and Gallo 1998). A heterozygous embryo showed an additional third band of intermediate mobility, which suggests dimeric structure of this enzyme. Such a suggestion has also been described for *T. orientalis* (Xie et al. 1991) and *A. chilensis* (Pastorino and Gallo 1998).

Malate dehydrogenase (MDH)

Two zones of activity with three variants were evident on gels stained for MDH. In our material we found one double heterozygous tree (Mdh 1 12, Mdh 2 12) and linkage analysis demonstrated independent segregations of bands (Table 2). This suggests that MDH in *J. phoenicea* was coded by two independent loci. In heterozygous trees the slowest allele of locus Mdh 1 (designated as 3) and the fastest allele of locus Mdh 2 (designated as 1) overlap, forming greater intensity of the overlapping band. A heterozygous embryo (13) for Mdh1 showed an additional third band of intermediate mobility between bands 1 and 3 which supports the idea about overlapping bands. Similar, dimeric structure of MDH was identified for *C. decurrens* (Harry 1986), *T. occidentalis* (Perry and Knowles 1989) and *Cupressus sempervirens* (Papageorgiou et al. 1993). Three activity zones of MDH were reported in *C. decurrens* (Harry 1983), *T. occidentalis* (Perry and Knowles 1989) and *C. sempervirens* (Papageorgiou et al. 1993). On the other hand, five zones of activity exist in *A. chilensis* (Pastorino and Gallo 1998).

Menadione reductase (MEN)

At least two zones of activity were on gels stained for MEN. Four double-banded variants were observed in the fastest migrating zone (Men1). The second zone was too indistinct and inconsistent for reliable scoring and therefore was not considered in this analysis. From one (Xie et al. 1991) up

TABLE 2. Numbers of trees tested for each pair of loci (upper right half) and results of statistical testing for detecting linkage (lower left half).

	Fle2	Gdh	Got 1	Idh	Mdh 1	Mdh 2	Men 1	Pgi	Pgm 1	Pgm 2
Fle 2		3	2	---	4	---	5	---	2	1
Gdh	NS		---	---	1	2	---	---	---	---
Got 1	NS	---		---	2	---	1	---	2	2
Idh	---	---	---		---	5	---	1	---	---
Mdh 1	NS	NS	NS	---		1	1	---	2	1
Mdh 2	---	NS	---	NS	NS		---	1	---	---
Men 1	NS	---	NS	---	NS	---		---	3	3
Pgi	---	---	---	NS	---	NS	---		---	---
Pgm 1	NS	---	NS	---	NS	---	NS	---		2
Pgm 2	NS	---	NS	---	NS	---	---	---	NS	

(---) – two locus combination not tested; NS – not significant

to four (Papageorgiou et al. 1993) zones of activity for this enzyme were observed in Cupressaceae.

Phosphoglucose isomerase (PGI)

This enzyme system shows one zone. In this zone two double-banded variants were observed. Heterozygous embryos showed an additional band, which suggests dimeric structure of the enzyme. Such a suggestion has also been described for some other members of the Cupressaceae family (Harry 1986; Xie et al. 1991; Papageorgiou et al. 1993). A single locus for this enzyme was reported also for *T. occidentalis* (Perry and Knowles 1989). However, in *C. deccurens* (Harry 1986), *Ch. lawsoniana* (Millar and Marshall 1991) and *T. orientalis* (Xie et al. 1991) two zones of PGI activity have been described.

Phosphoglucumutase (PGM)

Two zones of activity were detected on PGM gels. Pgm2 stained less intensively than Pgm1 but it was enough to score. Both of the loci contain three well distinguished variants. From one to three zones of PGM have been reported in Cupressaceae (Harry 1986; Perry and Knowles 1989; Xie et al. 1991; Papageorgiou et al. 1993). Heterozygous embryos displayed double-banded isozyme phenotypes, suggesting that PGM is a monomer in this conifer. Monomorphic structure of PGM has also been inferred in *C. deccurens* (Harry 1986), *T. occidentalis* (Perry and Knowles 1989), *T. orientalis* (Xie et al. 1991) and *C. sempervirens* (Papageorgiou et al. 1993).

Linkage

Out of the 45 possible two-locus combination, which can be formed from 10 polymorphic loci, 21 pairs of allozyme loci were compared in at least one tree. In our material these pairs do not appear to be linked. Table 2 shows the combinations of allozyme loci tested and the number of trees studied.

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ANALIZA SPOSOBU DZIEDZICZENIA ORAZ SPRZĘŻEŃ
POMIĘDZY LOCI ALLOZYMOWYMI U *JUNIPERUS PHOENICEA* L. (CUPRESSACEAE)

STRESZCZENIE

Analizowano osiem systemów enzymatycznych kodowanych w 14 loci u *Juniperus phoenicea*. Przez testowanie odchylenia segregacji alleli, w tkankach makrogametofitów heterozygotycznych drzew, od oczekiwanego stosunku 1:1 stwierdzono mendlowskie dziedziczenie allozymów kodowanych w 10 loci (Fle 2, Gdh, Got 1, Idh, Mdh 1, Mdh 2, Men 1, Pgi, Pgm 1, Pgm 2). Nie stwierdzono natomiast sprzężeń pomiędzy analizowanymi 21 parami loci.

SŁOWA KLUCZOWE: *Juniperus phoenicea*, Cupressaceae, allozomy, dziedziczenie, sprzężenia, elektroforeza.