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Marine biogeography of the Mediterranean Sea: patterns and dynamics of biodiversity

Seagrasses biodiversity (*Posidonia oceanica* and *Zostera marina*) in the Mediterranean Sea

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SUMMARY

Aim of this paper is to asses the genetic variability of Mediterranean seagrasses such as *Posidonia oceanica* and *Zostera marina* by Random Amplified polymorphic DNA (RAPD), using PCR (Polymerase Chain Reaction) technique, and to correlate it to primary productivity. The study was carried out for 11 populations of *P. oceanica* collected in different Mediterranean localities, along the coast, around the isles, and transplanted in the protected park of Port-Cros and for two populations of *Z. marina* sampled in Malamocco central basin of Venice lagoon (San Pietro in Volta and Alberoni).

Results of this research enable us to assess genetic variability for distinct groups of *P. oceanica* populations in the Mediterranean in relation to primary production. Instead, *Z. marina* revealed an high genetic variability within and among the two populations located to a distance of only 5 km in Malamocco basin confirming the role of this plant as cosmopolitan species for its phenotypic plasticity.

INTRODUCTION

Seagrasses contribute significantly to the productivity of coastal areas in both temperate and tropical waters (Phillips et al., 1983). The endemic Mediterranean species *Posidonia oceanica* (L.) Delile, *Cymodocea nodosa*, *Zostera marina and Z. noltii*, play the major role in the primary production of the beds in the species succession. In particular *Z. marina* and *Z. noltii* have long been reported in the lagoon of Venice and in various Mediterranean localities. In fact *Z. marina* comprises a significant structural element of both in the lagoon and coastal environments throughout the temperate northern hemisphere. It

is well know that population biology could benefit from a population genetic perspective because genetic data enable the extraction of useful demographic information such as isolation and gene flow between demes. Moreover, population genetic processes may contribute to the growing ecological risks of local population extinction (Reusch, 2001). In previous investigations, RAPD (Randomly Amplified Polymorphic DNA) technique revealed differentiation between populations of various species (Welsh and McClelland, 1990; Williams et al., 1990; Huff et al., 1993; Russel et al., 1993; Haig et al., 1994; Franconi et al., 1995; Peakall et al., 1995; Waycott, 1995; Burrows et al., 1996; Kimberling et al., 1996; Nusser et al., 1996; Steward and Excoffler, 1996).

In this paper we used RAPD technique to assess patterns of genetic diversity within and among two populations of *Z. marina* in the Central lagoon of Venice, Malamocco basin (Alberoni and S. Pietro in Volta), and in 11 different populations of *P. oceanica* transplanted at Port-Cros (Meinesz et al., 1993).

MATERIAL AND METHODS

After collection, individual plants were washed in distilled water and stored in liquid nitrogen (−180°C). According to the methodology of Dellaporta et al., (1983), DNA was isolated from the leaves of *P. oceanica* and *Z. marina* and, for only the plants of *Z. marina* sampled in Alberoni also from the flowers. PCR (Polymerase Chain Reaction) was applied (Echt et al., 1992; Franconi et al., 1995) and the amplification products, obtained by a thermal cycler (Perkin Elmer/Cetus), were separated by gel electrophoresis (agarose 1.4%) and photographed (Polaroid 667) under U.V. light illumination after Ethidium bromide staining.

The sequences of the primers utilized for the amplification of DNA of the two species (*P. oceanica* and *Z. marina*) are reported in Tab. I.

Cluster analysys

Cluster analysis (UPGMA) of the similarity indices was carried out using NT-SYS software (Rohlf et al., 1993). The method was used for determining the similarity among the samples. Fragments sizes of RAPD were estimated from the gel by comparison with a 1 Kb ladder marker. The bands were recorded as present (1) or absent (0) and assembled in a data matrix table.

RESULTS

Literature data on primary production (leaf production, mg (dw)/shoot y) of *P. oceanica* plants from the different Mediterranean localities are reported in Tab. II. These data were correlated with our results about genetic variability obtained using RAPD.

Tab. I - Sequences of the primers utilized for the PCR amplification.

Primer	Sequences	Species
BY 11	5'-ATCCACTGCA-3'	*P. oceanica, Z. marina*
BY 13	5'-CCTTGACGCA-3'	*P. oceanica, Z. marina*
BY 15	5'-CTCACCGTCC-3'	*P. oceanica, Z. marina*
BY 12	5'-GGTCGCAGGC-3'	*P. oceanica, Z. marina*
DN 4	5'-GTGGTGCTAT-3'	*P. oceanica*
DN 5	5'-CCGACGGCAA-3'	*P. oceanica*
DN 6	5'-TGGACCGGTG-3'	*P. oceanica*
UB 22	5'-AAGCCTCCCC-3'	*P. oceanica*
UB 24	5'-GGGTGAACCG-3'	*P. oceanica*
UB 26	5'-CGCCCCCAGT-3'	*P. oceanica*
UB 28	5'-GCTGGGCCGA-3'	*P. oceanica*

Tab. II - Literature data on leaf production of P. oceanica populations (mg (dw)/shoot y) from different localities of Mediterranean sea.

	Localities	Leaf production (mg (dw)/shoot y)
Pergent-Martini et al., 1994	Port-Cros Francia	1230
	Ischia	1320
	Banyuls	920
	Corsica	1530
	Sardegna	920
	Izmir Turchia	700
Marbà et al., 1996	Isole Medas	812
	Alboran-Front	548
	Costa Brava	815
Semroud Tesi di Dottorato 1993	Marsa Bay Algeria	3700
Panayotidis and Simboura, 1989	Atene	2310
Torricelli and Peirano, 1997	Monterosso Liguria	1170

Tab. III - Data on primary production (below-ground compartment and above-ground compartment) of Z. marina plants collected in the Malamocco basin.

Stations	Leaves (g/mq)	Roots and Rhizomes (g/mq)	Dead Material (g/mq)
San Pietro in Volta (February 2002)	80.2	124.0	33.8
Alberoni (May 2002)	125.0	101.0	25.9

Primary production data of *Z. marina* plants (g/mq) sampled in February at San Pietro in Volta and in May at Alberoni, are reported in Tab. III. These data concerned the biomass results of the below-ground compartment (roots and rhizomes) and the above-ground compartment (leaves).

GENETIC AND STATISTICAL RESULTS

Genomic fingerprints revealed by PCR technique, allowed us to obtain amplification products that, using similar index (Nei and Li, 1979), gave several molecular fragments ranging in size from 0.25 to 3.0. Using NT-SYS statistical program the relationships among DNA fragments generated by RAPD marker were further studied by generating a phenetic tree. Cluster analyses revealed the patterns of genetic distance in relation to physical distance (e.g geographical position) among two populations of *Z. marina* in Venice lagoon (Fig. 1). For *P. oceanica* data obtained enable us to assess genetic variability for distinct groups within the 11 populations in the Mediterranean sea (Fig. 2).

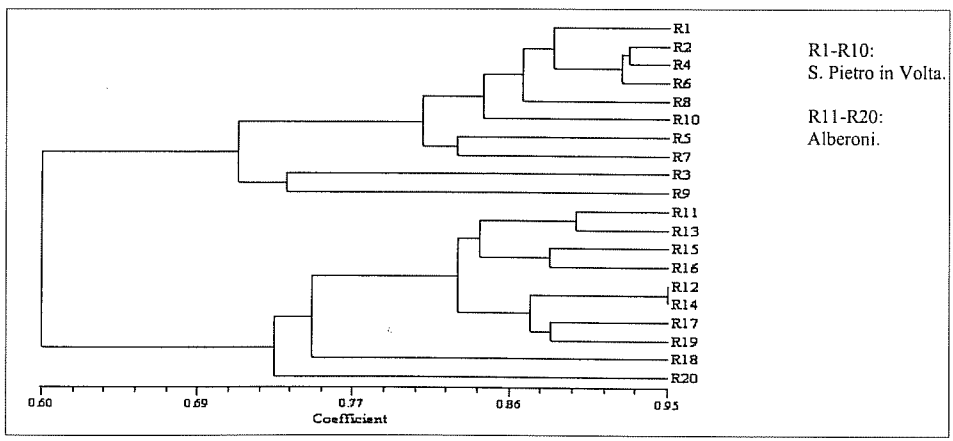

Fig. 1 - UPGMA phenogram constructed from matrix of RAPD-based genetic distances between two populations of *Z. marina* in Venice lagoon. R1-R10: samples collected at San Pietro in Volta; R11-R20: samples collected at Alberoni.

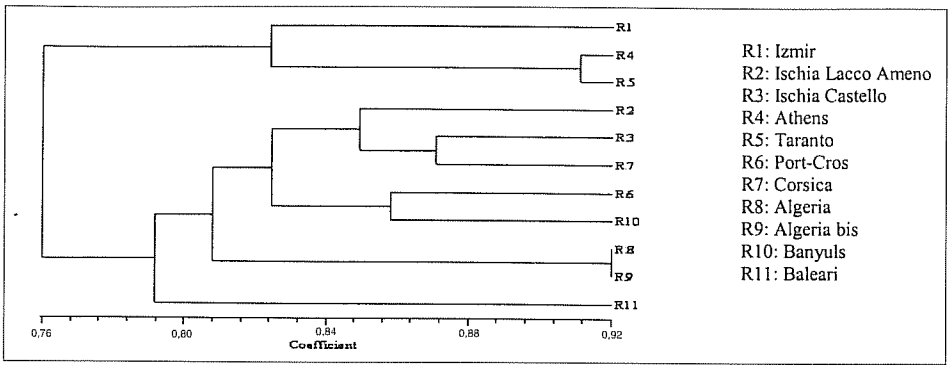

Fig. 2 - UPGMA phenogram constructed from matrix of RAPD-based genetic distances among 11 populations of P. oceanica transplantated at Port-Cros from different Mediterranean localities. R1: Izmir; R2: Ischia Lacco Ameno; R3: Ischia Castello; R4: Athens; R5: Taranto; R6: Port-Cros; R7: Corsica; R8: Algeria; R9: Algeria bis; R10: Banyuls; R11: Balearic Islands.

DISCUSSION AND CONCLUSIONS

This research allowed us to assess a degree of genetic diversity for seagrasses in the Mediterranean as revealed by RAPDs. *P. oceanica* data revealed genetic variability for distinct groups within the 11 populations in the Mediterranean sea, correlated with the primary production in relation to the different environmental parameters (temperature, light, currents), revealing a non-random pattern of distribution among the populations. Genetic, morphometric and production data enable us to confirm that the populations of *P. oceanica* of southern Mediterranean Sea (Algeria and Greece) show an high genetic variability, correlated to a high primary production, in comparison with the others that show homogeneity in the physiological and genetic features. In fact, even after transplantation in the protected park of Port-Cros, the populations of Greece and Algeria keep their typical size of the leaves, longer and wider than the other ones.

Also *Z. marina* revealed a high genetic variability within and between the two populations located to a distance of only 5 km in Malamocco basin, confirming the role of this plant as cosmopolitan species for its phenotypic plasticity. For *Z. marina* it appears from our analysis that sexual reproduction contributed to the expansion and maintenance of these populations. The RAPD analyses demonstrated significant genetic distinction among disjunct eelgrass populations and may offer some insights into the widespread distribution and ecological success of *Z. marina* in a diversity of temperate coastal habitats. The capacity for an high level of genetic variation in this cosmopolitan species probably also accounts for the diversity in leaf and shoot morphologies in different habitats and argues that these features may not be as useful as taxonomic characters at the species level as previously thought.

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