

## ZOSTERA MARINA IN VENICE LAGOON: A GENETIC STUDY

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### Abstract

With the aim of investigating the genetic variability of *Zostera marina* in Venice lagoon, two populations in Malamocco basin (San Pietro in Volta and Alberoni) were studied by the PCR (Polymerase Chain Reaction) technique. The results were correlated with environmental parameters and phenological and physiological features of the plants (leaves biometry, biomass and primary production) taken into consideration. Genomic fingerprintings obtained by RAPD (Random Amplified Polymorphic DNA) and confirmed by statistical analyses (NT-SYS), showed high genetic variability within and between two populations only 5 Km apart, confirming the role of *Z. marina* as a cosmopolitan species and its phenotypic plasticity.

**Key-words:** *Zostera marina*, RAPD, Venice lagoon

Since environmental stress represents an important factor which can induce changes in the physiology of the species (1), the genetic variability of *Zostera marina* from two different zones of the Venice lagoon was analysed, so that by comparing the results of molecular and environmental tests, a clearer assessment of the state of health of the two meadows could be made.

Samples of *Z. marina* were taken from two sites in the Malamocco basin (San Pietro in Volta and Alberoni) corresponding to an area of 162 km<sup>2</sup>. At the San Pietro in Volta site, *Z. marina* meadows are very extensive, mostly mixed with *Cymodocea nodosa*, whilst in proximity to Alberoni the populations are pure and less extensive.

The growth cycle of *Z. marina* favours the period April-June with regard to leaf production, even though it is a macrophyte devoid of conspicuous seasons (in contrast to *C. nodosa*) and therefore active all through the year.

Both sites present fairly good transparency values, which permit observation of the seabed (about 1,5 m) in nearly all conditions. The San Pietro in Volta site is characterized by more lasting transparency and sediment of a coarser consistency (2,3).

After collection, individual plants were washed in distilled water and stored in liquid nitrogen (-180 °C). The DNA of *Z. marina* was obtained using previous methodology (4). For the plants of *Z. marina* sampled in Alberoni only, DNA of the flowers, also, was recorded. PCR was applied (6) 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.

In these experiments four primers were used: BY 11 (5'-ATC-CACTGCA-3'); BY 13 (5'-CCTTGACGCA-3'); BY 15 (5'-CTCAC-CGTCC-3') BY 12 (5'-GGTCGCAGGC-3').

Cluster analysis (UPGMA) of the similarity indices was carried out using NT-SYS software (5) in order to determine similarities between samples. Fragment sizes of RAPD were estimated from the gel by comparison with a 1Kb ladder marker. The bands were recorded as present (1) or absent (0) and assembled in a data matrix table.

Genomic fingerprinting, revealed by PCR technique, gave several molecular fragments of varying sizes ranging from 0.25 to 2.5 Kb. Cluster analyses revealed the pattern of genetic distance in relation to physical distance (e.g. geographical position) between the two populations of *Z. marina*.

From a comparison of samples from San Pietro in Volta (A), an average similarity of 72% appears, while the average value of similarity for Alberoni (B) is 74%. Similarity drops to 43% when samples of group A and group B are compared.

The genetic variability we encountered may be correlated to the phenological and physiological variation of *Z. marina* in bloom at the Alberoni site. The morphological differences observed among plants of diverse provenance (length of leaves, presence of one or more leafy shoots, strength of stem) are a direct response to the environment, which at Alberoni presents greater environmental stress in general (sedimentary, turbidity, etc.) than at San Pietro in Volta.

The eelgrass *Z. marina* can reproduce both sexually and vegetatively. For species with two reproductive modes, molecular-genetic tech-

niques are useful in assessing the relative importance of the two modes in determining population distribution and structure. Such information is relevant to many basic and applied questions in marine ecology because colonizers resulting from vegetative reproduction all have identical genetic composition, whereas sexual reproduction results in genetically diverse individuals. Genetic variation within and between populations of the same species may, for example, indicate the sensitivity of a given population and its recovery from disturbance (7). Because of the vigorous rhizomatic growth of *Z. marina* and its wide distribution and ecological success, vegetative reproduction was expected to prevail. A high degree of genetic similarity within and even between not-too-distantly separated populations is expected. However, RAPD, used to quantify the genetic similarity of two geographically and morphologically distinct populations of *Z. marina* from Venice lagoon, showed just the opposite. *Z. marina* revealed a high genetic variability within and between the two populations located only 5 km apart, in Malamocco basin, confirming the role of this plant as a cosmopolitan species for its phenotypic plasticity. It appears from our analysis of *Z. marina* that sexual reproduction contributed to the expansion and maintenance of these populations. The RAPD analyses demonstrated significant genetic distinctions among disjunct eelgrass populations and may offer insight into the widespread distribution and ecological success of *Z. marina* in a diversity of temperate coastal habitats. The capacity for a high level of genetic variation in this cosmopolitan species probably also accounts for 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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