INVESTIGATION OF GENOMIC POLYMORPHISM IN *POSIDONIA OCEANICA* PLANTS COLLECTED IN DIFFERENT AREAS OF MEDITERRANEAN SEA USING RAPD MARKERS

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The monoic *Posidonia oceanica* (L.) Delile is a marine phanerogame endemic of Mediterranean sea which has a multifunctional role in the coastal ecosystem (BOUDOURESQUE *et al.*, 1984). During these last years the progressive reduction of *Posidonia* meadows claimed the attention toward the recovery of this marine phanerogame by means of experimental transplantation of different populations. (MEINESZ *et al.*, 1993). It is well known that vegetative reproduction appears to be the principal mode of proliferation for this species (MEINESZ and LEFEVRE, 1984), and it is correlated with environmental parameters (depth, light and temperature). However the sexual reproduction remains the principal way to create and to preserve genetic variability. With the aim of better knowing the genomic polymorphism in *P. oceanica*, we performed a study using molecular markers such as RAPDs (Random Amplified Polymorphic DNA) (WILLIAMS *et al.*, 1990). During may-november 1993, several plants of *P. oceanica* were collected from 7 different geografical areas of the Mediterranean sea : Giannutri (GR), Costa dell'Argentario (GR), Scoglio dell'Argentarola (GR), Civitavecchia (RM), Ponza (LT), Marina di Camerota (SA) and La Valette (Malta). After collection by SCUBA diving, individual plants were washed in distillated water and stored in liquid nitrogen at -80°C. Subsequently, all extraction steps of genomic DNA were carried out following the protocol reported by DELLAPORTA *et al.*, (1983). The PCR conditions used were similar to those described by ECHT *et al.*, (1992) with some modifications involving reaction buffer and temperature ramps. Amplification reactions were carried out in a thermal cycler (Perkin Elmer/Cetus), using 8 different oligonucleotide primers. The sequences (5'-3') of the primers are as follows : (DN4) GTGGTGCTAT; (DN5) CCGACGGCAA; (DN6) TGGACCGGTG; (BY11) ATCCACTGCA; (BY12) GGTCGCAGGC; (BY13) CCTTGACGCA; (BY14) GGACCCTTAC (BY15) CTCACCGTCC. The amplification products were separated by gel electrophoresis (Agarose 1.4%) and photographs (Polaroid 667) were taken under U.V. light illumination after ethidium bromide staining. The RAPD assay was able to generate informative genomic fingerprints of the *Posidonia* plants. The detected product sizes ranged from 0.25 to 1.95 Kb, while the number of amplification products varied from 2 to 12 (on average 5.6) for plant. The frequency distribution concerning the total number of amplification products detected with all of the primers is shown in figure 1. Most of DNA segments amplified from the *Posidonia* genomic DNAs were 0.26 to 1.50 Kb in length. This histogram also emphasized the different ability of primers to find homologous binding sites among *P. oceanica* templates. On the whole, primer DN5 produced complex electrophoretic banding patterns characterized by the largest number of amplification products and by the widest range of product size (Fig. 2). In addition, this primer resulted the best in discriminating *P. oceanica* plants and, therefore, in detecting genomic polymorphisms. The analysis of electrophoretic profiles allowed the identification of conserved and individual specific amplification products. In particular, primer DN4 amplified several genomic fragments which resulted population conserved (excepted for two products which were absent in plants P11 and P17 collected in Civitavecchia coast) (Fig. 3). One primer out of eight (BY14) was not able to generate scorable bands while a couple of primers (BY11 and BY13) supplied little informations. In conclusion, an appropriate choice of the oligonucleotide primers and the investigation of a larger number of plants would give a reliable estimation of the level of genomic polymorphism within and between *P. oceanica* populations. The results above reported confirm that RAPD markers represent a valuable tool for investigations such as phylogenetic analysis and that they could be used for monitoring the diffusion of single genotypes after transplantation programs.

REFERENCES

BOUDOURESQUE C.F., A. JEUDY DE GRISSAC and J. OLIVER (Eds.), 1984. International Workshop on *Posidonia oceanica* Beds. GIS Posidonie, Marseille, France: 454 pp.

DELLAPORTA S. L., J. WOOD and J.B. HICKS, 1983. A plant DNA mini-preparation : version II. P.M.B.R., (1) 4 : 19-21.

ECHT G. S., L. A. ERDAHL and T. J. MCCOY, 1992. Genetic segregation of random amplified polymorphic DNA in diploid cultivated alfalfa. *Genome* 35 : 84-87.

MEINESZ A., CAYE G., F. LOQUES and H. MOLENAAR, 1993. Polymorphism and development of *Posidonia oceanica* transplanted from different parts of the Mediterranean into the National Park of Port-Cros. *Botanica Marina*, 6 : 209-216.

MEINESZ A and J.R. LEFEVRE, 1984. Regénération d'un herbier de *Posidonia oceanica* 40 ans après sa destruction par une bombe dans la rade de Villefranche sur mer (Alpes Maritimes-France). In: C.F. BOUDOURESQUE, A. JEUDY DE GRISSAC and J. OLIVER (Eds.) (1984): International workshop on *Posidonia oceanica* beds. GIS Posidonie Publ., Marseille, France, 1 : 99-104.

WILLIAMS J.G.K., A.R. KUBELIK, K.J. L. J.A. RAFALSKI and S.V. TINGEY, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nuclei Acids Research*22 : 6501-6531.

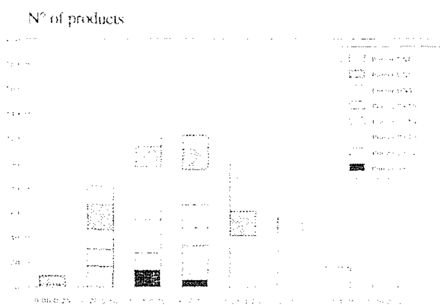

Figure1

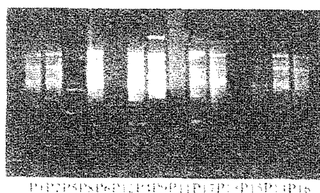

Figure 2

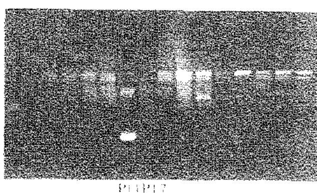

Figure 3