SHORT COMMUNICATION

DNA EXTRACTION FROM LEAVES OF *Vaccinium cylindraceum* SMITH (ERICACEAE). THE USE OF RAPD MARKERS TO DETECT GENETIC VARIATION. PRELIMINARY RESULTS.

JUAN MARTÍN-CLEMENTE, M. JOÃO PEREIRA, & CÉSAR PEREZ-RUIZ

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Vaccinium cylindraceum Smith is an endemic *Ericaceae* from Azores archipelago. This species is being produced by seed germination, micropropagation and stem cuttings. The produced plants are then reintroduced in their natural but disturbed environment and used to repopulate protected areas meanwhile infested with more or less invasive exotic flora. In order to protect the genetic variability within the species, a study of genetic variation between and within populations from different islands was started. DNA extraction was achieved on fresh, dried and criopreserved leaves and the First results using the PCR technique are also presented.

Juan Martin-Clemente & César Perez-Ruiz, Departamento de Biologia Vegetal, Universidad Politécnica de Madrid, Ciudad Universitaria, ES-28040 Madrid, Spain. - M. Jodo Pereira (e-mail: mjpereira@notes.uac.pt), , Universidade dos Açores, Departamento de Biologia, Rua da Mde de Deus 58, PT – 9501-801 Ponta Delgada, Portugal.

INTRODUCTION

One of the most difficult issues in conservation is how best to assess the minimal area needed for a communities survival. That implies at least the knowledge of the density of each species within their several types of natural communities and also their reproduction strategies. One way to assess the minimum area is to determine the area that includes those individuals that together possess most of the allelic variation within the species. This kind of knowledge is also necessary when reproduction *ex situ* is used as a tool to raise the number of individuals in a population and therefore raise the number of certain genotypes.

Information concerning these aspects is required to develop safe strategies for sampling, for example sampling the highest number of mother-plants, and introducing their descendants in the same proportions into the original populations. The assessment of genetic diversity in endangered plants can therefore help to enhance conservation efforts and strategies (HOLLINGSWORTH et al. 1998). Various molecular methods have been employed to investigate levels of variation within and between populations (BACHMANN 1994).

Vacciniunz cylindraceum Smith is an endemic shrub present only in seven of the nine Azorean islands, generally above 400 m and is a key species in different natural communities (DIAS 1996). Sampling all the individuals in a wild population for reproduction is often impossible due to their inaccessibility. Also, the obligation to introduce the produced plants in the same population of origin leads us to a considerable

effort of tagging the plants. Another aspect to consider is the possibility of raising the inbreeding depression, if the sampled population have already attained a reduced level of genetic variation (GUSTAFSSON & GUSTAFSSON 1994). These aspects lead us to the study of some natural reproduction aspects (breeding system: self fertilisation or outcrossing; flowers, fruits and seeds productions, seeds viability and seedlings vigour), and also to the study of genetic variation between and within populations. Spontaneous autogamy is not significant in this species, where fruit and seed development depends mainly on the pollinators activity (PERELRA et al. 1998).

In 1996 a preliminary investigation was made at 'Universidad Politécnica de Madrid' focused on the capacity to extract DNA from fresh and preserved material and also to survey the potential use of RAPD markers to assess genetic variation.

This preliminary investigation was presented at the 3rd Symposium of Fauna and Flora of the Atlantic Islands.

MATERIAL AND METHODS

The Random Amplified Polymorphic DNA (RAPD) is a Polymerase Chain Reaction (PCR) based technique, which has been used to assess genetic variation and kinship relationships in plants (CHALMERS et al. 1992; GUSTAFSSON & GUSTAFSSON 1994; HADRYS et al. 1992; HUFF et al. 1993,1994; NEWBURY & FORD-LLOYD 1993). Since almost all RAPD markers are dominant or rarely co-dominant (WILLIAMS et al. 1990), these markers are capable of distinguishing homozygous individuals but to distinguish heterozygous individuals other, more sensitive, molecular techniques are required (NEWBURY & FORD-LLOYD 1993). NYBOM (1993) states that the RAPD technique can be used in the study of population's genetic variability when the individuals are genetically distant due to xenogamy. Conceptually less complex and expensive than other PCR based techniques, this technique works on anonymous genomes and the profile of amplification products detects individual polymorphisms (HADRYS et al. 1992).

In this study, fresh leaves from nine seedlings of *Vaccinium cylindraceum*, all from the same population but with different parents, were used to determine the effectiveness of the DNA isolation technique and the leaf weight required to obtain profitable DNA extractions. Four other seedlings also from the same population, were used to assess the possible use of dried and crio-preserved leaves for DNA extractions.

DNA was isolated according the method described by TORRES et al. (1993) (except the final total of RNAse) and measured by electrophoresis in agarose gel, using samples with standard DNA weights. Nanograms of total genomic DNA were subjected to PCR using 3 different 10-mer nucleotide primers of random sequence from Operon Technologies Inc. (P3, P7 and P9, from kit 0) in the Perkin Elmer 480 DNA thermal cycler. The PCR was initiated at 94ºC for 1 minute and then cycled 35 times: 92ºC, 30 seconds + 37ºC, 1 minute + 72ºC, 2 minutes; with a terminator of 72°C for 3 minutes. After completion the reactions were kept at 5°C until electrophoresis. Fragments generated by amplification were separated on 1,2 % agarose gel run in 1XTBE (pH 8,0) and stained with ethidium bromide and made visible by illumination with ultraviolet light.

RESULTS AND DISCUSSION

We have been able to extract DNA successfully from *Vaccinium cylindraceum* following the method described by TORRES et al. (1993). From starting weights of 50 to 200 mg of fresh seedling leaves, we have been able to extract 1250 ng to 6500 ng of DNA, with a mean value of 34.2 ng I mg of fresh tissue (approximate values) (table I and figure 1). These extractions yielded sufficient amounts of DNA for RAPD reactions.

When fresh leaves are not available, criopreserved leaves can be used for DNA isolation and RAPD reactions while extractions from dried leaves do not yield sufficient amounts of DNA for RAPD reactions (figure 2).

The primers used revealed some polymorphism (figure 3) indicating the potential use of RAPD markers to assess genetic variation within this species. Further studies will be continued at the University of the Azores.

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Table 1
DNA quantification (estimated values).

Sample number (gel)	Plant	Leaves weight (mg)	Number of leaves used	Extraction Solution (µl)	DNA Concentration (ng/µl)	Total DNA (ng)	DNA (ng) / leaves (mg)
4	e	50	4	50	45	2250	45
1	a	52	3	100	35	3500	67
5	f	55	4	50	25	1250	23
3	c	95	3	50	65	3250	34
2	b	114	2	100	35	3500	31
6	d	140	3	100	55	5500	39
7	g	150	3	100	65	6500	43
8	h	200	3	50	45	2250	11
9	i	200	2	100	30	3000	15
Mean value							34.2

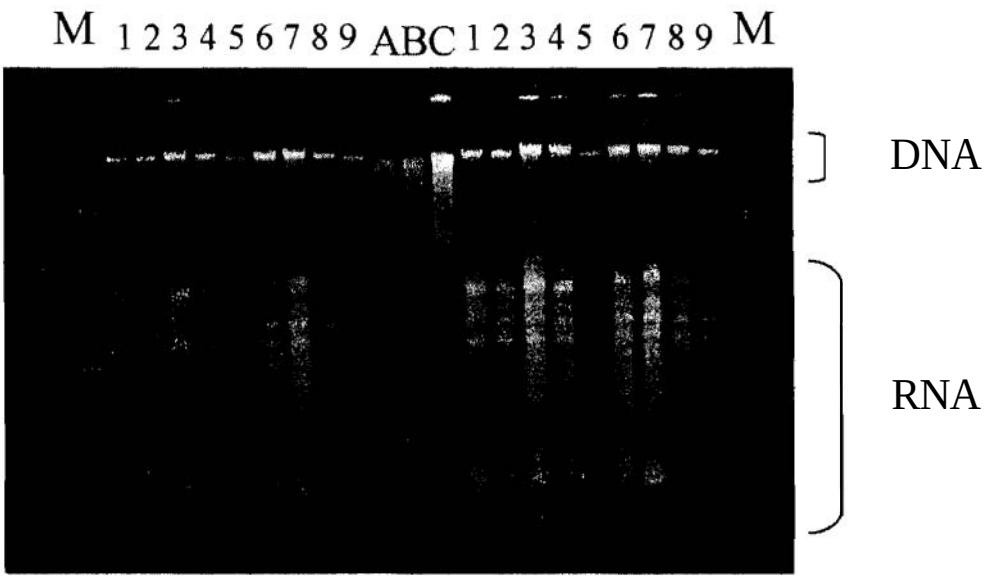

Fig.1 DNA isolated from fresh juvenile leaves. From the left to the right: M=Control size marker; samples number 1,2,3,4,5,6,7,8,9; A=100ng DNA; B=200ng DNA; C=400ng DNA; samples number 1,2,3,4,5,6,7,8,9; M=Control size marker. The samples placed at right are 2 times more concentrated then the samples placed at left.

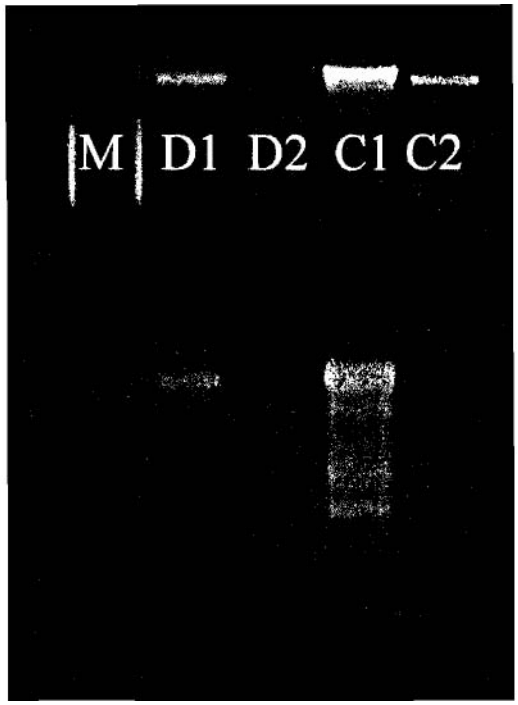

Fig. 2. Quantity of DNA isolated from dried and crio preserved leaves. M = Control size marker. D1 and D2 = DNA from dried leaves. C1 and C2 = DNA from frozen leaves. C1 and C2 samples are 3 times more diluted then D1 and D2 samples.

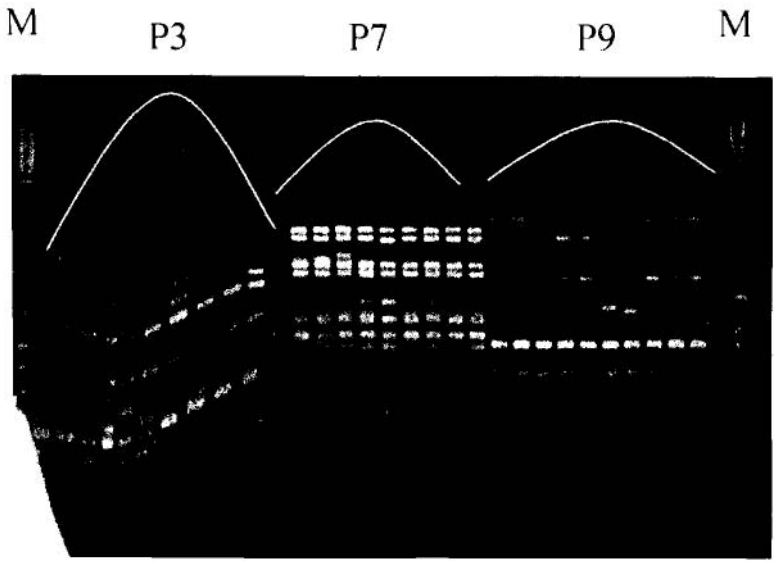

Fig. 3. Random Amplified Polymorphic DNA (RAPD). From the left to the right: M = Control size marker; P3 = Primer 3: samples 1,1,2,3,4,5,6,7,8,9; Negative control: P7 = Primer 7: samples 1,2,3,4,5,6,7,8,9; P9 = Primer 9: samples 1,2,3,4,5,6,7,8,9; Negative control, M = Control size marker. Kit O_from Operon 10-mer primers. 9 samples from 9 seedlings with origin in 9 different shrubs from de same population on S. Miguel Island.

REFERENCES

BACHMANN, K. 1994. Molecular markers in plant ecology. *New Phytologist* 126: 403-418.

CHALMERS, K.J., R. WAUGH, J.I. SPRENT, A.J. SIMONS & W. POWELL 1992. Detection of genetic variation between and within populations of *Gliricidia sepium* and *G. maculata* using RAPD markers. *Heredity* 69:465-472.

GUSTAFSSON, L. & P. GUSTAFSSON 1994. Low genetic variation in Swedish populations of the rare species *Vicia pisijormis* (Fabaceae) revealed with RFLP (rDNA) and RAPD. *Plant Systematics and Evolution* 189:133-148.

HADRYS, H.. M. BALICK & B. SCHIERWATER 1992. Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. *Molecular Ecology* 1:SS-63.

HOLLINGSWORTH, P.M., M. TEBBIT, K.J. WATSON & R.J. GORNALL 1998. Conservation genetics of an arctic species. *Saxifraga rivularis* L. in Britain. *Botanical Journal of the Linnean Society of London* 128: 1-14.

HUFF. D.R., R. PEAKALL & P.E. SMOUSE 1993. RAPD variation within and among natural populations of outcrossing buffalograss [*Buchloë dactyloides* (Nutt.) Engelm.]. *Theoretical and Applied Genetics* 86:927-934.

HUFF, D.R., T.F. BUNTING & K.A. PLUMLEY 1994. Use of random amplified polymorphic DNA markers for the detection of genetic variation in *Magnaporthe poae*. *Phytopathology* 84:1312-1316.

NEWBURY, H.J. & B.V. FORD-LLOYD 1993. The use of RAPD for assessing variation in plants. *Plant Growth Regulation* 12:43-51.

TORRES, A.M., N.F. WEEDEN & A. MARTÍN 1993. Linkage among isozyme. RFLP and RAPD markers in *Vicia faba*. *Theoretical and Applied Genetics* 85:937-945.

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