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The Assesment of Two Populations of *Gentiana nivalis* By RAPD Markers

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Abstract:

The amount of genetic variation in the rare annual herb *Gentiana nivalis* L was determined to explore its relation to population size. We surveyed two populations of *G. nivalis* found in Oriental Carpathians, by RAPD markers. Four decamer primers of arbitrary sequence were used in order to asses genetic variability within and between the two populations. Our studies revealed two different levels of genetic variability within populations in relation with the grazing policies. The mean genetic distances within the population found in overgrazed grassland decreased significantly in comparison with that growing in ungrazed grassland. Therefore, this method can by successfully used to asses genetic variability within and between *Gentiana* populations.

INTRODUCTION

The RAPD (Randomly Amplified Polymorphic DNA), a very cost effective and fast method, is suitable for the investigation of the genetic variability. We have used this method to investigate the genetic variability within and between two populations of alpine gentian *G. nivalis*, a rare montane annual growing plant in alpine grasslands. The small size populations have a negative effect over the genetic variability and reproductive capacity of the *Gentiana* species (MARC K 2000).

MATERIAL AND METHOD

Biological material was represented by leaves, collected from 20 individuals randomly selected from two populations, (ten individuals for each population) of *G. nivalis*, situated at about 110Km apart in Oriental Carpathians. Samples 106 to 115 were collected from Corongiş Peak – Rodnei Mountains population and samples 162 to 171 from Ceahlău Mountain plateau population. Both populations are situated at around 1800 m altitude. As an out group (O), a mixture of the DNA isolated from ten individuals of *Gentiana asclepiadea*, randomly selected from different populations, was used.

The DNA was isolated, using a Lodhi *et al*. 1994, protocol modified by Pop Rodica 2003. Prior to DNA isolation, the leaves were grinded in liquid nitrogen. DNA concentration and purity (absorbance ratios at $^{A260}/_{A280}$ and $^{A260}/_{A230}$) were estimated in a BioPhotometer Eppendorf.

The PCR amplification was performed in 25µl reaction volumes containing 5x reaction buffer (GoTaq Green Master Mix, Promega), 0.2 mM dNTPs, 2.5 mM $MgCl_2$,

0.5 μM primer, 2% PVP (Polyvinylpirolodone, Sigma), 1.0 unit of Taq DNA polymerase (GoTaq DNA Polymerase, Promega) and ~ 50 ng genomic DNA. Four decamer primers of arbitrary sequence (OP series, of the Operon Technologies Inc, CA, USA) were tested for PCR amplification (Table 1). PCR amplification was carried out in a Eppendorf Gradient termocycler programmed as follows: initial denaturation at 95°C for 1 min., followed by 45 cycles of 1 min. at 93°C, 1 min. at 34 °C, and 1.5 min at 72°C and final extension at 72°C for 10 min.

Table 1: Primers sequences

Primer	Sequence
OPA 01	5'- CAG GCC CTT C -3'
OPA 03	5'- AGT CAG CCA C -3'
OPB 10	5'- CTG CTG GGA C -3'
OPAB 11	5'- CTG CGC AAT G -3'

Amplification products were subjected to electrophoresis in a 1.4% (w/v) agarose gel TAE buffer (Sambrook et al., 1989) at 60v for 2h. A 100bp ladder (Promega) was used in all cases as the size marker. Gels were stained with $0.5\mu g/ml^{-1}$ ethidium bromide, visualized under UV light and photographed using a AlphaInnotech imager.

Polymorphic bands were scored as present (1) or absent (0) for each of the primer – sample combination. Based on these data, genetic distances were calculated by Jaccard coefficient and UPGMA trees were generated using FreeTree 0.9.1.50 software.

RESULTS AND DISCUSIONS

PCR amplifications revealed 26 polymorphic bands from a total number of 32 bands (81,25%). The bands were distributed as follows: primer OPA 01 - 10 bands (10 polymorphic) (Fig 1), OPA 03 - 8 bands (6 polymorphic), OPB 10 - 7 bands (7 polymorphic) and OPAB 11 - 7 bands (3 polymorphic).

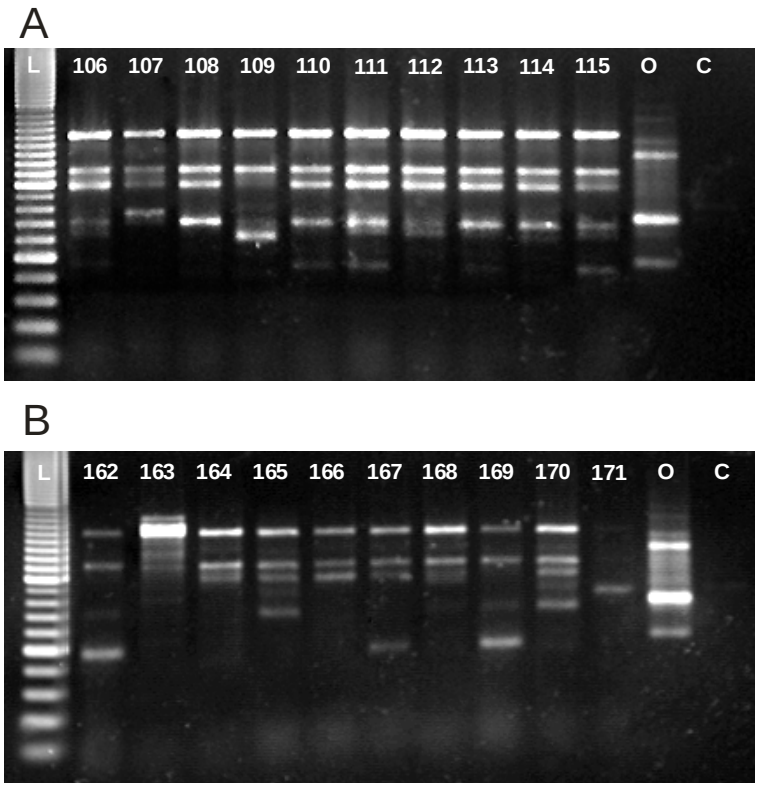

Figure 1: The amplification products obtained with primer OPA 01. (L = ladder, O = out group, C =

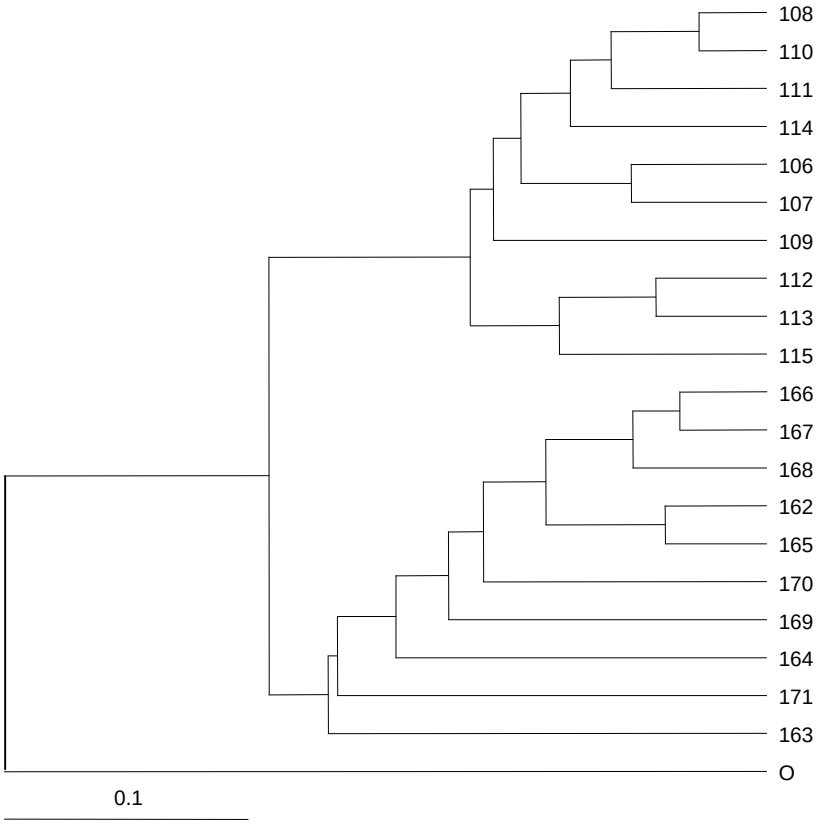

Figure 2: The graphic representation of the genetic distances and relations (cladogram) within accessions

negative control)

The cladogram generated based on distance matrix revealed two main clusters clearly separated. The distribution of the samples in each cluster corresponded with the distribution of the samples in the populations (Fig. 2). Furthermore, a high distance coefficient was found between the two populations of *G. nivalis*. This can be the result of the geographical isolation of the two populations.

The mean distance in population 1 was 0.209 with a standard deviation of 6.260×10^{-2}, for the population 2 the mean distance was 0.275 with a standard deviation of 9.941×10^{-2}. We assume that the higher mean distance in population 2 compared with population 1, was due to the genetic variability in population 2 which was significantly (✳✳✳) higher than in population 1 (t=3,76; df=88). A possible explanation for these results can be found in the sizes and density of the populations: population 1 is constituted by a number of approximately 30 individuals and the population 2 is constituted by several hundreds of individuals. The difference in population sizes can be explained by differences in the grazing policy of the populations areas. Ceahlău Mountain plateau is situated in a protected area where the grazing is forbidden. In contrast with this situation, Corongiş peak and surrounding areas are intensively grazed, and so, although the favorable environmental conditions are extended over the whole

area, the *G. nivalis* plants can only be found in few small areas protected from grazing by natural barriers.

Our study is in contradiction with a study conducted by Miller G. et al. - 1999. The authors of that study observed that the gentians on ungrazed plots grew taller and survived better than did plants in adjacent grazed plots. The density of plants on ungrazed plots was unaffected for the first years but thereafter declined. Perennial vegetation responded to protection from sheep grazing by growing taller and denser and progressively reduced the amount of bare soil in the ungrazed plots. They concluded that the loss of potential gaps for seedling establishment was probably the main cause of the decline in alpine gentian density on the ungrazed plots and so the presence of sheep helps to maintain alpine gentian colonies in grassland.

CONCLUSIONS

According to our results the RAPD technique can be successfully used for the survey of the genetic variability within and between populations of G. nivalis. We found that the genetic variability within the Corongiş Peak population is very low and therefor population can be considered endangered. The grazing of the alpine grasslands has a negative effect on the genetic variability and stability of the G. nivalis populations. Due to the results obtained, the method can be also extended to study the genetic variability in other population of *G. nivalis* and even other *Gentiana* species.

BIBLIOGRAPHY

1. Lodhi , M., A., Guang-Ning Y., N. F.Weeden, B.I. Reisch, 1994, A simple and efficient method for DNA extraction from grapevine cultivars, *Vitis* species and *Ampelopsis*, Plant Molecular Biology Reporter 12 (1): 6-13
2. MARC K, D MATTHIES and H.H.SPILLMANN, 2000, Reduced fecundity and offspring performance in small populations of the declining grassland plants Primula veris and Gentiana lutea. Journal of Ecology, 88, 17-30
3. Millera G. R., C. Geddesb and D. K. Mardon, 1999, Response of the alpine gentian Gentiana nivalis L. to protection from grazing by sheep, Biological Conservation, 87(3), 311-318.
4. Pop Rodica., M. Ardelean, D. Pamfil, Ioana Marina Gaboreanu, 2003, The Efficiency of Different DNA Isolation and Purification in Ten Cultivars of Vitis vinifera., Bul. Nr. 59 USAMV, seria ZB, 259-26
5. Sambrook, J., Fritsch, E.F., Maniatis, T. (1989) Molecular cloning: a laboratory manual. 2nd ed. New York: Cold Spring Harbor Laboratory Press.
6. Williams J., Kubelik A., Livak K., Rafalski J., Tingey S., 1990, Dna Polymorphisms Amplified by Arbitrary Primers are Useful as Genetic Markers, Nucleic Acids Research, 18 (22) 6531-6535.