THE USE OF ISOZYMES MARKERS FOR THE CHARACTERIZATION OF *Convolvulus persicus* L. INDIVIDUALS BELONGING FROM SULINA POPULATION

VOICHIȚĂ Carmen, HELEPCIUC Florența, HOLOBIUC Irina

Abstract. *Convolvulus persicus* L. is an endangered species, which grows in South –East of Romania, on the sandy area, near Sulina, Agigea, Mamaia, Eforie and Sf. Gheorghe towns. Our study deals on a population located near Sulina town, samples from several individuals were collected as fresh plant material represented by young fragment of shoots without flower buds. The aim of our study was to evaluate the isozymes electrophoretic spectra and to select the most suitable for intrapopulational diversity characterization. The enzymes analysed were peroxidases, catalases, esterases and alkaline phosphatases. The obtained electrophoretic spectra in the case of peroxidases and catalases showed polymorphism for individuals analysed. In the case of esterases and alkaline phosphatases, electrophoretic spectra did not detect a large variation, these enzymes being not suitable for characterization of the variability in this species.

Keywords: isozymes, elecetrophoretic spectra, intrapopulational variability.

Rezumat. Utilizarea markerilor izoenzimatici pentru caracterizarea indivizilor de *Convolvulus persicus* L. aparținând unei populații din Sulina. *C. persicus* este o specie critic pericliată, răspandită în sud-estul Romaniei, pe litoral, în zona oraşelor Sulina, Agigea şi a localităţilor Mamaia, Eforie şi Sf. Gheorghe. Cercetările noastre au vizat o populaţie situată în zona limitrofă a oraşului Sulina, fiind recoltate probe de la mai mulţi indivizi, reprezentate de fragmente de lăstari fără muguri florali. Scopul acestui studiu a fost evaluarea spectrelor electroforetice ale izoenzimelor şi selectarea celor adecvate pentru caracterizarea variabilitaţii intrapopulaţionale. Enzimele analizate au fost peroxidazele, catalazele, fosfatazele alcaline şi catalazele. Analiza spectrelor peroxidazelor şi catalazelor au evidenţiat un polimorfism ridicat în cazul indivizilor analizaţi. In cazul spectrelor electroforetice ale esterazelor şi fosfatazelor alcaline nu a fost detectată o plaja mare de variaţie, aceste enzime nefiind indicate pentru caracterizarea variabilităţii acestui taxon.

Cuvinte cheie: izoenzime, spectre electroforetice, variabilitate intrapopulaţională.

INTRODUCTION

Convolvulus persicus L. is a critically endangered plant species (DIHORU & NEGREAN, 2009) growing in the South-East of Romania at the seaside region in the neighborhood of Sulina, Agigea Mamaia, Eforie and Sf. Gheorghe. This taxon grows on drought sands, neutral, poor in nitrogen, in sunny areas, being a perennial species with sexual and asexual reproduction. The flowers are large and white and its high is below 50 cm and has white- pubescent leaves with petioles and seeds brown-black.

C. persicus is considered useful for decorative purpose having also a role in sand stabilization. This plant was used in the past as traditional homeopathic treatment by the indigenous peoples for insomnia and for respiratory diseases, being surnamed as "fisherman tea". It contains active calming and emollient principles.

The factors that restricted the area of this species is anthropization and mechanical cleaning of the beach, the ruderalization and parasitic fungi attack.

The conservation of this threatened species has to be made both *in situ* and also *ex situ*. To adopt different preservation strategies it is important to know the range of variation among the individuals from the natural populations.

In the case of *ex situ* conservation, it is also important to have a wide variation inside the population as if to ensure the possibility to survive and to adapt to various environmental conditions. The genetic variation inside a certain species or population is important because the heterozygosity is positively related to adaptative fitness (ALLENDORF & LEARY, 1986; BENSON, 1999). The use of biotechnologies as preservation mean also involved the evaluation of the stability of the plant material maintained during different period of time.

In this respect, biochemical and molecular markers are useful to characterize the variation among individuals and also the stability/variability of regenerants obtained after the use of biotechnological methods as mean of *ex situ* conservation (HALMAGY & BUTIUC-KEUL, 2007). Genetic markes are observable traits which can be detected at different levels: morphological, cytological, biochemical and molecular.

An ideal genetic marker has to show a qualitative and/ or quantitative variation, to not have environmental influences and to show a simple inheritance.

"…An efficient conservation of taxa must be based of a sound understanding of phylogeny; the amount and distribution of genetic variation; and the design of effective sampling and conservation methods" (HARRIS, 1999).

Despite of progress concerning molecular markers based on DNA-techniques, protein-based techniques (alloenzymes and izoenzymes) are still also used because their low cost and simplicity (WEEDEN &WEEDEN, 1990). The main advantages of isozymes are represented by codominant transmission in most cases (but not always), simplicity of identification technique (BUTIUC-KEUL, 2006). Isozymes detection is based on the staining of proteins with identical

function, but different electrophoretic mobilities. Several genes bcan codify different proteins with the same enzymatic function and different mollecular weight. As an disavantage of this technique we can mention the need of using fresh plant material and the fact that they may be not expressed in the same tissue and at the same time in development. This problem is solved if the same type of samples are used to detect enzymes activity.

Our aim was to detect the most appropriate izoenzyme marker to characterize the variation among several *C. persicus* L. individuals from Sulina population, using different peroxidases (POX), catalases (CAT), esterases (EST) and alkaline phosphatases (AKP) electrophoretic spectra analysis.

MATERIALS AND METHODS

At Sulina, *Convolovulus persicus* taxon has a well represented population located at the sandy beach close to Black Sea (Fig.1).

The plant material was collected from this seaside sandy area. Fragments of young shoots without flowers were detached from 16 individuals situated at least 3-5 m distance one from another.

The shoots were fragmented in small pieces to facilitate the extraction of total proteins. The enzymes extraction was carried out in 50mM phosphate buffer, pH=7, EDTA 2mM, 4% PVP. The tissues samples were ground with quartz sand and the homogenate was centrifugated at 15000 rpm, for 20 min, the supernatant was used for electrophoresis to detect several enzymes: peroxidases (POX), esterases (EST), catalases (CAT) and alkaline phosphateses (AKP). The polyacrylamide gel electrophoresis was prepared using a 10% (7% for catalases) running gel and the running buffer was 0.05M Tris-Gly, pH 8.3.

We used H_2O_2 as substrate in acetate buffer and benzidine for peroxidases detection (WANG & WANG, 1989). In the case of catalases, we used as substrate 0.003% H_2O_2 prepared in 0.01 M phosphate buffer, at pH=7, added with $K_3(Fe(CN)_6)$ and $FeCl_3$ (IORDACHESCU & DUMITRU, 1988). For esterases detection, we used as substrate a solution of α/β naphthyl acetate and Fast Blue RR prepared in 0.1 M phosphate buffer, at pH=6.5 (BACH, 1989 modified) and for alkaline phosphatases analyse, the substrate was Na α/β naphthyl phosphate prepared in Tris –citrate, at pH=8.3.

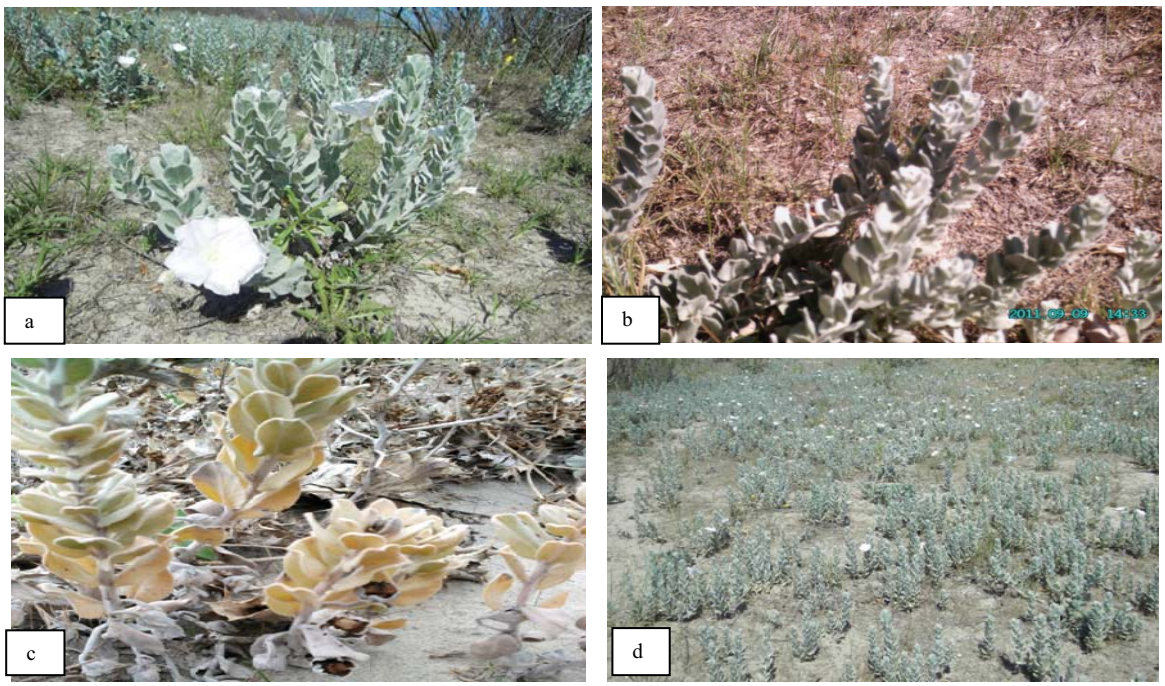

Figure 1. *C. persicus*: a. plant in anthesis, b. young shoots without flowers, c. plant with seeds, d. the population of *C. persicus* on the Sulina beach sands.

RESULTS AND DISCUSSIONS

In the case of POX, the electrophoretic spectrum analyzed in 16 individuals showed polymorphism (Fig. 2). There were detected two first bands common for all individuals. The number of bands of the POX isoenzyme patterns varied from two to seven bands.

The electrophoretic spectrum of individuals 10, 11, 13, 14, 15, 16 revealed 5 bands, while the individuals 1, 2, 3, 5, 6, 8 had just 2 bands. For 4 and 9 individuals were observed 3 bands. The individual number 12 presented 7 electrophoretic bands.

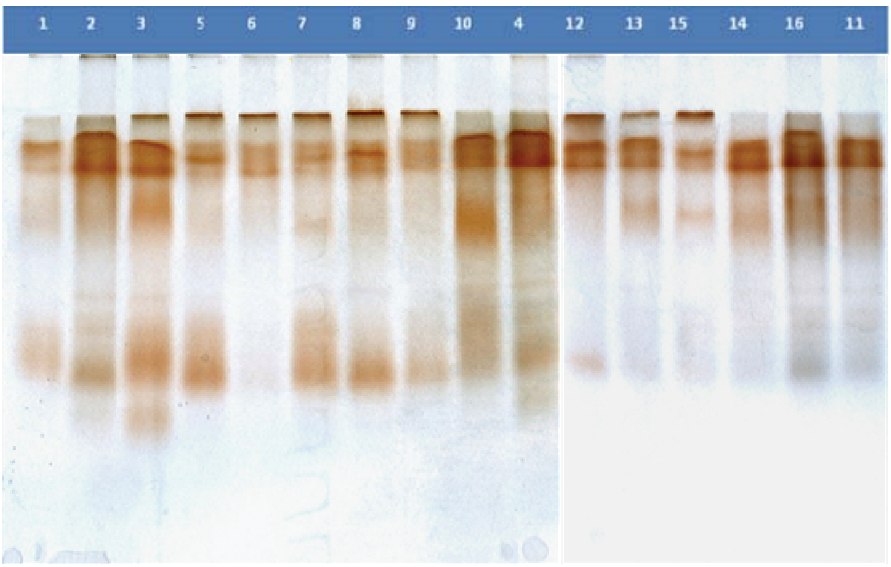

Figure 2. POX electrophoretic spectrum for 16 individuals of *C. persicus.*

Esterase spectrum showed several electrophoretic bands, but because they are very close one from the another it is difficult to estimate the real number and to appreciate the level of variability (Fig. 3).

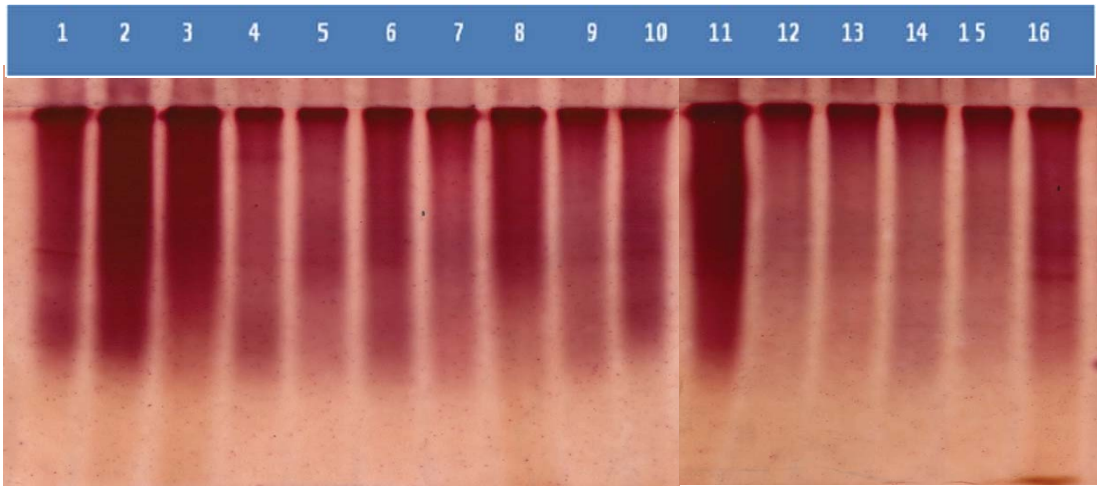

Figure 3. EST electrophoretic spectrum for 16 individuals of *C. persicus.*

In the case of AKP, electrophoretic spectrum showed the presence of a single band at individual number (Fig. 4).

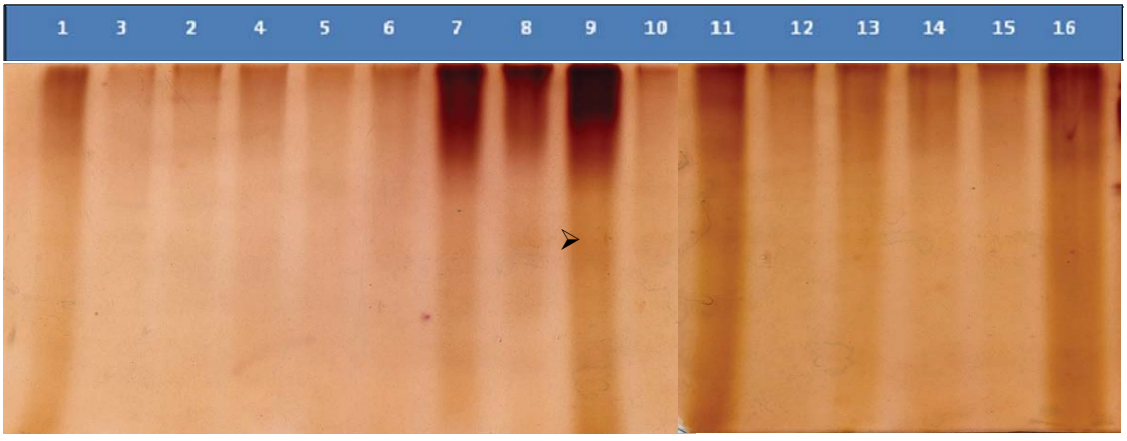

Figure 4. The electrophoretic spectrum of AKP for 16 individuals of *C. persicus.*

CAT electrophoretic spectrum is identically for individuals number 2, 3, 4, 5 and 6. In the case of individuals number 8, 9, 11, the expression of CAT was not detected (Fig. 5).

The individual number 7 showed a single band. The individual number 14 presents also a single band with the same Rf (Retention factor) like the second band of individuals number 2, 3, 4, 5, 6.

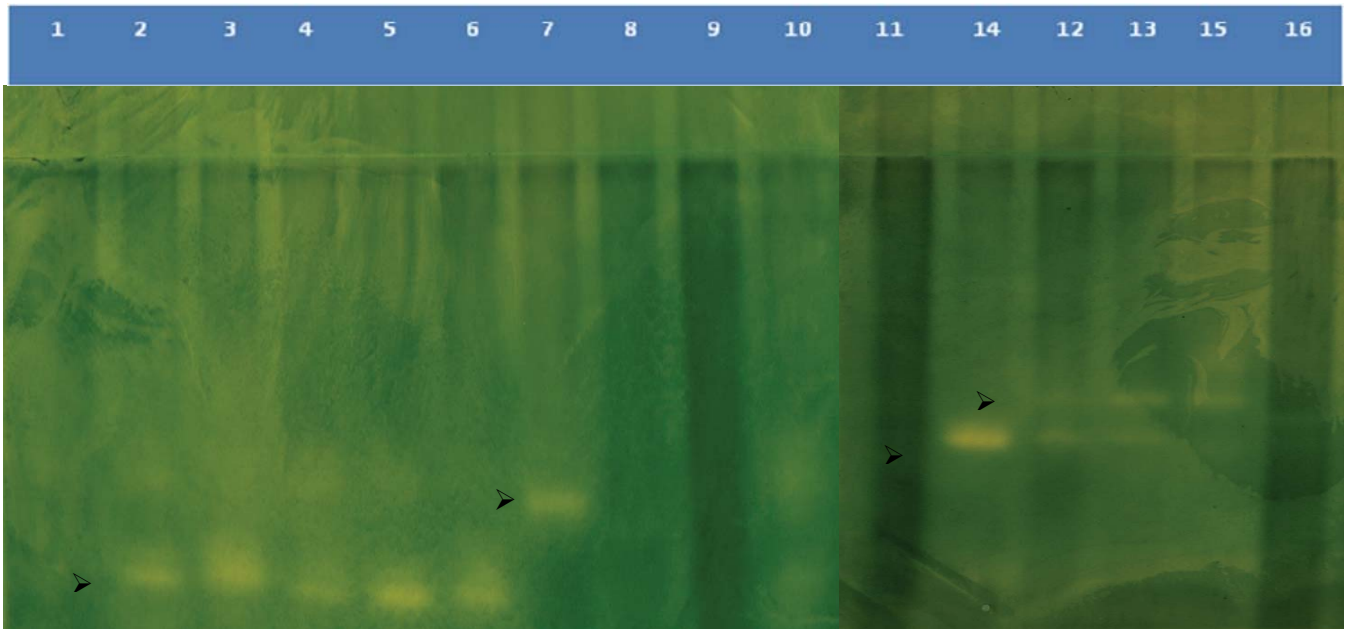

Figure 5. CAT electrophoretic spectrum for 16 individuals of *C. persicus.*

Some authors consider that are only few studies about genetic composition of wild species and the number is even lower when considering endangered or rare species (GONZALES-BENITO et al., 1999), but the studies regarding the involvement of isozymes in the assessment of genetic diversity are numerously mainly in crops. In this respect, our studies could be useful for intra-population variability characterization.

Despite this, there are some studies in which enzyme spectra was successfully used to detect the polymorphism in the case of some endangered or endemic plant species (ZHELEV et al., 2002; BORBA et al., 2007; KENEDY et al., 2007; KULL & OJA, 2010).

It is well known that peroxidases have specificity of tissue, their activity depending on the stage of plant development and growth. In our case, all the samples from the individuals of *C. persicus* were in the same stage of development- young shoots, being collected in the same time. KRZAKOVA (1996) also characterized the intra-populational diversity in the case of *Phragmites australis* using peroxidases spectra.

Other authors described that the isoperoxidases pattern in *Puccinella poissoni* showed a small intrapopulational diversity, while the isoesterases had a relevant pattern. The electrophoretic specta of POX and EST were identically to all plants analysed from *Jurinea molis* species.In the case of POX existed an acid isoperoxidase and for EST it was shown 2 acid isoesterases. These electroforetic bands are present to all plant analysed (BUTIUC-KEUL, 2006).

In our analyses concening the possibility to use isoenzyme spectrum to detect variation among the individuals of *C. persicus*, just POX and CAT are suitable for the evaluation of intra-populational diversity, while esterases and phoshatases were not optimally for this purpose.

Because of different expression of genes codifying these enzymes depending of taxon, it is necessary to carry out analyses to detect the best and cheaper marker. Despite molecular markers widely used, the enzyme detection could be more affordable.

Genetic polymorphism was described using others biochemical markers as: alcohol-dehydrogenase, superoxide dismutase, and malatdehydrogenase (BORZA et al., 1996).

There were using more enzymatic systems for identify the genetic polymorphism as well as: leucyl aminopeptidases, phosphoglucomutase, menadione reductase for *Quercus robur* and *Q. petrea* (MÜLLER-STARCK et al., 1993).

CONCLUSIONS

This study was focus on the biochemical characterization of several individuals of. *C. persicus* from Sulina population using different enzymes electrophoretic spectra.

These studies were for the first time carried on in this threatened, critically endangered species concerning the variation among individuals from a wild population.

There were analysed the electrophoretic spectra of peroxidases, catalases,esterases and alkaline phosphatases.

The results showed a polymorphism regarding catalases and peroxidases spectrum, while in case of alkaline phosphatases and esterases were not detected a relevant polymorphism.

The individuals analysed are different concerning the biochemical parameters, fact demonstrated by a different number of electrophoretic bands.

This study represents the first step in the characterization of intrapopulational variability.

When is adopted an *ex situ* conservation strategy it is important to have suitable methods to characterize the population genetic variability, for collecting the samples from the natural habitat and the plant material stability assesment after different preservation procedures.

ACKNOWLEDGEMENTS

The research was supported financial by Romanian Academy from project no. RO1567-IBB06/2011: **„Studies regarding ex situ conservation of natural genofond for establishing a bank for plant tissue and cell culture for biotehnological and conservative purpose”**.

REFERENCES

ALLENDORF F. W. & LEARY R. F. 1986. *Heterozygosity and fitness in natural populations of animal.* In: Soule, M. E. (Ed.), Sunderland, Massachusetts. Sinauer Associates. Conservation Biology: 57-76.

BORBA E. L., FUNCH R. R., RIBEIRO P. L., SMIDT E. C., SILVA-PEREIRA V. 2007. *Demography, and genetic and morphological variability of the endangered Sophronitis sincorana (Orchidaceae) in the Chapada Diamantina.* Plant Systematics & Evolution. **267**(1-4): 129-147.

BUTIUC-KEUL ANCA. 2006. *Markeri moleculari utilizaţi în genetica şi biotehnologia vegetală*. Edit. Mega Cluj-Napoca: 106-107.

BORZA T., COMAN N., DRAGOS N. 1996. *Preliminary study of allozyme polymorphism in the endemic species Astragalus peterfi*. Contributii Botanice: 197-201.

BACH E. E. 1989. *Utilização da eletroforese no estudo de alterações enzimáticas na interação planta-patógeno*. In Encontro sobre aplicações da eletroforese na agropecuária. Nova Odessa. **1**: 1-4.

DIHORU GH. & NEGREAN G. 2009. *Cartea Roşie a plantelor vasculare din Romania.* Edit. Academiei Romane Bucureşti: 178-179.

GONZALES-BENITO M. E., MARTIN C., IRIONDO J. M., PEREZ C. 1999. *Conservation of the rare and endangered plants endemic to Spain*. In: Erica E. Benson (Ed.) Plant conservation biotechnology. Edit. Taylor & Francis Inc. 325 Chestnut Street. Philadelphia. 260 pp.

HARRIS S. 1999. *Molecular approach to asssesing plant diversity*. In: Erica E. Benson (Ed.) 1999. Plant Conservation Biotechnology. Edit. Taylor & Francis Inc., 325 Chestnut Street, Philadelphia: 12-15.

HALMAGY ADELA & BUTIUC-KEUL ANCA. 2007. *Conservarea resurselor genetice vegetale*. Edit. Todesco, 2007. Cluj-Napoca: 79-80, 133-136.

IORDĂCHESCU DANA & DUMITRU I. F. 1988. *Biochimie practică*. Edit. Universităţii. Bucureşti. 114 pp.

KULL THEA & OJA TATJANA. 2010. *Allozyme Diversity and Geographic Variation among Populations of the Locally Endangered Taxon Carex magellanica subsp. irrigua (Cyperaceae).* Folia Geobotanica. **45**(3): 323 pp.

KENNEDY A. H. & WALKER G. L. 2007. *The Population Genetic Structure of the Showy Lady's-Slipper Orchid (Cypripedium reginae WALTER) in its Glaciated and Unglaciated Ranges*. Castanea. **72**(4): 248 pp.

KRZAKOVA M. 1996. *Review of genetic investigations in Poland.* Cryptogamie. Bryologie, lichenologie. **17**: 237-243.

MÜLLER-STARCK G., HERZOGAND S., HATTEMER H. H. 1993. *Intra- and interpopulational genetic variation in juvenile populations of Quercus robur L. and Quercus petraea LIEBL*. Annals of Forest Science. Edit. Elsevier. **50**(1): 233-244.

WANG H. C. & WANG Z. S. 1989. *The prediction of strain characteristics A. bisporus by the applications of the isoenzyme electrophoretic*. Mushroom Sci. **12**: 87-100.

WENDEL J. F. & WEEDEN N. F. 1990. *Visualisation and interpretation of plant isoyzmes*. In: SOLTIS D. E. and SOLTIS, P. S. Eds. Isoyzmes in Plant Biology. London. Chapman and Hall: 5-45.

ZHELEV P., GOMORY D., PAULE L. 2002. *Inheritance and Linkage of Allozymes in a Balkan Endemic, Pinus peuce Griseb.* Journal of Heredity. **93, 1**: 60 pp.

Voichită Carmen
Institute of Biology Bucharest, Romanian Academy, Romania.
E-mail: carmen.voichita@ibiol.ro

Received: March 31, 2013
Accepted: July 29, 2013