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MORPHOLOGICAL AND MOLECULAR GENETIC APPROACH OF *ELYMUS REPENS* AND ITS RELATED *POACEAE* SPECIES

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INTRODUCTION

Taxonomic classification is a major task of the biodiversity domain, which includes with great responsibility economic important plants, as medicinal species are. Up to now, taxonomy was conducted by the analysis of morphological characters, which frequently showed a rather limited variation leading to controversial discussions concerning the classification of related taxa [1, 13]. Modern, molecular genetic approaches, such as random amplified polymorphic DNA (RADP) analysis [1, 5, 9, 11] have been recently introduced for completing the peculiar information regarding genetic similarities of plant genomes.

In this paper we present a synthesis of our preliminary molecular taxonomic study of certain representatives of *Poaceae* (*Gramineae*) family, which includes a plant with known medicinal characteristics. A study case of a very common plant in our country, used as medicinal, *Elymus (Agropyron) repens* respectively and its relatives [2, 4,6], is presented as a model for the molecular approach in the domain of biodiversity. Our choice is based on the frequent problems encountered with the taxa of *Triticae tribe* from *Poaceae* (*Gramineae*) family, which come obvious from their numerous synonyms. Another motivation of this choice is based on the complex chorological and sozological aspects regarding the endemic and endangered position of some of these taxa in the Red List [2, 10].

The morphological features of spike and spikelets represented the main criteria routinely used up to now for the taxonomic classification for *Triticae tribe* such as:

1.Elymus repens (L.) Gould. Synonyms – *Agropyron repens* (L.) Beauv; *Triticum repens* L., *Elytrigia repens* (L.) Nevski. It is one of the most frequent *Elymus* species, found from the steppe zone up to the mountain area in bushes and grasslands. Our accessions were from the Botanical Garden, Bucharest [4].

2. Elymus elongatus (Host) Runemark subsp. *elongatus*. Synonyms- *Agropyron elongates* (Host.) Beauv.; *Triticum elongatum* Host. It is represented by numerous populations located in the South Romania and Dobrogea region, where is growing in grasslands and see shore sands.

3. Elymus farctus (Viv.) Runemark ex Melderis subsp. *bessarabicus* (Savul. &Rayss*)* Melderis. Synonyms- *Agropyron junceum (L.)* Beauv. Var. *bessarabicum* (Savul. &Rayss) Anghel & Morariu*, Agropyron bessarabicum* Savul. &Rayss, *Elytrygia juncea* subsp. *bessarabicum* (Savul.&Rayss) Tzvelev. It is represented practically only by few populations with a decreasing number of entities, which suggested that from the sozological point of view this species is critically endangered by the extensive touristic and building activities. Its accessions were made from Constanta (Eforie Sud/see shore sands).

4.Elymus sabulosus (Bieb.) Tzvelev. Synonyms- *Leymus racemosus* (Lam.) Tzvelev subsp. *sabulosus* (Bieb.) Tzvelev; *Elymus sabulosus* (Bieb.); *Elymus arenarius* L. subsp. *sabulosus*. It is a pontic species, which grows sporadicly in Romania in the Dobrogea see shores. It is considered an endangered species, considering the high antropic pressure in its habitats. Its accessions were made from Constanta (Eforie Sud).

5.Agropyron brandzae Pantu & Solac. Synonyms- *Agropyron cristatum (L.) Gaertner* subsp. *brandzae* (Pantu & Solac.) Melderis. It is related with *Agropyron ponticum* and is an endemic species for Dobrogea region, where it grows on sunny and stoney slopes. Its accessions were from Constanta-Cotul Vaii. The molecular approach partially confirmed such morphological hypotesis and suggested certain different arrangements of the taxa based on the polymorphic loci revealed by different primers used in PCR random amplifications (RAPD).

MATERIAL AND METHODS

Plant material. Herbarium specimens and fresh leaves from *Triticae* tribe accessions mentioned above (1 to 5) were used for DNA isolation.

DNA isolation. The Promega kit for DNA purification have been use for DNA preparation followed the protocol provided by company (Promega).

RAPD markers. RAPD profiles were obtained using 4 primers which amplify certain conserved minisatellite regions for core sequence minisatellite regions [14,15], in a standard PCR mixture reaction (50mM of each dNTP, 50 ng of genomic DNA, 1mM of each primer, Taq DNA polymerase and 10X standard PCR buffer). The PCR conditions reaction (Biometra thermocycler) was specifically set up for each primer and the PCR products were separated by electrophoresis through 1.5% agarose gels comparatively with a molecular marker (100/1000 bp ladder), stained with ethidium bromide and photographed under ultraviolet light with a Polaroid camera.

Data Analysis

Four RAPD profiles were obtained which showed different extent of polymorphism. The polymorphic bands were scored as a 0 (absence) and 1 (presence). The binomial matrix was used to calculate the level of polymorphism (percentage of polymorphic bands) for each sample and to compute similarities between individuals using the Jaccard's similarity coefficient, calculated as $J = a/(n-d)$, where a is the number of positive matches (i.e. the presence of a band in all samples), d is the number of negative matches (i.e. the absence of a band in all samples), and n is the total sample size including both the numbers of matches and „unmatches". The genetic distances were calculated as $GD = 1-J$ using the data from the Jaccard's similarity coefficient matrix (Table 1 to 4, represented the matrix for each sample and each primers) [16]. The minimum, maximum and mean genetic distance between the individuals for each population were compared in order to describe the molecular variance within the populations. A distance matrix was calculated using a simple Euclidian distance measure and all date were represented as a dendrogram (tree) that will give information's regarding the linkage between taxa.

RESULTS AND DISCUSSIONS

The study shows variable (3-11) extent of polymorphism depending on the type of the primers. The maximum number of polymorphic bands (11) corresponded to the primer 2. The genetic relatedness among species was obtained by dendrograms showing the cluster arrangements.

The four polymorphic patterns are represented in Fig.1 to 4. The informational potential of the resulted dendrograms are relatively good as they reproduce 2 of 4 the morphological based classification of the *Elymus* species. The results are based on the measurements of the RAPD generated DNA fragment length and the corresponding likelihood Jaccard coefficients, represented in the tables 1-4.

A.The RAPD pattern and the corresponding dendrogram generated by the primer 1.

Table 1. Jaccard coefficients calculated with the RAPD pattern generated by the primer 1

P1	1	2	3	4	5
1	0	0,858	0,775	0,834	0,66
2		0	0,25	0,834	0,858
3			0	0,66	0,775
4				0	0,834
5					0

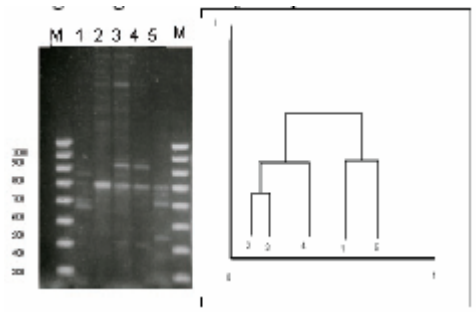

Fig.1. a) RAPD polymorphisms with primer 1. 1-5 samples*: 1. Agropyron repens, 2. Elymus elongatus. 3. Elymus bessarabicus, 4. Elymus sabulosus, 5. Agropyron brandzae, M-molecular marker. b)* dendrogram based on Jaccard similarity coefficient

B. The RAPD pattern and the corresponding dendrogram generated by the primer 2.

Table 2. Jaccard coefficients calculated with the RAPD pattern generated by the primer2

P2	1	2	3	4	5
1	0	0,637	0,88	0,5	0,77
2		0	0,363	0,446	0,63
3			0	0,77	0,88
4				0	0,72
5					0

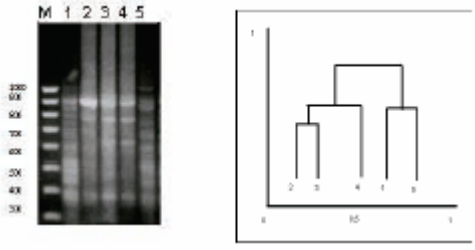

Fig. 2.a) RAPD polymorphisms with primer 2. 1-5 samples*: 1. Agropyron repens, 2. Elymus elongatus. 3. Elymus bessarabicus, 4. Elymus sabulosus, 5. Agropyron brandzae, M-molecular marker. b)* dendrogram based on Jaccard similarity coefficient

By scoring the presence of bands 1:1 for the 5 *Elymus* representatives which are compared in pairs, the following arrangements have been suggested: (*2,Elymus elongatus:3. Elymus bessarabicus*) and (*2.Elymus elongatus:3Elymus bessarabicus:4 Elymus sabulosus,*) and (*1.Agropyron repens*: *5. Agropyron brandzae*).

C. The RAPD pattern and the corresponding dendrogram generated by the primer 3

Table 3. Jaccard coefficients calculated with the RAPD pattern generated by the primer3

P3	1	2	3	4	5
1	0	0,875	0,78	0,75	0,705
2		0	0,756	0,67	0,5
3			0	0,67	0,9
4				0	0,56
5					0

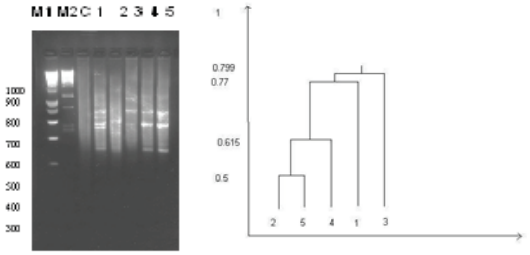

Fig. 3. a) RAPD polymorphisms with primer 3. 1-5 samples*: 1. Agropyron repens, 2. Elymus elongatus. 3. Agropyron brandzae,4. Elymus sabulosus, 5. Elymus bessarabicus, M1.-molecular marker,100bp ladder, λPstI, M2.- Smart. b)* dendrogram based on Jaccard similarity coefficient

D.The RAPD pattern and the corresponding dendrogram generated by the primer 4

Table 4. Jaccard coefficients calculated with the RAPD pattern generated by the primer 4

P4	1	2	3	4	5
1	0	0,57	0,8	0,57	0,66
2		0	0,50	0,33	0,2
3			0	0,8	0,33
4				0	0,2
5					0

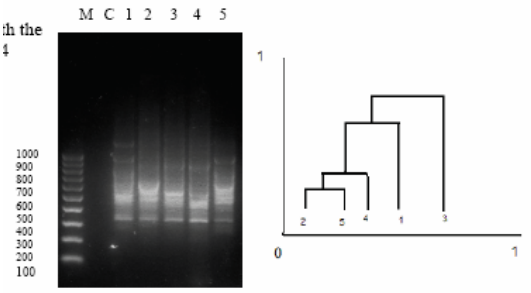

Fig. 4. a) RAPD polymorphisms with primer 4. 1-5 samples*:1. Agropyron repens,2. Elymus elongatus. 3 Agropyron brandzae, 4 Elymus sabulosus, 5 Elymus bessarabicus, M1.-molecular marker 100bp ladder. b)* dendrograme based on Jaccard similarity coefficient

By scoring the presence of bands 1:1 for the 5 *Elymus* representatives which are compared in pairs, the following arrangements have been suggested: (*2,Elymus elongatus: 5. Agropyron brandzae*), (*2,Elymus elongatus: 5. Agropyron brandzae: 4 Elymus sabulosus)* and (*1.Agropyron repens: 4 Elymus sabulosus: 5. Agropyron brandzae: 2.Elymus elongatus).* The accession *3, Elymus bessarabicus,* is suggested by the later two polymorphic pattern as a distinct entity.

SUMMARY

Certain representatives of Poaceae (Gramineae) family, which includes a plant with known medicinal characteristics, Elymus (Agropyron) repens, have been chosen for a study case of molecular taxonomic approach in the biodiversity domain. The resulted molecular variance has proven a relative good informational potential of the simple RAPD method: only one of the suggested arrangement of the Elymus taxa representatives was in accordance of the known morphological based classification.

CONCLUSION

Molecular methods have proven to be powerful tool in taxonomy to test morphological hypotheses at different taxonomic levels. In some cases, molecular data have confirmed previous morphological concepts or helped to decide in favor of one morphological hypothesis. In other cases, molecular studies have led to new arrangements of morphologically circumscribed entities, or were not at all supported by morphology.

The present study represents a first approach of taxonomy based on molecular methods. A simple RAPD marker showed rather relative informational potential as only two of four dendrograms reproduced the known morphological based classification.

Further investigations using new RAPD markers such codominant DNA markers, (microsatellites or AFLP's: amplified fragments length polymorphisms) would give elucidating information about the complete picture of genetic distances such as the levels of heterozygosity in the populations of the investigated plants.

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