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Original scientific paper

CHARACTERIZATION OF ONION GENOTYPES BY USE OF RAPD MARKERS

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In order to estimate, at the molecular level, the divergence of parental lines that were used in diallel crossbreeding for production of superior offspring (F1 generation hybrids) at the Institute for Vegetable Crops, the molecular analysis using five RAPD markers for five pairs of parents has been performed. It gives an insight into their genetic polymorphism and the possibility of their further use in breeding programs. Information from this research has pioneered the application of molecular markers of onion in Serbia.

Analyses were performed using the RAPD primers, which in previous studies established a high degree of polymorphism. In all five

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cases there was a corresponding amplification of DNA segments. From totally 50 bands analyzed, the length of fragments ranged from 500 to 3000 bp. Number of polymorphic band per example was 8 to 13. In our research at the level of the analyzed primers, a high degree of polymorphism between analyzed genotypes has been found. Based on UPGMA dendogram, analyzed genotypes were divided into two main clusters and two subclusters.

Key words: genetic polymorphism, RAPD, onion

INTRODUCTION

Onion is one of economically the most important vegetable species of the genus *Allium*. Breeders of the onion must focus on producing high quality bulbs, as one of the most important trait. The methods used to achieve the aims in breeding programs are a combination of quantitative genetics and the possibilities of modern molecular biology. Institute for Vegetable Crops in Smederevska Palanka has a large collection of vegetable species, which is a good basis for planning and modelling ideotypes vegetable species (PAVLOVIC *et al*., 2011, ZDRAVKOVIC *et al*., 2010).

Application of molecular markers in breeding has great features and the most important are: the choice of parents, increasing the efficiency of back crossing, selection based on marker-property relationship, identification of genotypes, detection of genes from other species, determining the stability of the genetic composition of cultivars and lines in time and space, protection of copyrights, etc.. Furthermore, RAPD markers are used to quickly and easily determine the genetic diversity of plant material within the population, breeding lines, as well as general collection germplasm, and also useful in genetic analysis of the resistance to the specific diseases of vegetable crops (CARVALHO *et al.*, 2004, CVIKIC *et al*., 2009, ZDRAVKOVIC *et al*., 2011).

MATERIALS AND METHODS

The subject of this research was the genetic divergency of five genotypes of onion (Makoi bronzi-MB, Piroska-PR, AC 101, Jasenicki crveni-JC and Bunkino beo-BB). DNA was isolated from the leaves of plants a few weeks old in accordance with the modified protocol for CTAB (*Cetyltrimethyl ammonium bromide*) for isolation of DNA (Soltis laboratorije, http://www.ihcworld.com/_protocols/lab_ protocols/soltis-lab-protocols.htm). About 50 mg of fresh leaf was crushed in liquid nitrogen into a fine powder that is then homogenized with 500µl extraction buffer (STAB buffer) and incubated 1 hour at 55ºC. Then, chloroform was added: isoamyl alcohol (24:1) and the mixture was centrifuged for 5 minutes at maximum speed (Eppendorf Microcentrifuge). Aqueous phase (top layer) was then transferred into new tubes and DNA was precipitated with cold ammonium acetate and isopropanol. After precipitation the DNA pellet was washed with cold 70% ethanol and dried well in the oven at 35ºC and then dissolved in 200µl TE buffer. All 5 designed primers were to assess the diversity of genotypes. Selection of primers for these markers was performed on the basis of previous study by WILKIE *et al* (1993).

RAPD primers 10 nucleotides long were used and their sequences were shown in Table 1.

Table 1. Sequences of RAPD primers

No	Primer	Sequence (5`-3`)
1	Oligo 1	5`-CTT CAC CCG A-3`
2	Oligo 2	5`-TCG GCG ATA G-3`
3	Oligo 3	5`-CAA TCG CCG T-3`
4	Oligo 4	5`-CAA ACG TCG G-3`
5	Oligo 5	5`-GTT GCG ATC C-3`

Arrangement of obtained bands was compared between the genotypes and the divergence was determined according to Nei and Li coefficient of genetic distance (1979):

$$Gd = 1\text{-}dxy/dx+dy\text{-}dxy$$

Where:
Gd = genetic distance between the two genotypes; dxy = total number of bands for two genotypes; dx = total number of lanes in genotype 1; dy = total number of bands in genotype 2.

In order to construct dendogram method of average connection *UPGMA* (*Unweighted pair-group method*, *arithmetic average*) was used. This method (WARD, 1963) starts from similarity index matrix (D_1) among all researched genotypes (n), therefore form $n \cdot n$.

Determination of the cluster and the graphical representation of dendogram in these studies were performed using Windows SPSS, the option Agglomeration schedule, using the Between-groups linkage and interval measures Euclidiean Squared distance.

RESULTS AND DISCUSSION

Onion genotypes were analyzed using five RAPD primers, for which WILKIE *et al.* (1993) found high degree of polymorphism. Amplification of DNA segments was found in all five cases and polymorphism was found in all five analyzed markers. A total of 50 bands were read and the length of fragments was ranging from 500 to 3000 bp. Number of polymorphic bands per primer ranged from 8 to 13. The highest polymorphism was found in the primer Oligo02 and the lowest in Oligo03 (Table 2).

Genetic distance between onion genotypes were calculated at the individual and common level for all five primers.

At the level of all five analyzed primers a high degree of polymorphism among genotypes was identified. Pairs of genotypes JC-BB and AC - JC had the greatest genetic distance (73.52 and 70.59%, Tables 3), i.e. their genetic similarity is

only 26.48% and 29.41%. Their mutual genetic polymorphism was confirmed by the results of UPGMA cluster analysis (Fig. 1).

High level of genetic polymorphism: 91,24% was found by KUTTY *et al.* (2006).

Table 2. Number of bands detected after amplification of five RAPD primers for five onion genotypes

Primer	Number of bands
Oligo01	11
Oligo02	13
Oligo03	8
Oligo04	9
Oligo05	9
Total:	50

Table 3. Genetic distances of five onion genotypes for five analysed primers

Genotype	PR	AC 101	JC	BB
MB	0,6667	0,4138	0,6667	0,5000
PR	-	0,5938	0,6857	0,6364
AC 101	-	-	0,7059	0,6765
JC	-	-	-	0,7353

At the individual level, high values of genetic distances for genotype pairs (comparing to other genotype pairs for given primer) were confirmed for primers Oligo1, Oligo2, Oligo3 and Oligo4, while Oligo5 had lower genotype distances JC-BB и ZG-JC.

The lowest genetic distance at the level of all five genotypes was found for genotype pairs MB-ZG and MB-BB (41,38 and 50,00%). Low values of genetic distances for studied genotypes were found for primers Oligo2, Oligo3 and Oligo5. Low genetic distance for genotype pair MB-BB was found in primer Oligo1, but not for genotype MB-ZG. For primer Oligo4 situation is reverse.

Table 4. Genetic distances of five onion genotypes for primer Oligo01

Genotype	PR	AC 101	JC	BB
MB	1,0000	0,6667	0,8333	0,3333
PR	-	0,8333	0,3333	0,7500
AC 101	-	-	0,8333	0,7500
JC	-	-	-	1,000

Differences in genetic distances of some genotypes pairs were found among some different primers can be explained with low number of RAPD primers and genotypes used in our research (Table 4, 5, 6, 7 and 8). Necessity of using large number of primers for total identification of onion genotypes were emphasized in researches conducted by MADOKA *et al.* (2004).

Table 5. Genetic distances of five onion genotypes for primer Oligo02

Genotype	PR	AC 101	JC	BB
MB	0,5000	0,1667	0,6250	0,3750
PR	-	0,5556	0,7000	0,5000
ZG	-	-	0,6667	0,6000
JC	-	-	-	0,6000

Table 6. Genetic distances of five onion genotypes for primer Oligo03

Genotype	PR	ZG	JC	BB
MB	0,6000	0,3750	0,5000	0,3333
PR	-	0,4286	0,6000	0,4000
AC 101	-	-	0,7500	0,6250
JC	-	-	-	0,6000

Furthermore, values of genetic distances established for all analysed genotypes and primers are higher than values obtained within the same species *Allium cepa* L. (ARIFIN *et al.* 2000, TANIKAWA *et al.* 2002), which proves that precise definition of genotype relations requires the use of large number of RAPD primers with polymorph products.

Characteristic profiles of DNA amplification enabled differentiation of individual genotypes in case of few markers. Primer Oligo01 enables precise differentiation of MB and PR genotypes, such as JC and BB, since for these genotype pairs genotype distances were 100% (Table 4). Maximal values of genetic distances were found for genotypes AC 101 and JC, AC 101 and BB (primer Oligo04), while for primer Oligo05, 100%genetic distance was found for genotypes PR and BB (Table 7 and 8).

Table 7. Genetic distances of five onion genotypes for primer Oligo04

Genotype	PR	AC 101	JC	BB
MB	0,5714	0,5000	0,8000	0,8571
PR	-	0,6667	0,8571	0,5714
AC 101	-	-	1,0000	1,0000
JC	-	-	-	0,8000

Table 8. Genetic distances of five onion genotypes for primer Oligo05

Genotype	PR	AC 101	JC	BB
MB	0,8750	0,5000	0,6250	0,5000
PR	-	0,7143	0,8571	1,0000
AC 101	-	-	0,4286	0,5000
JC	-	-	-	0,6667

Classification of genotypes in clusters based on DNA fingerprinting and agronomic traits in order to research genetic and phenotype diversity became the leading method in praxis (FRANCO *et al*., 2001).

According to UPGMA dendogram, analysed genotypes divided in 3 basic clusters (cluster I and cluster II). Two genotypes (BB and JC) were in two clusters, which were the farthest according to RAPD (73%, Table 3). Only one genotype (Jasenicki crveni), was in cluster II while cluster I is further divided in two subclusters. Genotype Bunkino beo is in subcluster 1 while three other genotypes are in subcluster 2, further divided in subsubcluster 1 (AC 101 and Makoi bronzi) and subsubcluster 2 (Piroska). RAPD analysis for all five primers proved the lowest genetic divergence (41,38%) of members of subsubcluster 1 (AC 101 and MB), while genotype PR had higher genetic distance comparing to previous two (Table 3), so he was in the special subsubcluster 2 (Fig. 1).

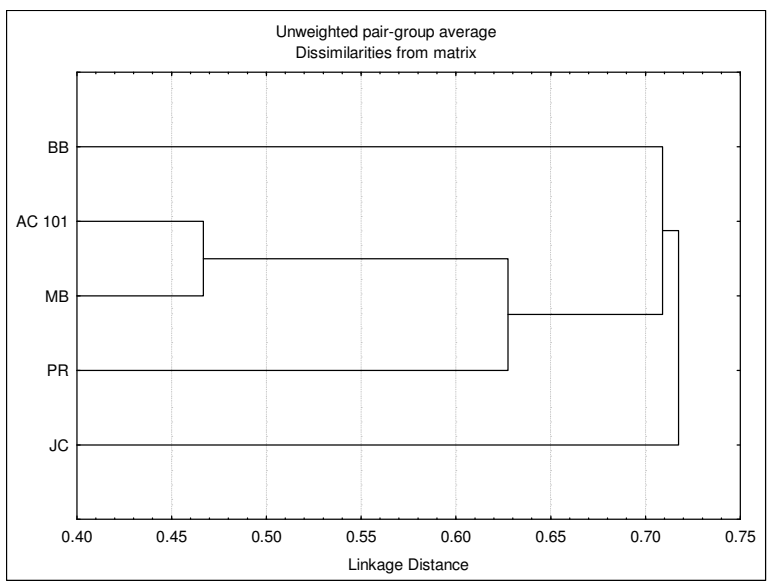
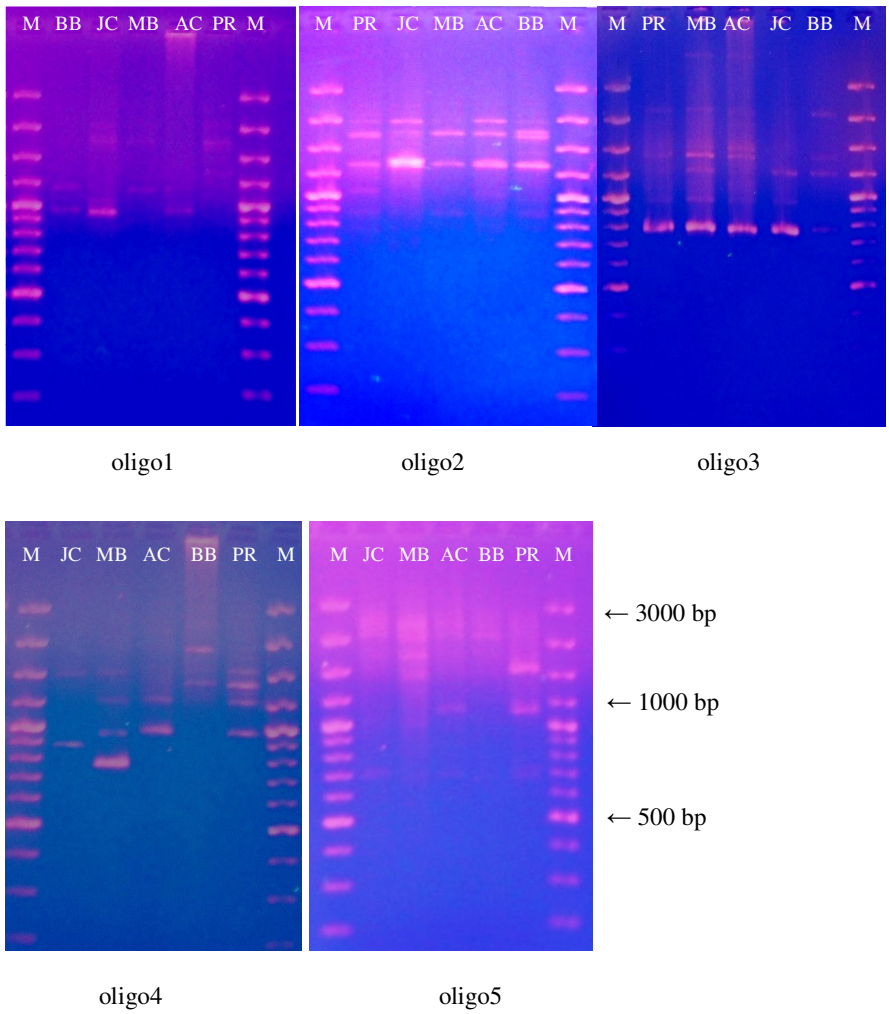


Fig. 1. Dendogram constructed according to UPGMA cluster analysis of genetic distances of five onion genotypes



M – GeneRuler 100bp Plus DNA ladder; BB – Bunkono beo; JC – Jasenicki crveni; MB – Makoi bronzi; AC 101; PR – Piroska

Fig. 2. PCR profiles of five genotypes of *Allium cepa L.* obtained using five RAPD primers (oligo 1-5)

CONCLUSION

Some markers with characteristic profiles of DNA amplification enable differing of individual genotypes. Primer Oligo01 enables precise differing of genotype Makoi bronzi and Piroska, as well as Jasenicki crveni and Bunkino beo, since for these genotype pairs genetic distances were 100%. Maximal values of genetic distances wer found for genotypes AC 101 and Jasenicki crveni, AC 101 and Bunkino beo (primer Oligo04), while 100% genetic distance for primer Oligo05 was found among genotypes Piroska and Bunkino beo.

According to UPGMA dendogram, analysed genotypes were divided in two basic divergent clusters (cluster I and cluster II). Genotypes with higher resemblance were placed in less divergent subclusters and subsubclusters. Such classified genotypes were different in agronomic traits. Further analysis could prove the connection of polymorphism of researched RAPD markers and morphological/agronomical onion traits.

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MOGUĆNOST PRIMENE MOLEKULARNIH RAPD MARKERA U SELEKCIJI CRNOG LUKA

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U cilju procene divergencije na molekularnom nivou roditeljskih linija koje su korišćene u dialenom ukrštanju sa ciljem dobijanja superiornog potomstva (hibrida F_1 generacije) u Institutu za povrarstvo, urađana je molekularna analiza primenom pet RAPD markera kod pet roditeljskih parova. Ovim putem je sagledan njihov genetički polimorfizam kao i mogućnost njihove dalje primene u oplemenjivačkim programima. Informacije dobijene ovim istraživanjem predstavljaju pionirski poduhvat u primene molekularnih markera na crnom luku u Srbiji.

Analize su izvršene upotrebom RAPD prajmera, za koje je u ranijim istraživanjima utvrđen visok stepen polimorfizma. U svih pet slučajeva je došlo do amplifikacije odgovarajućih DNA segmenata. Ukupno je očitano 50 bendova, dužine fragmenata u rasponu od 500 do 3000 bp. Broj polimorfnih bendova po prajmeru se kretao od 8 do 13. U našem istraživanju na nivou svih analiziranih prajmera, utvrđen je visok stepen polimorfizma između analaziranih genotipova. Na osnovu UPGMA dendograma analizirani genotipovi su razvrstani u dva osnovna klastera i dva subklastera.

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