DNA POLYMORPHISM OF THE DROUGHT TOLERANT LOCAL VARIETIES OF SOYBEAN (*Glycine max* (L.) Merr.) FROM REPUBLIC OF MOLDOVA

Ana BÎRSAN, Anna MOLDOVAN, Hoa TRAN

Introduction. Soybean (*Glycine max* (L.) Merr.) is a major source of plant protein and oil worldwide. Due to its high economic value, it is important to identify suitable traits and genotypes associated with drought tolerance in soybean [6]. It can take almost 12 to 15 years to develop new crop varieties but with biotechnology the time has been shorten to 7 to 10 years for new crop varieties development. The PCR

method is one of the most sensitive, fast, effective and reproducible methods of modern science and is increasingly used for solving theoretical and applied problems in many fields [5, 9].

Using Simple Sequences Repetitive Markers (SSR) may highlight the genetic diversity of the species *Glycine max* L. revealed the specific fragments, which can be used as molecular markers in soybean genotyping. Genotyping of soybean germplasm is useful tool for modern breeding programs which are aimed at the development of new cultivars with desirable traits such as drought resistance [3]. Eight alleles per SSR locus among a group of 43 soybean and wild soybean genotypes was identified [1]. In order to analyze the genetic polymorphism of local soybean varieties with different tolerance to drought two varieties of soybean were studied.

Materials and methods. In order to analyze the genetic polymorphism of local soybean varieties with different tolerance to drought, the genetic material was obtaned from yang roots of Dorinta and Albisoara genotypes. DNA was extracted using the protocol described by Vallejos et. al. 1992 [10] with some modifications. Amplification of DNA fragment was conducted using Sat_175 primer pair specific for genes related to drought resistance.The sequences of the primers were 5'-GCG TCA TTG TTA AAA CTT AGT GTC TAA TAA A-3 ' (forward) and 5'-GCG GTC AAA GTC AGT TAG TGA AAG AGA-3 '(reverse). PCR reaction was performed in 25 µl volume: 15,75 µl water; 2,5 µl buffer; 2,5 µl MgCl2; 0,5 µl dNTPs; 1,25 µl primer F;1,25 µl primer R; 0.25 µl Taq polymerase.PCR reactions were performed in SimpliAmp ™ Thermal Cycler Applied Biosystems automated amplifier according to the developed protocol (initial denaturation, 4 min at 94°C; denaturation, 1 min. at at 94°C, annealing, 1 min at 50°C, extension, 1 min. 30 sec. at 72°C, 35 cycles and a final extension step for 7 min.)The PCR product was visualized on 2.5% agarose gel [2].

Results and discussions. The total genomic DNA electrophoresis in 1% agarose gel showed that genomic DNA was acceptable for PCR. Plant materials are among the most difficult for high quality DNA extractions. The key is to properly prepare the tissues for extraction. The age and growth conditions of the plant material influence the isolation efficiency of high quality DNA. The resultant validity depends on many factors: the protocol, the accurate of equipment, the

skill of the experimenter. These factors play an important role in DNA quality obtained. Good quality DNA extracted from soybean can be used in later research.

DNA amplification performed using Sat_175 primer pair sin allowed us to highlight 3 DNA fragments of approximately 150 bp, 250 bp and 900 bp length, common to both Albisoara and Dorinta genotypes (Figure 1). At the same time, electrophoresis of PCR product revealed the presence of a weak band of approximately 1300 bp in Dorinta genotype which could be seen better under UV light, which was absent in Albisoara genotype.

Moreover, Jabalpur (2007) obtained some similar result and observed that primer Sat_175 provide 3 polymorphic bands with the length: 130 bp, 150 bp, 200 bp. They used 51 SSR marker and 94% of them provide polymorphic loci while only 6 % provide monomorphic loci [8].

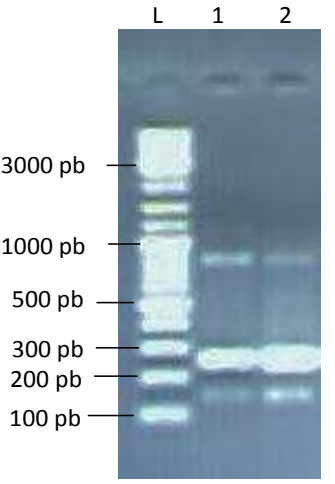


Fig. Electrophoresis of the PCR product (2-Sat_175) in agarose gel

Conclusions and recommendations. Soybean roots are a successful material for extraction of large amount of high-quality amplifiable DNA. In soybean, high levels of SSR allele length polymorphism have been reported. Present study demonstrated the opportunity and importance of using genetic-molecular analysis for knowledge of genetic resources of the soybean gene pool of the Republic of Moldova, in order to preserve its purity, as well as to capitalize on results in improving soybean germplasm source.

Based on the research carried out and on the results obtained with regard to the study of genetic polymorphism in soybean G. max L., we can conclude that the level of genetic polymorphism differs from one genotype to another.

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