Assessment of genetic diversity among barley varieties with different origin using simple sequence repeat (SSR) markers

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INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the principal cereal crops in the world, because it's used as raw material in beer production, animal feed and human consumption. By area and production, barley is ranked as the fourth most important variety, followed by wheat, rice and corn. Barley is considered one of the best adapted cereals due to it's tolerance to salinity, low temperatures and lower water demand. The primary goal in any breeding programme is the grain yield, but the basic requisite it is determine the distinguish among varieties of crop plants and establish their purity (Matus & Hayes, 2002). Assessment of the genetic variability within cultivated varieties has major role in plant breeding and conservation of genetic resources. In recent years a lot of researches used a molecular markers to evaluate genetic diversity at molecular level. Plant breeders usually use multi locus techniques based on PCR and DNA molecular markers as tools for directly evaluating the genetic variation among related varieties without effect of environmental factors. In addition, DNA techniques allow the assessment of unlimited number of polymorphic marker loci (Nguyen et al., 2004). The most informative polymorphic markers available are microsatellite or simple sequence repeats (SSR). SSRs markers are the choice of many genetics researches for barley varieties because of their multi allelic nature, codominant inheritance (Deric et al., 2005), highly variable and able to distinguishes closely related plant cultivars, easily assayed by PCR and cost effective to use and available for barley (Ramsey et al., 2000). The propose of this research was to determine the genetic diversity among barley genotypes developed in different counties using the SSRs marker.

MATERIALS AND METHODS

As an experimental material were used twenty-one two row barley varieties with different origin. Five varieties were Macedonian, two varieties were Serbian, two varieties had Croatian origin and the other twelve varieties were developed in Bulgaria. Total DNA was isolated from young leaf tissue of a single plant from each genotype. DNA was extracted by CTAB method (Doyle and Doyle, 1990). Quantification of the DNA concentration and its quality were made by spectrophotometer and gel electrophoresis. 18 microsatellite primers pairs were selected on the basis of their chromosomal location. Their names, sequences and chromosomal locations are given in Table 1. The amplified PCR products were separated by electrophoresis using 3% agarose gel 1xTBA buffer, than stained with ethidium bromide (10mg/ml) and visualized under UV light.

RESULTS AND DISCUSSION

Nine of them showed monomorphic bands (Bmag13, GMS1, HVB23D, Bmac0213, Bmac0013, HVM 3, HVM 4, HVM 7 and HVM 9), three markers had high polymorphisms (MGB391, MGB402 and MGB318) and the rest of them didn't show bands. The calculated polymorphism information content (PIC) values range from 0.163 to 0.574 with an average of 0.380 which shows the importance of the markers for future diversity analysis of barley. Locus MGB318 show the highest PIC and locus MGB391 shows the lowest PIC in this characterized barley genotypes (Fig. 1 and 2).

Figure 1. Agarose gel showing the alleles in barley genotypes obtained of MGB318 marker

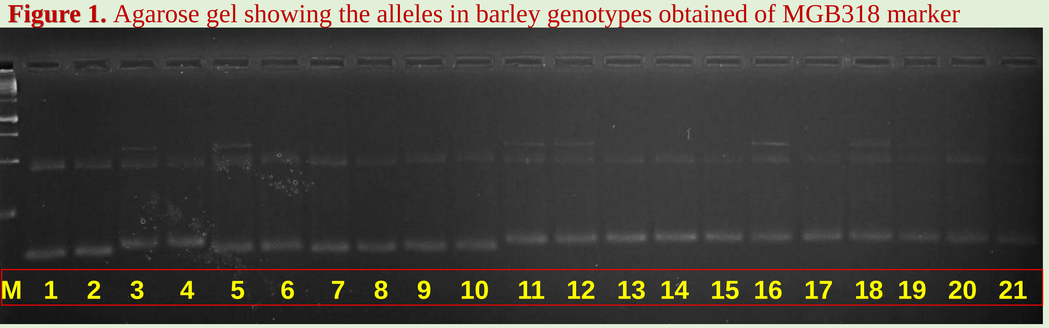
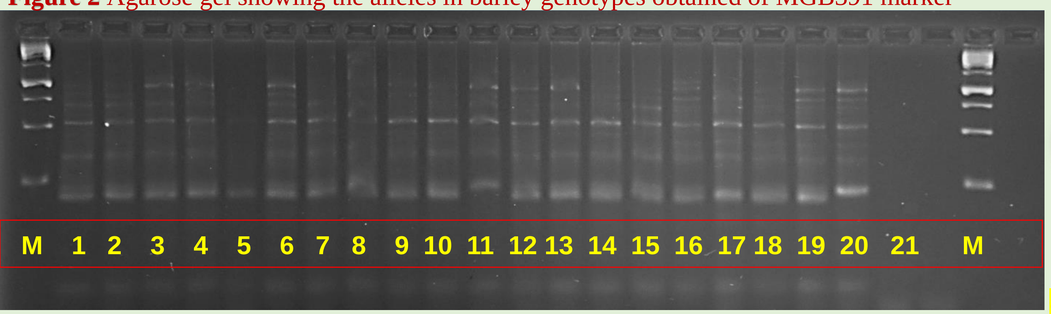


Figure 2 Agarose gel showing the alleles in barley genotypes obtained of MGB391 marker



CONCLUSION

The good information content of the markers used and estimated extent of diversity among the studied barley varieties are basic outcomes of this study upon which a more comprehensive study can be built. Actually, studies such as this are useful for the establishments of genetic relatedness and molecular characterization of barley germplasm. These results can be useful for barley germplasm management and design of new crosses for future breeding propose.

Table 1. Barley SSRs primers and their sequence (Von Korff et al., 2004)

Primer	Sequence	Annealing temperature (°C)	Chromosome location
MGB391	For 5' –AGCTCCTTTCCTCCCTTCC-3'	54	2 (2H)
	Rev 5'- CCAACATCTCCTCCTCCTGA-3'		
HVITR1	For 5' –CCACTTGCCAAACACTAGACCC-3'	55	3 (3H)
	Rev 5'- TTCATGCAGATCGGGCCAC-3'		
Bmag13	For 5'-AAGGGGAATCAAAATGGGaG-3'	54	3 (3H)
	Rev 5'- TCGAATAGGTCTCCGAAGAAA-3'		
HV13GEIII	For 5' –AGGAACCCTACGCCTTACGAG-3'	56	3 (3H)
	Rev 5'- AGGACCGAGAGTGGTGGTGG-3'		
HVB23D	For 5' –GGTAGCAGACCGATGGATGT-3'	54	4 (4H)
	Rev 5'- ACTCTGACACGCACGAACAC-3'		
MGB396	For 5' –CGCTAGCTTGTTTCTCGTTTG-3'	-	4 (4H)
	Rev 5'- TCGCATGGCATCAACTACAG-3'		
MGB402	For 5' –CAAGCAAGCAAGCAGAGAGA-3'	55	5 (1H)
	Rev 5'- AACTTGTGGCTCTGCGACTC-3'		
Bmag 149	For 5' –CAAGCCAACAGGGTAGTC-3'	-	5 (1H)
	Rev 5'- ATTCGGTTTCTAGAGGAAGAA-3'		
HVGLUEND	For 5' –TTCGCCTCCATCCCACAAAG-3'	-	5 (1H)
	Rev 5'- GCAGAACGAAAGCGACATGC-3'		
MGB371	For 5' –CACCAAGTTCACCTCGTCCT-3'	56	6 (6H)
	Rev 5'- TTATTCAGGCAGCACCATTG-3'		
MGB 356	For 5' –TGGTCTGGAGCTCTCAACAG-3'	-	6 (6H)
	Rev 5'- AAGCCACATTGAAGGAGCAC-3'		
EBmac624	For 5' –AAAAGCATTCAACTTCATAAGA-3'	54	6 (6H)
	Rev 5'- CAACGCCATCACGTAATA-3'		
Bmag 210	For 5' –ACCTACAGTTCAATAGCTAGTACC-3'	-	6 (6H)
	Rev 5'- GCACAAAACGATTACATCATA-3'		
MGB 384	For 5' –CTGCTGTTGCTGTTGTCGTT-3'	-	7 (5H)
	Rev 5'- ACTCGGGGTCCTTGAGTATG-3'		
BMS02	For 5' –AGAGTAGTGGAAAGAAAGTT-3'	-	7 (5H)
	Rev 5'- TGGTAGTGAGATGAGGTGAC-3'		
MGB318	For 5' –CGGCTCAAGGTCTCTTCTTC-3'	55	7 (5H)
	Rev 5'- TATCTCAGATGCCCCTTTCC-3'		
MGB357	For 5' –GCTCCAGGGCTCCTCTTC-3'	52	7 (5H)
	Rev 5'- AGCTCTCTCTGCACGTCCTT-3'		
GMS1	For 5' –CTGACCCTTTGCTTAACATGC-3'	55	7 (5H)
	Rev 5'- TCAGCGACAAACAATAAAGG-3'		