UDC 575.22; 633.11
Original scientific paper

DETERMINATION OF POLYMORPHISM OF MICROSATELLITE PRIMERS IN HEXAPLOID WHEAT

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Galović V., S. Denčić, and D. Jelovac (2005): *Determination of polymorphism of microsatellite primers in hexaploid wheat*. – Genetika, Vol. 37, No. 3, 217-223.

The objective of this paper was to examine the functionality of two microsatellite primers as their polymorphism levels were determined for select Novi Sad wheat genotypes. Chosen as representatives of Gatersleben wheat microsatellites (GWM) were two sets of microsatellite primers, GWM165 and GWM539, which had been described according to RODER *et al*. (1998a; 1998b). Twenty five wheat genotypes from the World Collection of the Institute of Field and Vegetable Crops in Novi Sad were used in the study. Genomic DNA was isolated from the plant materials using a modification of the PLASCHKE *et al*. (1995) method. PCR amplification of the desired fragments was carried out in a volume of 30 ul (Eppendorf thermocycler) according to RÖDER *et al*. (1998b). The PAGE conditions were implemented according to GALOVIĆ *et al*. (2004). The GWM539 set, with six different alleles, showed a higher level of polymorphism than GWM165, in which three different alleles were detected for the locus concerned.

Key words: wheat, microsatellites, polymorphism

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INTRODUCTION

Getting to know wheat genetics and genomic organization using molecular markers is of great importance for the advancement of wheat breeding. Wheat is an allohexaploid (2n=6x=42) with three genomes (A, B, and D) that is extreme in size ($16x10^9$ bp) and contains more than 80% of repetitive DNA sequences. Given that this species is characterized by a very limited level of polymorphism, its gene and genome mapping requires the use of populations obtained from crosses between very distant parents. However, in order to achieve the most important goal of a breeding program—the mapping of agronomically important genes (QTL)—highly informative marker systems need to be provided in the context of a high level of polymorphism. The discovery of special kinds of repetitive sequences, possessed by any eukaryotic genome (LITT and LUTY, 1989), made it possible to design microsatellites, or SSRs (TAUTZ *et al*., 1986). This molecular system has proven to be highly informative and locus-specific in many plant species. In hexaploid wheat, microsatellites have shown a greater level of polymorphism and informativeness than any other marker system (PLASCHKE *et al*., 1995), so, as codominant and in most cases chromosome-specific, the microsatellite system has found extensive application in the hexaploid genome of this species. Still, due to the sheer size of the wheat genome, developing microsatellite markers for it is a highly demanding and costly endeavour, as only 30% of all primer sets developed from microsatellite sequences are functional and usable for genetic analysis (RODER *et al*., 1997).

The objective of this study was to examine the functionality of two microsatellite primers designed as Gatersleben wheat microsatellites (GWM165 and GWM539) and thereby determine their polymorphism levels for select Novi Sad wheat genotypes.

MATERIALS AND METHODS

Twenty five wheat genotypes from the World Collection of the Institute of Field and Vegetable Crops in Novi Sad were used in the study. Twenty four of the 25 genotypes (10 cultivars and 14 promising lines) are part of the breeding program of the Institute's Small Grains Department. The single remaining genotype, the Chinese cultivar Chinese Spring, was used in the study as the standard. Total, genomic DNA was isolated from the genotypes using a modified version of the method by PLASCHKE *et al*. (1995). The Gatersleben wheat microsatellites (GWM) were represented by two sets of microsatellite primers, GWM165 and GWM539, which had been described according to RODER *et al*. (1998a; 1998b). The cultivar Chinese Spring was chosen as the standard for analyzing parameters such as GWM designation, fragment size according to the CS standard, and allele size rank and location on the chromosome (Table 1). PCR amplification of the desired fragments was carried out in a volume of 30ul (Eppendorf thermocycler) according to RODER *et al*. (1998b). The checking of PCR products on agarose gel, their preparation for

application to the PAA (polyacrylamide) gel, electrophoresis conditions and PAA gel staining were all carried out according to GALOVIĆ *et al*. (2004).

Statistical data processing was done using the NTSYSpc software package and its SIMQUAL module for genetic distance calculations (NEI and LI, 1979). UPGMA dendrogram representation was made with the SAHN (Sequential agglomerative hierarcical nested cluster analysis) module according to SNEATH and SOKAL (1973).

RESULTS AND DISCUSSION

When optimizing the PCR reaction for GWM165 and GWM539, we used the gradient option for determining the optimal annealing temperature for each primer set separately (Fig. 1). PCR products were checked on a 2% agarose gel and it was determined that both primers yielded the most specific PCR products at 65.5ºC (the default, recommended temperature is 60ºC).

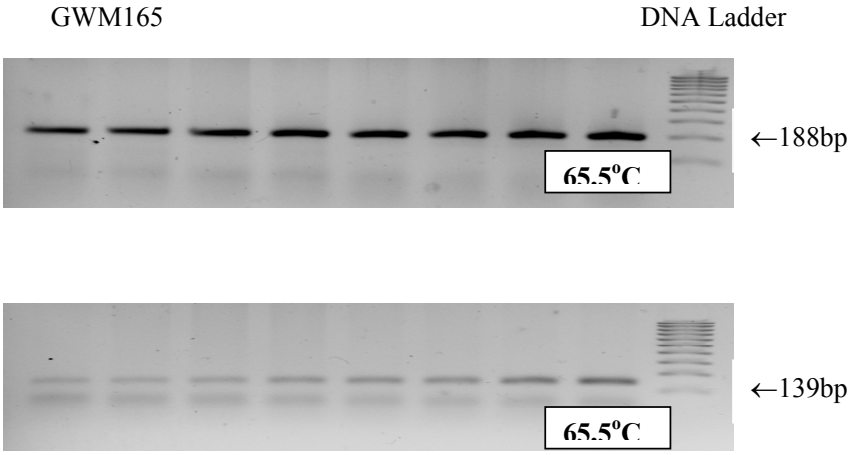

Fig. 1. Gradient annealing temperature and PCR product check on a 2% agarose gel for primer sets GWM165 and GWM539

Table 1. Characterization of microsatellite primers

Locus	Primer set sequence	Repeat	An. Temp.	CS (bp)
Xgwm165-4A	TGC AGT GGT CAG ATG TTT CC CTT TTC TTT CAG ATT GCG CC	(GA)20	65.5	188
Xgwm539-2D	CTGCTCTAAGATTCATGCAACC GAGGCTTGTGCCCTCTGTAG	(GA)27	65.5	139

The temperature of 65.5ºC was found to produce the best DNA yield. DNA sequence data read from the PAGE profile were used to determine allele size

rank (Table 1 and Table 2) for both primer sets. The rank values were 188 - 192 for GWM165 (Fig. 2) and 135 -145 for GWM539 (Fig. 3, Table 1 and Table 2).

Table 2. Wheat microsatellites and allele location, number and size

Primer set	Location on chromosome	Allele number	Fragment size in 'CS' (bp)	Allele size rank (bp)
GWM165	4AS	3	188	188-192
GWM539	2DL	6	139	135-145

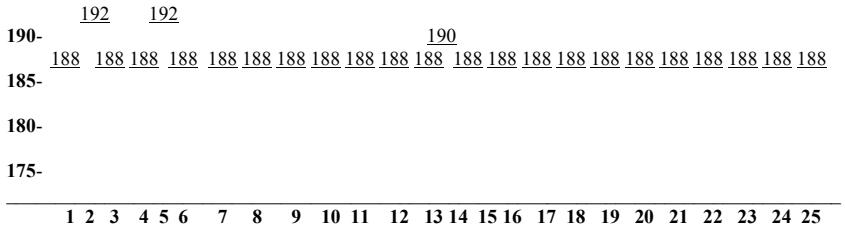

Fig. 2 PAGE for GWM165 in 25 Novi Sad cultivars and lines of wheat 195 (1.GK-17, 2.GK-11, 3.GK-78, 4.GK-81, 5.GK-80, 6.GK-90, 7.GK-104, 8.GK-106, 9.GK-136, 10.GK-137, 11.GK-158, 12.GK-174, 13.GK-175, 14.GK-227, 15.GK-228, 16.GK 243, 17.GK-250, 18.GK-269, 19.GK-276, 20.GK-279, 21.GK-509, 22.GK-520, 23.GK-701, 24.GK-199, 25.CS-127)

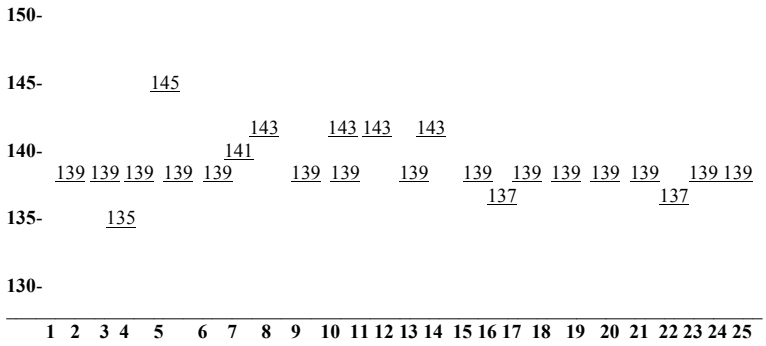

Fig.3 PAGE for GWM539 primer set in 25 Novi Sad cultivars and lines of wheat (1.GK-17, 2.GK-11, 3.GK-78, 4.GK-81, 5.GK-80, 6.GK-90, 7.GK-104, 8.GK-106, 9.GK-136, 10.GK-137, 11.GK-158, 12.GK-174, 13.GK-175, 14.GK-227, 15.GK-228, 16.GK-243, 17.GK-250, 18.GK-269, 19.GK-276, 20.GK-279, 21.GK-509, 22.GK-520, 23.GK-701, 24.GK-199, 25.CS-127)

PAA gel readings for primer set GWM165 produced three different alleles (**a**, **b** and **c**, Fig.1). Allele **a** appeared in 88% of the cases, i.e. 88% of the genotypes. Allele **b,** with a contribution of 8%, was found in only two genotypes ("Kolubara" and "Jugoslavija"). Allele **c** (with its 4% contribution) was observed only in the cultivar "Pesma". The dendrogram for the locus in question, distin-

guished only two genotypes, Partizanka (GK17) and Kolubara (GK11). All the rest, however, exhibited small genetic distance. These results may be indicative of their low genetic distance for the locus concerned.

In the case of primer set GWM539, six different alleles (**a**, **b**, **c**, **d**, **e**, **f**) were identified based on the PAA electrophoretic profile. Allele **a** had a contribution of 58.3%, which is a significantly lower frequency of occurrence compared with what the same allele had for the gwm165 locus. Allele **b** occurred in line NSR-2, allele **c** was found only in the cultivar Rana Niska, and allele **d** appeared in line NSP187. Allele **e** had a greater frequency of occurrence (16.7%) and was found in three of the lines (NS37/90, NS90/92, and NSP199) and the cultivar Pesma. Allele **f** was recorded in the cultivars Sremica and Lira. After statistical data processing, the dendrogram discriminated between three different groups of genotypes. One was comprised of the genotypes Kolubara and Jugoslavija, which had the narrowest genetic distance. The second group included all the other genotypes except the cultivar Pesma, which appeared as completely independent with its large genetic distance.

CONCLUSION

The results of our study showed that both of the wheat-specific primer sets, GWM165 and GWM 539, amplified the expected fragments (according to the DNA sequence data). Both primer sets were also found to produce the most specific PCR products at an annealing temperature of 65.5°C.

Based on the PAGE profiles, allele size rank was found to be 188-192 bp for primer set GWM165 and 135-145 bp for primer set GWM539.

The GWM539 set, with six different alleles, showed a higher level of polymorphism than GWM165, in which three different alleles were detected for the locus concerned.

Received March 29th, 2005
Accepted June 1st, 2005

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UTVRĐIVANJE POLIMORFIZMA MIKROSATELITSKIH PRAJMERA KOD HEKSAPLOIDNE PŠENICE

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I z v o d

Cilj ovoga rada bio je da se ispita funkcionalnost dva mikrosatelitska prajmera, pri odredjivanju njihovog nivoa polimorfnosti za odabrane Novosadske genotipove pšenice. Kao predstavnici Gatersleben wheat microsatellites (GWM) za rad su izabrana dva seta mikrosatelitskih prajmera, GWM165 i GWM539 koji su opisani po RÖDER *et al*. (1998a; 1998b), U radu je korišćeno 25 genotipova pšenice iz Svetske kolekcije Instituta za ratarstvo i povrtarstvo u Novom Sadu. Genomska DNK izolovana je iz biljnog materijala po modifikovanoj metodi PLASCHKE *et al*. (1995). PCR umnožavanje željenih fragmenata je izvedeno u zapremini od 30ul (Eppendorf thermocycler) po metodi RODER *et al*. (1998b). Uslovi PAGE sprovedeni su po GALOVIĆ *et al*. (2004). Na osnovu dobijenih rezultata ustanovljeno je da je kod ispitivanih genotipova pšenice prajmer set GWM539 sa 6 razlicitih alela pokazao viši nivo polimorfnosti u poređenju sa prajmer setom GWM165 kod koga su detektovana 3 različita alela za posmatrani lokus.

Primljeno 29. III 2005.
Odobreno 1. VI 2005.