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SSR MARKER TSM106 IS A CONVENIENT TOOL FOR IDENTIFYING WHEAT-RYE 1AL.1RS TRANSLOCATION

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ABSTRACT

Wheat continues to be one of the most cultivated cereals in the world. The research undertaken to find new genetic resources and new favourable alleles are a priority in wheat breeding programs. Increasing genetic diversity is necessary to cope with the social, natural and economic challenges.

Rye (*Secale cereale* L.) represents an important genetic resource for wheat breeding. The short arm of the rye chromosome 1 (1RS) contains several genes inducing resistance to biotic and abiotic stresses, increase yield and better adaptation to various environments. Therefore, identification of new tools for detection/selection of favourable alleles can help obtaining fast and reliable results. Molecular markers-SSRs are such convenient tools.

In our study, conducted on 17 genotypes (rye cultivar - Harkovskaya; five Romanian wheat cultivars; four 1AL.1RS translocation genotypes, including "Amigo" derived from "Insave" rye, and seven 1BL.1RS translocation), we used SSR markers to detect the presence of 1RS translocation.

We found that the SSR-TSM $106_{(\sim 170bp)}$ marker clearly distinguished the wheat-rye 1AL.1RS translocation, coming from "Insave" or related rye chromatin, from the wheat-rye 1BL.1RS translocation, coming from other rye sources. Therefore, SSR-TSM marker could be used in MAS (Marker-Assisted Selection) for 1AL.1RS translocation.

Key words: molecular markers, wheat-rye translocation, 1R, 1AL.1RS, TSM.

INTRODUCTION

Development of new wheat cultivars with resistance to biotic and abiotic stresses, better yields and good quality will be always an objective for breeders from entire world. To accomplish this objective, the breeders have to find new genetic resources. Finding and transferring genes that are effective against a wide range of constraints, is ideal.

Increased genetic diversity in wheat breeding is desirable for dealing with present and future challenges caused by the need for increased yields, by climate change, and by higher consumer concerns about food safety (Saulescu et al., 2011).

Rye (*Secale cereale* L.) is and continues to be such a resource. Today, there are many wheat cultivars that carry rye translocation, mainly 1BL:1RS and 1AL:1RS that have provided resistance genes to rusts (Li et al., 2016; Crespo-Herrera et al., 2017), powdery mildew (Lu et al., 2014) greenbug (Porter et al., 1991), common bunt (Ciuca, 2011), barley yellow dwarf virus (BYDV) (Crespo-Herrera et al., 2013) etc. These translocations have also brought positive impact on wheat yield, adaptability and drought (Howell et al., 2014). Saulescu et al., (2011) identified variation for first and second leaf width among lines derived from triticale × wheat crosses.

The wheat-rye chromosomal translocations 1BL.1RS and 1AL.1RS are widely reported to have detrimental effects on hard wheat quality (McKendry et al., 2001). Graybosch et al. (1993) found the effects of 1AL:1RS to be less severe on quality than those of 1BL:1RS. Kofler (2008) developed SSR (Simple Sequence Repeat) marker primer pairs, named

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TSM (Tulln Secale Microsatellites) specific for the short arm of rye chromosome 1. This study aimed to search for molecular markers that highlight rye chromatin and can be used to distinguish different wheat-rye chromosomal translocations.

MATERIAL AND METHODS

Plant material

Our study was conducted on 17 genotypes (rye cultivar - Harkovskaya; five Romanian wheat cultivars; four 1AL.1RS translocation genotypes, including "Amigo" derived from "Insave" rye, and seven 1BL.1RS translocation genotypes).

Molecular analysis

Genomic DNA was isolated from two seeds using SDS 3 method (Cristina et al., 2017). Five set of primers (four sets specific for 1RS and one for 1AS) were used to detect 1RS or 1AS arms chromosomes. One of them, O-SEC5'-A/OSEC3'-R, is special for *Sec-1* locus (Shimizu et al., 1997). Other three sets SCM9 (Saal and Wricke, 1999), TSM106 and TSM123 ("Tulln Secale Microsatellites" Koefer et al., 2008) are specific for 1R chromosome and gwp7072 (Nicot et al., 2004: wheat.pw.usda.gov/cgi-bin/…/name=GPW7072) is specific for 1A chromosome. DNA amplification with TSM106 primers was performed in a 15 µl volume containing 1X buffer (My Taq Red DNA polymerase kit - BIOLINE), 0.5 µM for each primer, 80 ng genomic DNA and 0.3 µl of 360 GC enhancer solution. The following amplification parameters were used: initial denaturation at 95°C-3 min, and then 35 cycles of 95°C-15 sec., 55°C-15 sec., 72°C-15 sec. and final extension 72°C-5 min. PCR was performed in Gene Amp PCR system 9700 thermal cycler. The PCR products were separated on 1.5% agarose for routine use, in 0.5X TBE buffer, stained with ethidium bromide and photographed under ultraviolet light with Vilber Lourmat system.

RESULTS AND DISCUSSION

Characterization of 1AL:1RS and 1BL:1RS translocation in wheat has important practical value for wheat improvement (Yediay et al., 2010), and identification of convenient tools for detection/selection of favourable rye alleles can help obtaining fast and reliable results. Molecular markers-SSRs are such tools.

In this study, the marker gwp7072 gave PCR product in all genotypes apart of T1AL:1RS. Based on previous research, SCM9 and O-SEC5'-A/OSEC3'-R markers differentiated these two wheat-rye translocation (1A:1R and 1B:1R).

Furthermore, amplification with TSM123 marker gave PCR product in all genotypes with wheat-rye translocation and rye cultivar Harkovskaya. In contrast to the TMS123, TSM106 showed PCR product only in 1AL:1RS translocation and Harkovskaya cultivar (Table 1, Figure 1). We tested this result in three repetitions. The locus Xtsm106 is in distal position on 1RS (Koefer et al., 2008).

Therefore, SSR-TSM106 is a convenient tool that can be used in MAS (Marker-Assisted Selection) for 1AL:1RS wheat-rye translocation. The disadvantage of this marker is that it does not make difference between genotypes with 1BL:1RS translocation and genotypes without rye chromatin.

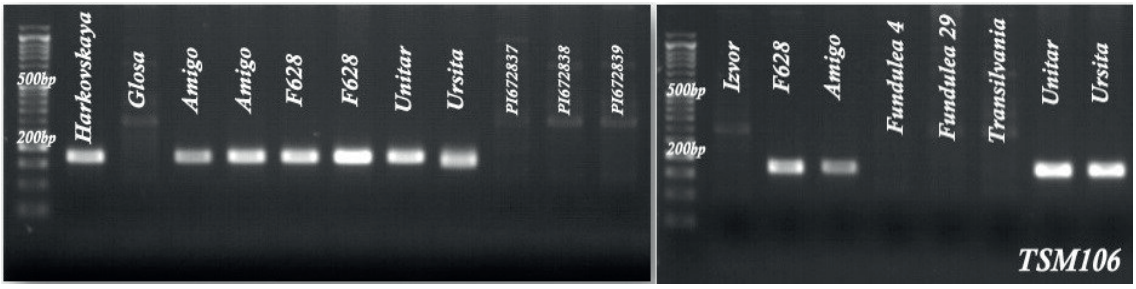

Figure 1. Detection of 1AL.1RS wheat-rye translocations in wheat background by rye-specific DNA marker TSM106

Table 1. Evaluation of molecular markers used in this study

Cultivars/ Lines	Rye source	Seeds source	Rye specific markers/wheat specific marker							
			Scm9		O-SEC5'-A/OSEC3'-R			Tsm123	Tsm106	Gpw7072
			220bp	200bp	1500bp	1100bp	700bp	250bp	170bp	230bp
T1AL:1RS										
F00628G34-1 (F628)	unknown	NARDI Fundulea	+	-	+	+	-	+	+	-
Unitar	F00628G34-1	NARDI Fundulea	+	-	+	+	-	+	+	-
Ursita	F00628G34-1	NARDI Fundulea	+	-	+	+	-	+	+	-
Amigo	Insave FA	IPK Gatersleben, Germany	+	-	+	+	-	+	+	-
T1BL:1RS										
Fundulea-29	Avrora (Petkus)	NARDI Fundulea	-	+	+	-	+	+	-	+
Transilvania-1	Avrora (Petkus)	NARDI Fundulea	-	+	+	-	+	+	-	+
Fundulea-4	Avrora (Petkus)	NARDI Fundulea	-	+	+	-	+	+	-	+
C429-99	unknown	NARDI Fundulea	-	+	+	-	+	+	-	+
PI 672837 WW	Petkus (Howell et al., 2014)	National Small Grains Collection	-	+	-	-	-	+	-	+
PI 672838 WR	Petkus	National Small Grains Collection	-	+	-	-	-	+	-	+
PI 672839 RW	Petkus	National Small Grains Collection	-	+	+	-	+	+	-	+
NO-Rye										
Izvor	NO	NARDI Fundulea	-	-	-	-	-	-	-	+
Glosa	NO	NARDI Fundulea	-	-	-	-	-	-	-	+
Pitar	NO	NARDI Fundulea	-	-	-	-	-	-	-	+
Miranda	NO	NARDI Fundulea	-	-	-	-	-	-	-	+
Litera	NO	NARDI Fundulea	-	-	-	-	-	-	-	+
Rye cultivar										
Harkovskaya			+	-	+	+?	+	+	+	-

CONCLUSIONS

In our study we found that the SSR-TSM 106 (~170 bp) marker clearly distinguished the wheat-rye 1AL:1RS translocation, coming from "Insave" or related rye chromatin, from the wheat-rye 1BL.1RS translocation coming from other rye sources, behaving like a dominant marker. Thus, SSR-TSM 106 marker could be used in MAS (Marker-Assisted Selection) for highlighting the 1AL:1RS translocation. Therefore, with improved molecular tools for detection of rye chromatin in wheat, we expect new translocation lines to be developed, new superior genes to be discovered and used in wheat breeding.

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