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1RS translokacija u hrvatskome sortimentu ozime pšenice

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Izvorni znanstveni članak

SUMMARY

The translocation between the short arm of chromosome 1R of rye and the long arm of chromosome 1A, 1B or 1D of wheat represents practically the most common introduced foreign genes into the genome of hexaploid wheat. 1RS chromosome arm represents a source of different useful genes, associated with increased disease resistance, improved adaptability, stress tolerance, and increased stability and level of yield. On the other hand, translocation is associated with poor technological quality of wheat as a result of the presence of secalin and reduced number of gluten loci. The aim of this study was to determine the prevalence of 1RS translocation among some Croatian winter wheat varieties using molecular markers. The study included 40 varieties of which 23 Croatian. Four pairs of primers: RIS, SCM9, RYE-NOR and PAW161 were used for determination of translocation. The presence of translocation was determined in 12 of 40 (30%) varieties, while among the Croatian wheat varieties translocation had 8 of 23 (34.78%) varieties (Zlatna Dolina, Barbara, Nova Žitarka, Marija, Prima, Kuna, Koleda and Dea).

Key-words: wheat, rye, translocation, molecular marker

INTRODUCTION

The main goal of wheat breeders is improving yield and quality, including the development of resistance to biotic and abiotic stress. A way for increasing the genetic variability, as a basic premise of successful breeding, was found through the exploitation of rye genome (Riley and Kimber, 1966). Of particular interest is the short arm of chromosome 1R of rye established that carries many desirable genes increasing the stability and level of yield, water use efficiency, resistance to certain pests diseases and tolerance to different stress conditions (McIntosh, 1983, Rajaram et al., 1983, Villareal, 1994, Mirzaghaderi et al., 2011). The translocation between the short arm of chromosome 1R of rye and the long arm of chromosome 1A, 1B or 1D of wheat represents practically the most common introduced foreign genes into the genome of hexaploid wheat (Denčić et al., 2008). More than 16 different wheat–rye translocations are known among which 1BL/1RS has been most widely used in wheat breeding (Schneider and Molnar – Lang, 2008, Bagherikia et al., 2014).

Spontaneous translocation of short arm of chromosome 1R of rye in place of the short arm of chromosome 1B of wheat was first mentioned by Kattermann (1937). The translocation has been unintentionally transferred to number of wheat genotypes by conventional breeding, while selection for disease resistance without cytological control has favoured precisely those genotypes (Mettin et al., 1973). The mentioned translocation was introduced into the wheat cultivars worldwide using Russian wheat cultivars Kavkaz and Aurora in wheat breeding programs (Schlegel and Korzun, 1997, Rabinovich, 1998).

Beside its positive effects, translocation is sometimes associated with poor technological quality of wheet, manifested in terms of reduced tolerance of dough during mixing, increased stickiness of dough and reduced volume of bread. It is considered that the presence of rye secalins, as well as the reduced number of gluten loci causes the negative impact of translocation on the technological quality of wheat (Moonen and Zeven, 1984, Dhaliwal and MacRitchie, 1990, Martin and Carrillo, 1999). However, research results on the

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translocation influence on agronomic traits of wheat are sometimes very contradictory. Jošt et al. (1989) studied the effects of 1B/1R translocation on bread making quality of high yielding Yugoslavian wheat. Their results indicated that good quality bread wheat could possess 1B/1R translocation without significant detrimental effect on quality, probably due to good HMW glutenin subunits composition.

Ren et al. (2012) report that the main reasons for inconsistent effect of 1BL/1RS translocation are different genetic bases of wheat varieties and different sources of rye chromosome. Also, they claim that the genetic diversity of 1BL/1RS lines is the result of not only different genetic basis of wheat and different sources of rye chromosome, but also of their interactions and of the translocation itself.

Since the translocation plays an important role in wheat breeding programs it is necessary, for practical reasons, to develop methods for its fast and reliable identification (Weng et al., 2007). Small chromosomal segments of rye, being the most desirable in wheat breeding, are difficult to identify using cytological methods. Therefore, there is a growing interest in molecular identification methods, based on the use of repetitive sequences specific for rye (Tiwari et al., 2002, Iuoras et al., 2006). Molecular markers have been widely used in wheat breeding for germplasm characterization, genetic diversity assessment, genetic loci identification etc. (Petrović et al., 2012a, 2012b, Rukavina et al., 2012).

The aim of this study was to determine the prevalence of 1RS translocation among some Croatian winter wheat varieties using molecular markers.

MATERIAL AND METHODS

The study included 40 varieties of hexaploid winter wheat (*Triticum aestivum* ssp. *vulgare* L.) and two rye varieties (*Secale cereale* L.). Among 40 varieties of hexaploid winter wheat, 23 are Croatian and 17 foreign (Table 1). Some foreign wheat varieties (Kavkaz, Chinese Spring) and two rye varieties (Picasso, Albedo), served as a control in the identification of translocation.

The DNA isolation from leaves was performed by the CTAB method (Doyle and Doyle, 1987, modified according to Grljušić, 2003). For the identification of 1AL/1RS and 1BL/1RS translocation and PCR analysis, four rye specific molecular markers were used: RIS, SCM9, RYE-NOR and PAW161. Primer sequences, expected amplification products and references of the above mentioned molecular markers are listed in Table 2. Final concentrations of the reagents used in PCR amplification were, for RIS and RYE-NOR - DNA 25 ng, Taq 0.02 U/μl, each primer 0.04 μM, dNTP 0.08 mM, $MgCl_2$ 2 mM; for SCM9 - DNA 25 ng, Taq 0.125 U/μl, each primer 0.5 μM, dNTP 0.2 mM, $MgCl_2$ 1.75 mM and for PAW161 - DNA 25 ng, Taq 0.05 U/μl, each primer 0.2 μM, dNTP 0.2 mM, $MgCl_2$ 2.0 mM. Annealing temperatures for RIS, SCM9, RYE-NOR and PAW161 primers were 65°C, 63°C, 65°C and 60°C, respectively. PCR analysis was performed using Eppendorf Mastercycler® Thermal Cyclers 5333. The products of PCR analysis were applied on 2% agarose gel for separation and further analysis. After the completion of electrophoresis agarose gel with PCR products was photographed using Syngene® G: BOX F3 imaging system.

Table 1. List of analyzed wheat varieties

Tablica 1. Popis analiziranih kultivara pšenice

Nr. *Br.*	Variety *Kultivar*	Pedigree *Rodoslovlje*
1	U1	Carlotta Strampeli/Marquis
2	Osječka 20	Osk. 6.9-1-64/V-188-M
3	Slavonija	Osječka 20/Osk.4.216-2-76
4	Žitarka	Osk.6.30-20/Slavonka/3/Eph. M68/Osk.154-19/Kavkaz
5	Srpanjka	Osk. 4.50.-1-77/Zg-2696
6	Demetra	Osk. 4.216-2-76/Zg 2877-74
7	Superžitarka	GO 3135/Žitarka
8	Zlatna Dolina	Zg 414-57/Leonardo
9	Golubica	Slavonija/Gemini
10	Janica	Osk. 5.36-9-91/Srpanjka
11	Barbara	GO 3135/Žitarka
12	Nova Žitarka	FS-800/89/Žitarka
13	Sana	Mura/CI 14123//Zg 2413-72
14	BC Elvira	Bc 2377-79/MV-C2-33//Irena
15	Adriana	ZG 1758/70/TpR-349
16	Marija	Venera/NSJP-49
17	Prima	Sana/Gala

Nr. *Br.*	Variety *Kultivar*	Pedigree *Rodoslovlje*
18	Kuna	St 563/Skopljanka
19	Cerera	Divana//Zlatna Dolina//Kavkaz
20	Koleda	Divana//Zlatna Dolina//Kavkaz
21	Divana	Favorit/5/Cirpiz/4/J.Kwang/2/Atlas66/Comanc,/3/Velvet
22	Gabi	Srpanjka/GK 32-38
23	Dea	Srpanjka/Brutus
24	Kavkaz (Russia)	Lutescens-314-h-147/Bezostaya-1
25	Florida (Germany)	Caribo/Disponent
26	Mv 14 (Hungary)	Mir-808/Bez-1//Kavkaz/Rana-1//Zl.Dolina/Arthur
27	Pesma (Serbia)	NS-51-37/Balkan
28	NS 602 (Serbia)	S-13, ITA/Aobakomugi
29	Renesansa (Serbia)	Yugoslavia/NS-55-25
30	Sofia NS (Serbia)	GKGRA-965-2/Panonija
31	Sava (Serbia)	Fortunato*2/(CI-13170)Redcoat
32	NS Rana 1 (Serbia)	Bezostaya-1/NS-262//Mironovskaya-808/3/NS-435
33	San Pastore (Italy)	Balilla/Villa gloria
34	Libellula (Italy)	Tevere/Giuliari//San Pastore
35	Mironovskaya 808 (Ukraine)	(T)Artemovka
36	Bankuti 1205 (Hungary)	Marquis/Bankuti-5
37	SW Maxi (Germany)	-
38	Chinese Spring (China)	LV/Sichuan
39	Golin (Switzerland)	EXSR-400/Sonett/3/Sappo//B-580/B-664/4/EXSR-400/Selepek
40	Mv 18 (Hungary)	Bezostaya-2/Krasnodarskii-Karlik-1

Table 2. Primer sequences and expected amplification products

Tablica 2. Sekvence početnica i očekivani produkti amplifikacije

Marker *Marker*	Primer sequences (5´→ 3´) *Sekvence početnica (5´→ 3´)*	Expected products *Očekivani produkti*	Reference *Literaturni izvor*
RIS	F: TAA TTT CTG CTT GCT CCA TGC R: ACT GGG GTG CAC TGG ATT AG	110 bp	Koebner (1995)
SCM9	F: TGA CAA CCC CCT TTC CCT CGT R: TCA TCG ACG CTA AGG AGG ACC C	206 bp (1BL/1RS) 226 bp (1AL/1RS)	Saal and Wricke (1999)
RYE-NOR	F: GCA TGT AGC GAC TAA CTC ATC R: CCC AGT TTT CCA TGT CGC	360 bp	Koebner (1995)
PAW161	F: TGA GGG CCC AGA CGG CCC TTT TTG R: TTA TCG CAA TTA CAA CTC AAA TTT	400, 600, 800 bp	Guidet et al. (1991)

RESULTS AND DISCUSSION

Among 40 analyzed wheat varieties translocation was, using RIS primers, determined in nine of them of which five Croatian (Nova Žitarka, Marija, Kuna, Koleda and Dea) and four foreign varieties (Kavkaz, Mv 14, San Pastore and Mv 18). Using SCM9 primers, translocation was determined in six wheat varieties of which three Croatian (Marija, Kuna and Koleda) and three foreign varieties (Mv 14, San Pastore and Mv 18). Using RYE-NOR varieties, translocation was determined in four of them of which two Croatian (Kuna and Koleda) and two foreign varieties (Mv14 and San Pastore). Using PAW161 primers, translocation was determined in 10 varieties, six Croatian (Zlatna Dolina, Barbara, Marija, Prima, Kuna and Koleda) and four foreign varieties (Kavkaz, Mv 14, San Pastore and Mv 18).

The presence of the translocation in Kavkaz variety was confirmed with two pairs of primers (RIS and PAW161), in varieties Mv 18 and Marija with three pairs of primers (RIS, SCM9 and PAW161) and in varieties Mv 14, San Pastore, Kuna and Koleda with all pairs of primers (RIS, SCM9, RYE-NOR and PAW161). In other varieties translocation was confirmed with only one pair of primers (Figure 1).

Generally, translocation was determined in 12 of 40 (30%) analyzed wheat varieties, while among Croatian wheat varieties translocation had eight of 23 (34.78%) varieties (Zlatna Dolina, Barbara, Nova Žitarka, Marija, Prima, Kuna, Koleda and Dea). Villareal et al. (1998) reported that in some countries 90% of the sown wheat has a 1B/1R translocation, also over 50% of CIMMYT wheat varieties possess the mentioned translocation (Merker, 1982), in Hungary translocation was determined in 53% wheat varieties (Koszegi et al., 2000), in India in 78.94% (Tiwari et al., 2002), in Bulgaria in 54% (Landjeva et al., 2006), in Iran according to different sources from 11% to 21% (Mirzaghaderi et al., 2011, Tabibzadeh et al ., 2013, Bagherikia et al., 2014), in Serbia in 25% varieties (Denčić et al., 2008) etc.

Sinkovič (2011) reports that indirect sources of 1BL/1RS translocations in Yugoslavian breeding programs were the substitution lines Weique and Neuzhücht, which carried chromatin from Petkus rye. 1B/1R translocation was introduced at these areas using Russian wheat varieties Aurora, Kavkaz and Skorospelka in breeding programs of former Yugoslavia (Dimitrijević et al., 2008, Jošt and Samobor, 2008). In most studies precisely rye varieties Petkus and Salmon and wheat varieties Aurora and Kavkaz are used as positive controls for the identification of the mentioned translocation. In this study wheat variety Kavkaz and rye varieties Picasso and Albedo were used as positive controls, while Petkus seed was not available. As a negative control we used wheat variety Chinese Spring, referred in many papers as a control variety not possessing the translocation (Weng et al., 2007, Yediay et al., 2010, Tabibzadeh et al., 2013). The expected amplification products of all primers were present by two rye varieties while in case of the wheat variety Kavkaz only products of two primers were present. It could be caused by DNA degradation, unadjusted PCR conditions or unspecific markers. No amplification products were present by wheat variety Chinese Spring.

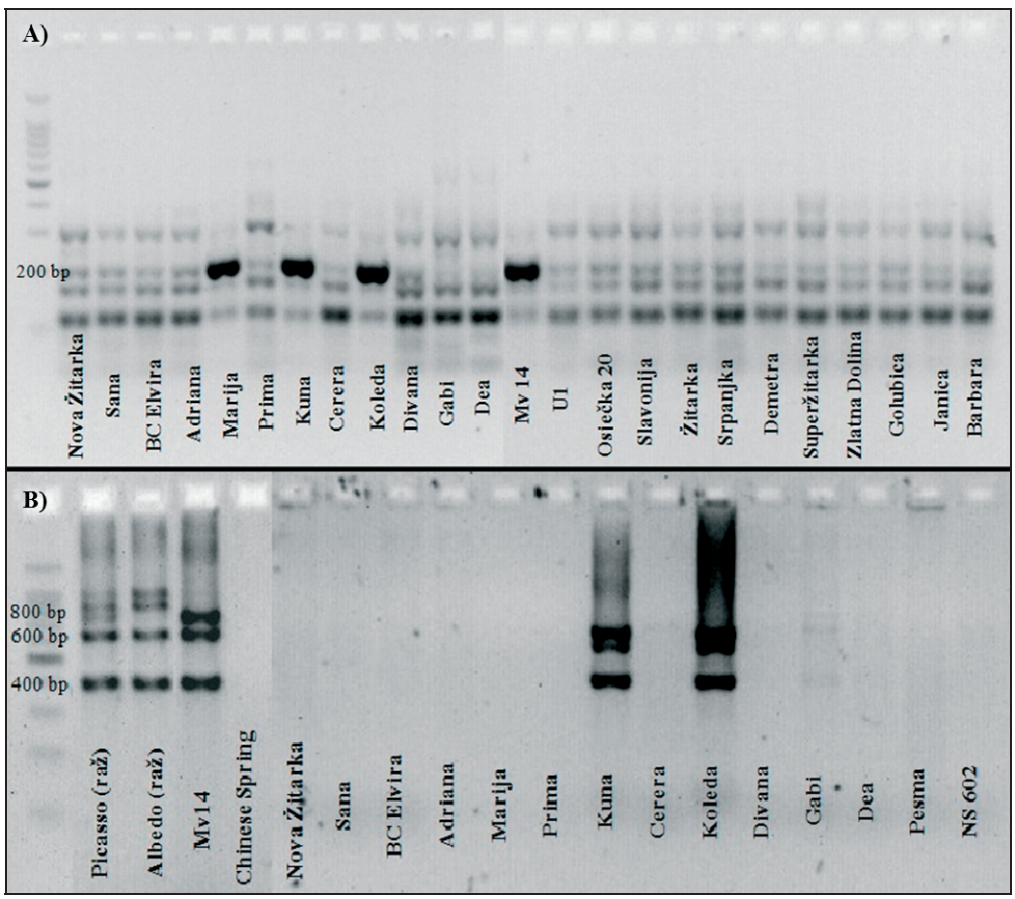

Figure 1. Amplification products of: A) SCM9 primers, B) RYE-NOR primers (photo original; S. Guberac)

Slika 1. Produkti amplifikacije: A) SCM9 početnica, B) RYE-NOR početnica (foto original; S. Guberac)

By comparing the obtained results with genealogy of some cultivars it can be concluded that genealogy is not sufficient indicator of the presence of translocation and that it is necessary to combine information on the origin with data of molecular analysis. For example, in wheat variety Žitarka the presence of the translocation has not been established although one of its parents is Kavkaz, 1B/1R translocation carrier. It is interesting that of the two sister lines, Cerera and Koleda, according to our results, one possesses the translocation and one does not. Also, there is a disagreement between pedigrees of wheat varieties San Pastore and Zlatna Dolina

and obtained results. This could be due to the incomplete pedigrees, seed contamination through cross-fertilization or marker error. Therefore, further analysis of those varieties should be carried out.

In order to facilitate and speed up the identification of translocation, the previously used methods are increasingly being replaced by molecular and number of different molecular markers for the identification of rye genetic material in wheat genome has been developed. However, Weng et al. (2007) claim that despite the fact that large number of molecular markers has been developed there are very little research and data on their evaluation and usefulness for marker assisted selection.

Considering that the translocation is associated with important agronomic traits of wheat, the information on inheritance and distribution of translocation would be very helpful for breeders in planning the breeding programs.

CONCLUSION

Using different pairs of primers, the presence of translocation was determined in 12 of 40 (30%) varieties, while among Croatian wheat varieties translocation had 8 of 23 (34.78%) varieties (Zlatna Dolina, Barbara, Nova Žitarka, Marija, Prima, Kuna, Koleda and Dea).

Based on the existing research results on this issue in the world, there are a number of advantages, but probably and disadvantages of local winter wheat varieties possessing 1RS translocation, in contrast to varieties without translocation. It is therefore necessary to do additional research on the above mentioned varieties and determine their reaction to various agroecological conditions through multi-year research trials on several locations.

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1RS TRANSLOKACIJA U HRVATSKOME SORTIMENTU OZIME PŠENICE

SAŽETAK

Translokacija između kratkoga kraka kromosoma 1R raži i dugoga kraka kromosoma 1A, 1B ili 1D pšenice predstavlja praktično najzastupljenije unesene strane gene u genom heksaploidne pšenice. 1RS krak kromosoma predstavlja izvor različitih korisnih gena, koji se povezuju s povećanom otpornošću na bolesti, povećanom adaptabilnosti, tolerantnosti na stres te povećanom stabilnošću i visinom prinosa. S druge strane, translokaciju se povezuje i s lošom tehnološkom kvalitetom pšenice, kao posljedicom prisutnosti sekalina i smanjenja broja glutenskih lokusa. Cilj ovoga istraživanja bio je utvrditi zastupljenost 1RS translokacije u hrvatskom sortimentu ozime pšenice korištenjem molekularnih markera. U istraživanje je bilo uključeno 40 kultivara pšenice, od čega 23 hrvatska kultivara. Za identifikaciju translokacije korištena su četiri para početnica: RIS, SCM9, RYE-NOR i PAW161. Istraživanjem je utvrđena prisutnost translokacije u 12 od 40 (30%) ispitivanih kultivara, dok je među hrvatskim kultivarima pšenice translokaciju imalo 8 od 23 (34,78%) kultivara (Zlatna Dolina, Barbara, Nova Žitarka, Marija, Prima, Kuna, Koleda i Dea).

Ključne riječi: pšenica, raž, translokacija, molekularni marker

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