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MOTSNY I.I.[1] \ SUDARCHUK L.V.[1] \ GALAEV A.V.[1] \ CHEBOTAR S.V. [1,2]

*[1] Plant Breeding and Genetics Institute - National Center of Seed and Cultivar investigations, Ukraine, 65036, Odessa, Ovidiopolskaya dor., 3, e-mail: motsnyyii@gniail.com **

[2]Odessa National Mechnikov University, Department of Genetics and Molecular Biology, Ukraine, 65026, Odessa, Dvoryanskaya str., 2

APPLICATION OF PCR MARKERS FOR DETECTING $1B_L.1R_S$ WHEAT-RYE CHROMOSOME TRANSLOCATIONS AND (1 B) 1R SUBSTITUTIONS

Nikolai Vavilov was the first to recognize the utilization of wheat relatives is a promising source for wheat improvement [1]. As an development of Vavilov's ideas a number of wheat introgression stocks with a high resistance to powdery mildew, leaf and stem rusts, frost tolerance, high protein content and some *morphological* characters has been obtained as a result of wide crosses [2, 3]. For a successful practical application the stocks require an identification of the alien introgressions. DNA markers become a useful tool for gene or chromosome identification, especially being valuable in respect of new for wheat an alien genetic material.

This paper deals with PCR marker assisted detection of (IB)IR wheat-rye chromosome substitution and $1B_L$. 1 R_s translocation, their meiotic behavior and genetic analysis of certain alien characters. incorporated into wheat. The investigation was carried out within a program for the development of a genetic collection of bread wheat lines with qualitative characters.

Material and methods

A set of original primitive introgression stocks (*2n* = 42): Erythrospermum 200_97-2 (in further E200 97-2), Erythrospermum 217_97
(E217 97). Hostianum 242 97-1 (H242_97-l).
Hostianum 242 97-2 (H242_97-2), Hostianum 273_97 (H273 97), Hostianum 274_97 (H274_97) and OH232_03, collection sib-strains H74 90-245 and H74 90-258. w inter bread wheat cv. Odesskaya 267 (Od267) and F| hybrids between Od267 and all the lines have been investigated. The majority of the stocks were developed from a cross: triticale (8x) cv. AD825/7'. *durum* Desf. cv. Chernomor and spontaneous hybridization of the F_3 hybrids with the strain H74 90-245 or H74 90-258. or without it. Triticale AD825 is a primary amphidiploid (*T. aestivum* L. cv. Hostianum 237*IS. cereale* L. cv. Voronezhskava SHI) [4]. The strains H74 90-245 and H74 90-258 were derived in Dobroudja Agricultural Institute (General Toshevo, Bulgaria) from the step cross: Dr. Savov's synthetic *(T. timopheevii* Zhuk*JAe. tauschii* Coss.)/Tom Pouce

Blanc//Avrora/3/Rusalka and received from Dr. Ivan Panayotov. The stock OH232_03 was obtained from a cross Od267/H74_90-258.

All lines were analyzed by using DNA- markers. DNA was isolated from leaf material of adult plants and seedlings according to standard CTAB-methods. Because IRs chromosome presence, as well as some target gene location were supposed. the molecular markers: rye microsatellites: *Xremsl303. SR1R003* [5], a secalin-specific STS-marker — ωo-sec-P3 + ωsec-P4 [6] and wheat microsatellites:Xgwm18-1B$_l$, Xgwm-lBs, *Xgwml40-\B$_L$ Xgwml53-1B$_L$, Xgwm357-* 1A$_L$ [7], *Taglut-* 1A$_S$ [8] were chosen for the analysis. PCR amplification was carried out in a thermocycler 'Tercik' (Russia), and a standard electrophoresis procedure in 10 % poly acrylamide gel (PAAG) was applied for differentiation of PCR products [9]. Fragment sizes were calculated by comparison with molecular weight marker pUC19/MspI. IRs chromosome presence was detected with the rye microsatellites and the secalin-specific STS-marker. Substitution or translocation was identified by the absence of IB chromosome corresponding arm via application of the wheat microsatellites.

Resistance to powdery mildew, leaf and stem rusts, hairiness of the glumes and leaves was evaluated within researched material to contain. Moreover, the stocks, cv. Od267 and the F$_1$S were studied cytologically with routine acetocarmine methods. The chromosome substitution or translocation presence in the stocks and the strains was confirmed cytologically for meiotic configurations at metaphase I (MI) in pollen mother cells (PMCs) of the Fi hybrids.

Plant pathogen resistance was evaluated at the adult plant stage in field with use of an international universal scale. Furthermore, powdery mildew resistance was noted in field in later autumn at the seedling stage. Leaf and stem rust resistance were scored both at natural epiphytoty conditions and under an artificial infection pressure. Herewith, population mixtures of the most aggressive local races of both diseases were used. All phenotypical evaluations were conducted under field conditions at the heading and flow'ering stages. Hairiness (pubescence) was searched on the glumes, upper (adaxial) and lower surfaces of a leaf blade, as well as on the leaf margin at the culm node using a magnify ing glass.

Results and discussion

The presence of 1R$_s$chromosome was detected in the introgression stocks and sib-strains by the presence of specific products of: *Xremsl303. SR1R003, ω-sec-P3 + ω-sec-P4* markers. The absence of PCR products with the markers *Xgwml8* (IBs), *Xgwm550* (1B$_S$), as well as *Xgwml40* (IB$_L$) and *Xgwml53* (1B$_L$.) permitted to identify IB chromosome translocation or substitution. The detection of PCR-products of the *Taglut* (1As) and *Xgwm357* (1A$_l$.) markers proved the presence of intact 1A chromosome in the lines. The amplification products with the markers *Xgwml40* and *Xgwml53* were not detected for the stocks H273 97 and H274 97, but were obtained within collection sib-strains and for the stocks E200_97-2, H217_97. H242_97-I, H242_97-2 and OH232_03. as well (Table 1). Thus, the stocks H273_97 and H274_97 carry (IB)IR substitution, and all other lines carry I B$_L$.. IR$_S$ translocation chromosome.

Table 1. Results of PCR-analysis of the lines studied for the marker loci alleles, bp

Marker locus	Od267	H74_90-245	H74_90-258	E200_97-2	E217_97	H242_97-1	H242_97-2	H273_97	H274_97	OH232_03
Xrems1303 (1R$_S$)	-*	290	290	290	290	290	290	290	290	290
SR1R003 (1R$_S$)	-	97	97	97	97	97	97	97	97	97
ω-sec-P3/P4 (1R$_S$)	-	400	400	400	+#/-	400	400	400	400	400
Xgwm18 (1B$_S$)	186	-	-	-	188	-	-	-	-	-
Xgwm550 (1B$_S$)	195	-	-	-	-	-	-	-	-	-
Xgwm140 (1B$_L$)	223	223, 233	223, 233	223, 233	223, 233	223	223	-	-	223, 233
Xgwm153 (1B$_L$)	195	195	195	195	195	195	195	-	-	195
Taglut (1A$_S$)	126	137	134	135	131	128	128	128	128	131
Xgwm357 (1A$_L$)	124	124	124	124	124	124	124	124	124	124

Notes: * – the primer product absence; # Size of DNA amplification fragment in PAAG is more than 400 bp at the stock E217_97.

In general there was no polymorphism of rye DNA markers among the lines with the introgressions. Only by using the secalin-specific ω-sec-P3 + ω-sec-P4 primers a genetic polymorphism has been detected supposing a new allele of *Seel* locus in the stock E217_97. The presence of the product 188 bp w ith the *Xgwml8* marker simultaneously with rye DNA fragments (Table 1) has proved the translocation heterozygosis in that stock.

Meiotic observations have supported the molecular-genetic evidence and have revealed 20 closed bivalents (the maximum) plus an open bivalent (2^{11}c + l"o) at MI in the F_1 hybrids Od267/translocation stocks (fig. 1, a). Similarly 20 bivalents and 2 univalents ($I9^{11}_c$- + 1^{11}o + 2^1) were observed in the F_1S Od267/substitution stocks (fig. 1, b). The translocation $1B_L$| .IRs heterozygosis has also been confirmed in the stock E217 97: some F_1, plants Od267/E217 97 had 2^{11}_c +1 ^{11}o as the highest meiotic association and the others- $2l^{11}$c(fig. l, c).

The pairing between short arms of IR and IB chromosomes has not been well documented in literature. In this study there was no pairing between IR and IB chromosomes in any 322 PMCs studied in the H273_97/Od267 and H274_97/Od267 crosses. In the contrast. 21^{11}c were observed in 3 meiotic PMCs of 894 (0.3 %) studied in the F|S between Od267 and the introgression stocks E200_97-2. E217_97 (plants with $1B_L$.IRs translocation), H242_97-l and H242_97-2. Therefore, the IB_L,1 R_s translocation of the stocks might rarely pair with IBs chromosome.

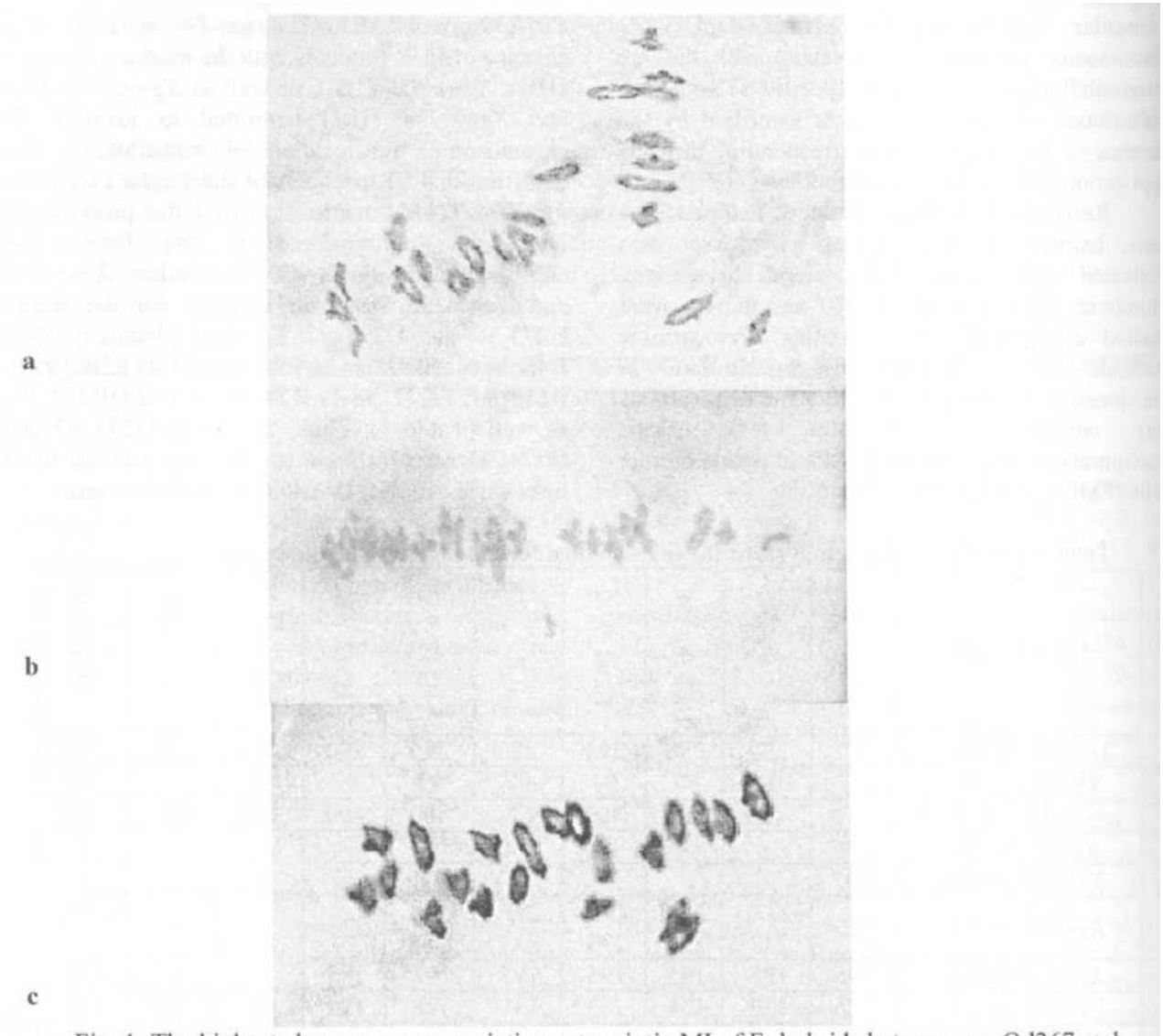

Fig. 1. The highest chromosome associations at meiotic MI of F_1 hybrids between cv. Od267 and **(a)** line E242_97-1: $20^{II}_C + 1^{II}_O$; **(b)** line H274_97: $19^{II}_C + 1^{II}_O + 2^I$; **(c)** line E217_97 (plants without $1B_L.1R_S$ translocation): 21^{II}_C (590×)

Thereby, investigated introgression stocks and the collection sib-strains have (IB)1R wheat-rye chromosome substitution or IB_L. 1 R_s translocation. That was determined and identified with use of PCR-markers (Table I) and confirmed cytologically (fig. 1). The translocation was contributed by the collection sib-strains H74_90- 245 and H74_90-258 and derived from Russian wheat cv. Avrora. Therefore, the rye $1R_S$ chromosome is originated from Petkus rye. This chromosome arm transferred to bread wheat genetic background carries genes, important for the adaptation of wheat varieties, particularly closely linked the genes *PmS,* Tr9. *Lr26* and *Sr3l* [10]. The intact rye chromosome IR for the substitution was contributed by triticale (8x) cv. AD825 and, therefore, originated from *S. cereale* L. cv. Voronezhskaya SHI. Evidently, such chromosome rearrangements are known to occur in wheat-rye or wheat-triticale crosses [II].

Due to their agronomic advantages trans locations with IR_S are usually widespread in cultivars from Forest-Steppe zone of Ukraine, but not from South. In South Ukraine IRs chromosome has not been used in wheat breeding, because of traditional to PBGI — NCSCI storage protein composition selection for the high technological quality [12]. However, nowadays a program for wheat-rye translocation use in wheat breeding has been started [13] and the cvs Zhitnitsa (with IA_I. IRs translocation, leaf and stem rust resistance and middle quality) and Schedrisf (with IB_L. $1R_S$ translocation and low quality) have been developed.

Depending on karyoty pe structure the stocks were considerably distinguished by powdery mildew, leaf and stem rust resistance and by the presence of morphological characters (hairy spike or leaf). The lines E20097-2, H242 97-1, H242_97-2. H74 90-245 and H74_90-258. carry ing the 1 Bi. I R_s translocation from cv. Avrora, had high resistance to all the diseases. There were three and two genes for resistance, respectively, to leaf and stem rusts in the lines, and *Lr26* and *Sr3l* among them [14]. Cv. Od267 was susceptible. The stocks H273_97 and H274 97 were moderately infected by powdery' mildew and stem rust (MS) and did not have any leaf rust resistance (S-VS). E217_97 was somewhat resistant (MS-MR) to powdery mildew only at the adult plant stage, and OH232_03 was susceptible (MS) to stem rust.

As to a pubescence, the presence of typical wheat *Hgl* gene (short and week glume hairiness like in cv. Ulyanovka) in the stocks of *Hostianum* species (H242_97-l, H242_97-2, H273_97 and H274 97) is determined. The gene is located in I A_s chromosome [10] and is originated from old cv. Hostianum 237 - a parental form for the octoploid triticale AD825. The *Hgl* gene coding hairiness of glumes Mendelian mode of inheritance was determined: 63 haired: 16 not haired (jf_3 t = 0.95) Ft hybrids in a test-cross with Od267.

As for leaf blade hairiness, the stocks E217 97. H273 97 and H274 97 were identified as glabrous ones, and cv. Od267 had a thin layer of hairs on the adaxial surface. In contrast, the stocks E20097-2. H242_97-l and H242_97-2, as well as the collection strains H74 90-245 and H74 90-258 were found to carry hairiness on upper and lower surfaces, as well as on leaf margin at leaf base. Three major linked genes determining hairiness of the leaf upper surface *(HI^T),* lower surface (////„„.) and leaf margin *(Him)* were revealed with location, supposedly, on the long arm of chromosome 4D. The genes were contributed by a synthetic *(T. timopheevii* Zhuk *JAe. tauschii* Coss) and, therefore, were originated from *T. timopheevii* or *Ae. tauschii.* The *Hr^p, HI„w* and *Him* loci are non-allelic to *HI* gene. In wheat the alleles *Hg* and *HI* determine hairiness of glumes or leaf pubescence which allows them to avoid drought and high temperatures during the vegetation or grain filling [10].

Conclusion

With use of molecular-genetic and cytological analyses (1B) 1R wheat-rye chromosome substitution or IB_L.IRs translocation were detected in the original primitive introgression stocks. The pairing between $1R_S$ and IBs chromosomes was revealed with very low frequency. Three and two genes for resistance, respectively, to leaf and stem rusts were revealed, and *Lr26* and *Sr31* among them have been recognized and determined to be somewhat effective. The genes were identified with the molecular markers *Xremsl303, SR1R003.* ω-sec-P3 + ω-sec-P4. contributed by cv. Avrora and originated from Petkus rye.

The *Hgl* gene coding hairiness of glumes Mendelian mode of inheritance was determined. Three major linked genes determining hairiness of the leaf upper surface *(Hl^{up}),* lower surface (HL_{low}) and leaf margin *(Him)* were revealed. The glume hairiness gene was contributed by the old cv. Hostianum 237. The leaf pubescence genes were contributed by a synthetic *(T. timopheevii* Zhuk *JAe. tauschii* Coss) and. therefore, originated from *T. timopheevii* or *Ae. tauschii.* The *Hl^{up}. Hl_{low}* and *Him* loci are non-allelic to *H11* gene.

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MOTSNY I.I. SUDARCHUK L.V. \ GALAEV A.V. CHEBOTAR S.V. [12]

[1] Plant Breeding and Genetics Institute - National Center of Seed and Cultivar investigations, Ukraine, 65036, Odessa, Ovidiopolskaya dor., 3, e-mail: motsnyyii@gmail.com

' Odessa National Mechnikov University, Department of Genetics and Molecular Biology, Ukraine, 65026, Odessa, Dvoryanskaya sir., 2

APPLICATION OF PCR MARKERS FOR DETECTING 1B,.1R$_S$ WHEAT-RYE CHROMOSOME TRANSLOCATIONS AND (1B)1R SUBSTITUTIONS

Aims. Molecular-genetic and cytological analyses were carried out to detect the alien genes in original introgression stocks and to investigate their inheritance. ***Methods***. Rye (*Xremsl303, SRIR003)* and wheat *(XgwmJ8*-1 B$_s$, *Xgwm550-1B$_s$, XgwmI40*-1B$_L$ *Xgwml53*-IB$_L$, *Xgwm357*-IA$_L$, *Taglut*-1A$_s$) microsatellites and secalin-specific STS-marker (ω-sec-P3+ ω-.sec-P4) have been applied. ***Results***. The (IB)1R wheat-rye chromosome substitution and 1B$_L$.IR$_S$ translocation have been identified. The pairing between short arms of the 1 B$_L$ .1 Rs translocation and of bread wheat chromosome 1B was observed with very low frequency (in 0.3% PMCs). ***Conclusions***. The stocks have (1B)1R wheat-rye chromosome substitution or 1B$_L$. 1Rs translocation. The translocation was contributed by the collection strains, derived from wheat cv. Avrora and originated from Petkus rye. The intact rye chromosome IR for the substitution was contributed by triticale (8x) cv. AD825 and originated from rye Voronezhskaya SHI. The substitution stocks were susceptible to leaf and stem rusts because of another origination of the 1R chromosome. Three major linked genes determining hairiness of the leaf upper surface (*HL^{up}*). lower surface (*HL^{low}*) and leaf margin *(Hlm)* were revealed. The genes were contributed by a synthetic (*T. timopheevii/Ae. tauschii*) and were non-allelic to *Hll* gene.

Key words: Triticum aestivum, (1 B) IR substitution. I B$_L$. I R$_S$ translocation, hairy leaf, PCR-markers.