P L A N T   B R E E D I N G   A N D   S E E D   S C I E N C E
Volume 46 no. 2 2002

Mirosław Tyrka, Grażyna Stefanowska, Czesław Tarkowski

University of Agriculture in Lublin, Institute of Genetics and Plant Breeding, Akademicka 15, 20–934 Lublin, Poland

IDENTIFICATION OF 1BL/1RS TRANSLOCATION IN INTERSPECIFIC HYBRIDS BETWEEN *AEGILOPS* AND *TRITICUM*

ABSTRACT

The aim of studies was testing for presence of 1BL/1RS translocation in 17 lines derived from wide crosses of *Aegilops ventricosa* and *Aegilops juvenalis* with durum and common wheats (including translocation carrying Lanca and CZR1406), along with parental forms. PCR analyses detected translocated chromosome in Lanca and CZR1406 wheats and in 4 hybrid lines (JCPC, JCCP, JCC and JCCC). The lack of 1RS arm in lines (VGL and VGLL) derived from crosses with Lanca suggests that another chromosomal rearrangements occurred. We confirmed the usefulness of applied STS–PCR assays for fast and robust identification of 1BL/1RS translocation in breeding materials.

Key words: 1BL/1RS translocation, *Aegilops spp.*, STS–PCR, *Triticum durum*, *Triticum aestivum*

INTRODUCTION

1BL/1RS translocation is very important in common wheat breeding programmes as 1RS chromosome arm carry genes responsible for resistance to rusts (*Yr9*, *Sr31*, *Lr26*), powdery mildew (*Pm8*) and Hessian fly (*Schizaphis graminum* Rondani) – *Gb6* (McIntosh 1983). The presence of 1BL/1RS translocation can positively affect the yield (Carver and Rayburn 1994; Moreno–Sevilla *et al.* 1995; Villareal *et al.* 1995), but sometimes has deleterious effects on it's quality (Lee *et al.* 1995; Bullrich *et al.* 1998). According to Lee *et al.* (1995) improvement of yield quality in 1BL/1RS lines obtained after crossing with cultivars containing high gluten quantities, low level of water–soluble proteins, and possessing good baking properties is possible.

There are many methods of identification of 1BL/1RS translocation. They rely on detection of changes in chromosome morphology (Rayburn and Caver 1988), protein electrophoresis (Mitteilung 1986,

Communicated by Andrzej Anioł

Koebner 1990, Gupta and Shepherd 1992, Hussain and Lukow 1994), hybridisation techniques as RFLP (*Restriction Fragment Lenght Polymorphism*) or GISH (*Genomic in Situ Hybridisation*) (Heslop–Harrison *et al.* 1990), and PCR (*Polymerase Chain Reaction*) based methods (Rogowsky *et al.* 1992, Iqubal and Raybrun 1995, Francis *et al.* 1995, de Froidmont 1998). According to Francis *et al.* (1995) markers detecting gene product are specific to defined locus while, hybridisation methods based on probing with rye DNA are too time–consuming to be routinely used for analyses of big number of plants usually obtained in breeding populations.

The aim of presented study was the identification of 1BL/1RS translocation in hybrid materials obtained from multiple crosses of common wheat cultivars with *Aegilops ventricosa* and *Aegilops juvenalis* along with parental forms. Two PCR based methods were used (Iqubal and Raybrun, 1995; de Froidmont, 1998) to detect the presence of 1RS chromosome arm; Van Campenhout *et al.* (1995) method was exploited for testing for 1BS presence. Through application of recently developed PCR–based methods of identification of translocated chromosome 1BL/1RS on materials derived from crosses between *Aegilops* and various wheat cultivars with and without translocation (Tarkowski and Apolinarska, 1992) we: 1) verified the usefulness of the PCR–based methods tested on common wheat cultivars to identification of 1BL/1RS translocation in complex intergeneric hybrids with possible rearrangements in coding and deletions in non–coding sequences (Liu *et al.*, 1998 a,b) and 2) verified the presence or absence of 1BL/1RS chromosome in hybrid materials as that were expected to carry translocation on the basis of descent.

MATERIALS AND METHODS

Plant materials

Plant material used in studies is presented in Table 1. Hybrid materials were obtained in result of crosses with wheat cultivars possessing 1BL/1RS translocation (cvs. Lanca and CZR1406) or without this translocation (cvs. Panda, Begra, Arda and Grandur). Methods of obtaining hybrids and breeding history were described previously (Stefanowska, 1995; Stefanowska *et al.*, 1995). In subsequent generations material was cytogenetically controlled and selected for fertility and yield–affecting traits. Material was sampled from 10 of field–grown plants. On the base of yield–affecting traits and molecular analyses (Tyrka and Stefanowska, 2001; Tyrka and Stefanowska, submitted) material selected was found to represent higher diversity when compared to parental wheat cultivars.

Table 1

Hybrid lines and parental forms

Cultivar / Line	Pedigree [a]
VGL	F_8 *Ae.ventricosa*/Grandur/2/Lanca
VGLL	F_7 Ae.ventricosa/Grandur/2/2 × Lanca
VGPP	F_7 *Ae.ventricosa*/Grandur/2/2 × Panda
VGPPP	F_6 *Ae.ventricosa*/Grandur/2/3 × Panda
VGPB	F_7 *Ae.ventricosa*/Grandur/2/Panda/3/Begra
VGPBP	F_6 *Ae.ventricosa*/Grandur/2/Panda/3/Begra/4/Panda
VGPA	F_7 *Ae.ventricosa*/Grandur/2/Panda/3/Arda
VGPAA	F_6 *Ae.ventricosa*/Grandur/2/Panda/3/2 × Arda
JCP	F_7 *Ae.juvenalis*/CZR1406/2/Panda
JCPC	F_6 *Ae.juvenalis*/CZR1406/2/Panda/3/CZR1406
JCCP	F_5 *Ae.juvenalis*/2 × CZR1406/3/Panda
JCCPC	F_6 *Ae.juvenalis*/2 × CZR1406/3/Panda/4/CZR1406
JCB	F_7 *Ae.juvenalis*/CZR1406/2/Begra
JCBB	F_6 *Ae.juvenalis*/CZR1406/2/2 × Begra
JCBC	F_6 *Ae.juvenalis*/CZR1406/2/Begra/3/CZR1406
JCC	F_8 *Ae.juvenalis*/2 × CZR1406
JCCC	F_6 *Ae.juvenalis*/3 × CZR1406
T. aestivum L. cv.Lanca	Nadzieja/Pluto
T. aestivum L. cv.Panda	Dana/Flevina
T. aestivum L. cv.Begra	Grana/Bezostaja
T. aestivum L. cv.Arda	CJ 12633/Capelle–Desprez/2/C474/73
T. aestivum L. CZR 1406	Lanca/S.cereale L506/2/Lanca
Ae. juvenalis Thell. Eig.	
Ae. ventricosa Tausch.	
T. durum Desf. cv.Grandur	Adur/mutation of Capelli

[a] Generation since last cross

DNA extraction

DNA was extracted from leaves of 10 plants representing parental forms and hybrid lines as in Milligan (1992). DNA quantity was mea–sured on agarose after comparison with Low DNA Mass Ladder (Gibco). Analyses were performed on bulked DNA for each accession used in concentration of 10 ng/mL.

PCR reaction

1BL/1RS translocation was identified on the basis of three PCR re–actions. Two were specific to short arm of 1R chromosome:

a). de Froidmont (1998) with minor modifications relying on using of one primer set: SECA2 5'–GTT TGC TGG GGA ATT ATT TG–3' and SECA3 5'–TCC TCA TCT TTG TCC TCG CC–3'. 1 X

PCR buffer (75mM Tris pH 8.8, 20mM $(NH_4)_2SO_4$, 0.01% Tween 20), 250 nM of each primer, 200 µM dNTP, 2.5 mM MgCl2, 0.4 U Taq DNA Polymerase (Fermentas, Lithuania) and 50 ng DNA in 20 mL volume.

b). Iqbal and Rayburn (1995) with primers J07IF1 5'–TAA GCC GTA AAG CAT GGT GCA C–3' and J07IR1 5'–CTT CAA CGA AAT GTT TTC CTC TTC–3' with minor modifications. We used 0.5 U DNA Taq polymerase (Fermentas, Lithuania) and 30 ng DNA in 20 ml of reaction volume.

c). The third method detected fragment of LMW glutenin gene in Glu–B3 locus on wheat 1BS chromosome arm (van Campenhout *et al.* 1995) with primers O11B3 (5'–GTT GCT GCT GAG GTT GGT TC–3') and O11B5 (5'–GGT ACC AAC AAC AAC AAC CC–3').

Obtained PCR products were separated on 1.5% agarose with 0.01% EtBr and photographed on Polaroid. Bands size was counted after photography scanning and coordinates of bands were measured in pixels with Scion Image software.

RESULTS AND DISCUSSION

Binary matrix obtained in result of performed analyses is shown in Table 2. In all cases results obtained with both methods for identification of 1RS arm were in agreement. The presence of 1RS chromosome arm was always connected with the absence of 1BS wheat chromosome. This confirms that 1RS arm replaced 1BS arm leading to formation of 1BL/1RS chromosome. As expected, we found no amplification when *Aegilops* DNA were used as a template.

De Froidmont (1998) exploited multiplex PCR for one–step testing for presence of 1BL/1RS translocation. This system was sensitive enough to detect presence of both 1RS and 1BS chromosome arms. However using primers targeting secalins we observed three PCR products (Fig. 1.) thus we decided on separating of assays to avoid misinterpretation. Modifications of method did not affected quality of results obtained suggesting small sensitivity of PCR methods to changes in reaction conditions. This is advantage of STS–PCR methods increasing their value. The method of Iqubal and Rayburn (1995) yielded one main product (Fig. 2.).

Performed analyses enabled identification of 1RS chromosome arm in Lanca and CZR1406 wheats, thus confirming earlier cytological evidence (Tarkowski and Apolinarska, 1992). 1RS detected in plants representing combinations JCPC, JCCP, JCC and JCCC was presumably introduced from CZR 1406 line. There was no sample where both arms 1RS and 1BS were simultaneously present (Fig. 3.), thus we can conclude that genotypes tested do not segregate for pres–

Table 2

Binary matrix summarizing analyses performed

No	Genotype[a]	Presence of 1RS		Presence of 1BS
		SECA2 + SECA3	J07IF1 + J07IF1	O11B3 + O11B5
1	*Ae. ventricosa*	0	0	0
2	'Grandur'	0	0	1
3	VGL	0	0	1
4	VGLL	0	0	1
5	VGPP	0	0	1
6	VGPPP	0	0	1
7	VGPB	0	0	1
8	VGPBP	0	0	1
9	VGPA	0	0	1
10	VGPAA	0	0	1
11	*Ae. juvenalis*	0	0	0
12	JCP	0	0	1
13	JCPC	1	1	0
14	JCCP	1	1	0
15	JCCPC	0	0	1
16	JCB	0	0	1
17	JCBB	0	0	1
18	JCBC	0	0	1
19	JCC	1	1	0
20	JCCC	1	1	0
21	Lanca	1	1	0
22	Begra	0	0	1
23	CZR 1406	1	1	0
24	Panda	0	0	1
25	Arda	0	0	1
26	Mass	412 bp	1 kbp	636 bp

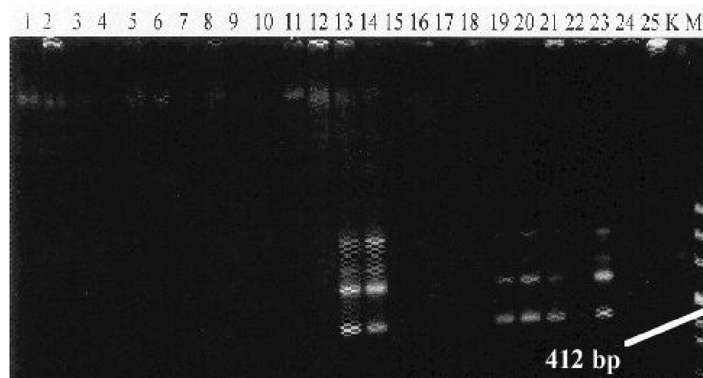

Fig. 1 Three PCR products (897, 605 and 412* bp – specific) obtained with method of de Froidmont (1998). Specific band marked with arrow. Genotype order as in Table 2

Fig. 2 Amplification products obtained with method of Iqubal and Rayburn (1995)

Fig. 3 Amplification products obtained with method of Van Campenhout *et al.* (1995)

ence/absence of 1BL/1RS translocation. Surprisingly, translocated chromosome derived from 'Lanca' wheat was not detected in VGL and VGLL lines. These lines possess different morphological characteristics (Tyrka and Stefanowska, 2001) but are cytologically stable. This suggests that another chromosomal rearrangements can be present in these lines what can be further studied using microsatellite markers.

CONCLUSIONS

Our studies suggest that:

1. The PCR methods used in present study for identification of 1BL/1RS translocation in common wheat cultivars can be applied to complex hybrids of wheat with *Aegilops ventricosa* and *Aegilops juvenalis.*
2. 1BL/1RS translocation present in CZR1406 was also identified in hybrid lines (JCPC, JCCP, JCC and JCCC) obtained in effect of crosses with this wheat.
3. 1BL/1RS translocation present in Lanca was not found in respective hybrids obtained by the use of this cultivar.

REFERENCES

Bullrich L., Tranquilli G., Pfluger L. A., Suarez E. Y., Barneix A. J. 1998. Bread–making quality and yield performance of 1BL/1RS wheat isogenic lines. Pl. Breed. 117: 119–122.

Carver B. F., Rayburn A. L. 1994. Comparison of related wheat stocks possessing 1B or 1RS.1BL chromosomes: agronomic performance. Crop Sci. 34: 1505–1510.

Francis A., Leitch A. R., Koebner R. M. D. 1995. Conversion of a RAPD generated PCR product, containing novel dispersed repetitive element, into a fast and robust assay for the presence of rye chromatin in wheat. Theor. Appl. Genet. 90: 636–642.

Froidmont de D. 1998. A co–dominant marker for the 1BL/1RS wheat–rye translocation via multiplex PCR. J. Cereal Sci. 27: 229–232.

Gupta R. B., Shepherd K. W. 1992. Identification of rye chromosome 1R translocations and substitutions in hexaploid wheats using storage proteins as genetic markers. Pl. Breed. 109: 130–140.

Heslop–Harrison J. S., Leitch A. R., Schwarzacher T., Anamthawat–Jonsson K. 1990. Detection and characterization of 1B/1R translocations in hexaploid wheat. Heredity 65: 385–392.

Hussain A., Lukow O. M. 1994. Characterization of the 1B/1R translocation in wheat using water extractable protein concentrate. Euphytica 78: 109–113.

Iqubal M. J., Rayburn A. L. 1995. Identification of the 1RS rye chromosomal segment in wheat by RAPD analysis. Theor. Appl. Genet. 91: 1048–1053.

Koebner R. M. D. 1990. Subtilisin inhibitor – a polymorphic protein produced by gene on the short arms of wheat homeologous group 1 chromosomes. J. Genet. Breed. 44: 49–52.

Lee J. H., Graybosch R. A., Peterson C. J. 1995. Quality and biochemical effects of a 1BL/1RS wheat–rye translocation in wheat. Theor. Appl. Genet. 90: 105–112.

Liu B., Vega J. M., Feldman M. 1998a. Rapid genomic changes in newly synthesized amphiploids of *Triticum* and *Aegilops.* II. Changes in low–copy coding DNA sequences. Genome 41: 535–542.

Liu B., Vega J. M., Segal G., Abbo S., Rodova M., Feldman M. 1998b. Rapid genomic changes in newly synthesized amphiploids of *Triticum* and *Aegilops.* I. Changes in low–copy noncoding DNA sequences. Genome 41: 272–277.

McIntosh R. A. 1983. A catalogue of gene symbols for wheat. W Sakamoto S. (wyd.) Proc. Int. Wheat Genet. Symp. 6th Kyoto, Japan, 28 Nov. – 3 Dec 1983, pp. 1197–1254.

Milligan B. G., 1992. Plant DNA isolation. In: Hoelzel A. R. (Ed.) Molecular analysis of populations: a practical approach. IRL Press, Oxford, UK, pp 59–88.

Mitteilung K. 1986. Comparison of electrophoretic peroxidase patterns of 1A–1R and 1B–1R wheat–rye translocation. Biochem. Physiol. Pflanzen 181: 425–429.

Moreno–Sevilla B., Baenziger P. S., Peterson C. J., Graybosch R. A., McVey D. V. 1995. The 1BL/1RS translocation: agronomic performance of F_3–derived lines from a winter wheat cross. Crop Sci. 35:1051–1055.

Rayburn A. L., Caver B. F. 1988. Cytological identification of 1B/1R wheat–rye translocation in winter wheat breeding lines. Euphytica 38: 237–240.

Rogowsky P. M., Shepherd K. W., Langridge P. 1992. Polymerase chain reaction based mapping of rye involving repeated DNA sequences. Genome, 35: 621–626.

Stefanowska G. 1995. Charakterystyka niektórych cech morfologicznych i plonotwórczych mieszańców *Triticum aestivum* L z *Aegilops juvenalis* (Tell.) Eig. i *Aegilops ventricosa* Tausch. Biul. IHAR, 194: 35–43.

Stefanowska G., Prażak R., Strzembicka A., Masłowski J. 1995. Transfer genów z *Aegilops ventricosa* Tausch. i *Aegilops juvenalis* (Tell.) Eig. do *Triticum aestivum* L. Biul. IHAR, 194: 45–52.

Tarkowski Cz., Apolinarska B. 1992. The use of chromosome substitutions and translocations in the breeding of triticale, wheat and rye. Hereditas 116: 281–283.

Tyrka M., Stefanowska G. Genetic diversity in hybrids of *Aegilops ventricosa* and *Aegilops juvenalis* with wheats by RAPD markers. (in preparation).

Tyrka M., Stefanowska G. 2001. Ocena zróżnicowania cech plonotwórczych mieszańców *Aegilops juvenalis* i *Aegilops ventricosa* z pszenicą. Biul. IHAR. 218/219: 57–68.

Van Campenhout S., Vander Stappen J., Sagi L., Volckaert G. 1995. Locus–specific primers for LMW glutenin genes on each of the group 1 chromosomes of hexaploid wheat. Theor. Appl. Genet. 91: 313–319.

Villareal R. L., Del Toro E., Mujeeb–Kazi A., Rajaram S. 1995. The 1BL/1RS chromosome translocation effect on yield characteristics in a Triticum aestivum L. cross. Pl. Breed. 114: 497–500.