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RESEARCH ARTICLE

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Identification of the improved genotypes with 2NS/2AS translocation through molecular markers for imparting resistance to multiple biotic stresses in wheat

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Abstract

Alien introgression is one of the techniques to impart stress tolerance if a broad spectrum resistance source is not available in cultivars. Several introgressions have been utilized in wheat improvement, which has provided multiple stress tolerance in high-yielding varieties. The 2NS/2AS translocation introgressed from *Aegilops ventricosa* providing resistance to multiple biotic stresses has gained attention after being found to carry resistance to wheat blast. The introgression was already known to carry resistance to leaf rust (*Lr37*), Stem rust (*Sr38*), and yellow rust (*Yr17*), along with resistance to several other biotic stresses. The presence of several resistance genes in an introgression size of 32–33 mb makes it a preferred choice to utilize in the breeding program. Therefore, the present study was conducted to identify the superior genotypes carrying 2NS/2AS translocation. The twelve superior genotypes *viz.,* HD2967, HD3043, HD3059, HD3171, HD3249, HD3293, HD3334, HD3349, HD3368, HI1605, HI1653 and HI1654 have been identified to carry this introgression with superior agronomic traits. The lines are already released or in the advanced stage of testing and hence can be utilized in hybridization programs to transfer resistance to multiple stresses in new varieties.

Keywords: 2NS/2AS translocation, multiple biotic stresses, wheat, resistance.

Introduction

Wheat is one of the most important cereal crops serving the dietary requirement of the large population. The wheat encounters several biotic and abiotic stresses, leading to significant yield losses (Wheeler et al. 2013; Zampieri et al. 2017; Juliana et al. 2019). Biotic stresses such as rusts, powdery mildew, Karnal bunt, spot blotch, loose smut, and head scab are common under different climatic conditions (Singh et al. 2016). In recent years, the wheat blast caused by fungal pathogen *Magnaporthe oryzae* pathotype *Triticum* emerged in several parts of the world, which potentially can cause yield losses of up to 100% under severe epidemic conditions (Cruz and Valent 2017). To cope with wheat diseases, several alien introgressions have been developed to transfer resistance from wild relatives of wheat. Alien introgressions have been widely utilized in wheat to attain resistance/tolerance from several stresses, including biotic and abiotic stresses. The gene pool of the *Aegilops* genus has been used widely as a source of agronomically important genes in wheat breeding. It could be traced back to 1956 when Sears transferred a leaf rust resistance gene, *Lr9* from *Aegilops umbellulata* Zhuk into wheat. Subsequently, many genes have been introgressed into wheat from *Aegilops* species for resistance/tolerance against different stresses.

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Some of the major genes introgressed from *Aegilops* species and are being used in the Indian wheat breeding program are *Lr28* from *Aegilops speltoides* (McIntosh1982), *LrM* from *Aegilops markgrafii* (Rani et al. 2020), *Lr37+Yr17+Sr38* from *A. ventricosa* (Bariana and McIntosh 1993), *Sr39+Lr35* from *A. speltoides* (Kerber and Dyck 1990), and many more. Although many of the introgressions provide resistance against one or two major wheat diseases (Omkar et al. 2019), only a few translocations have been reported that provide resistance to multiple diseases. Among alien introgressions, 1BL/1RS translocation from rye provides resistance to leaf rust, stripe rust, stem rust, and powdery mildew (Crespo-Herrera et al. 2017). This translocation has been characterized, and *Lr26+Yr9+Sr31+Pm8* genes have been identified on the rye segment utilized intensively in the wheat breeding program (Tomar et al. 2014). Similarly, 2NS/2AS translocation carrying introgression from the *Ae. Ventricosa* provides resistance to leaf rust, yellow rust, stem rust, powdery mildew, eyespot, root-knot nematode, and blast resistance (Helguera et al. 2003; Gao et al. 2021).

Recently, wheat blast disease has been reported in Bangladesh and has also been determined as the most devastating disease, causing severe loss in crop yield (Islam et al. 2016). Although wheat blast was first reported in 1985 in Brazil, it has also been reported in South America (Goulart et al. 1992, 2007; Kohli et al. 2011). However, with the emergence of this disease in Bangladesh and its chances of spread in other countries, there is a need for the development and identification of varieties resistant to blast disease and other biotic stresses.

A 2NS segment of *A. ventricosa* has been characterized and has been found to carry several resistance genes in a 32–33 Mb translocation in wheat (Gao et al. 2021). A small segment having so many disease resistance genes is a bonus to breeders as it lessens the work of breeders by eliminating the need for stacking of genes for different stresses and diseases. Hence identification of the agronomically superior lines having 2NS/2AS translocation, which can serve as parents in crossing programs to develop new varieties with multiple disease resistance is important. Therefore, the present work was undertaken to identify the released varieties and advanced stage genotypes carrying 2NS/2AS translocation, which can be used intensively in the breeding programs.

Materials and methods

Thirty-one wheat lines comprising released varieties (RV) and entries in the advance varietal trial of AICRP testing (AVT) were selected for the present study. Two bread wheat Indian landraces, Agra Local and Kharchia Local were included in the study as negative checks for 2NS/2AS translocation, while near Iso-genic line of Thatcher, RL6081 carrying *Lr37* was taken as a positive control. The details of genotypes along with their adaptation ecology are provided in Table 1. DNA was extracted from 15-days-old seedlings using the CTAB method. DNA was quantified on 0.8% (w/v) agarose gel using Lambda Uncut DNA as standard and also confirmed with NanoDrop Lite spectrophotometer (THERMO FISHER SCIENTIFIC INC., USA). DNA samples were diluted to the working concentration of 25 ng/µL and stored at -20°C. To characterize the wheat lines, the two primer pairs were taken for identifying the translocation 2NS/2AS (Helguera et al. 2003). The sequence of primers is given in Table 2. The PCR reactions were performed according to the profile described by Helguera et al. (2003). After amplifying the 2AS CAPS marker, the PCR product was digested with the restriction enzyme *mspII* as described by Helguera et al. (2003). In addition to identifying 2NS/2AS translocation, the lines were also evaluated for leaf rust resistance at the seedling stage against 77-5 and 77-9, currently the most prevalent pathotypes of leaf rust in India. Ten-days-old seedlings of each genotype was inoculated with both the pathotypes separately following the method of Joshi et al. (1988) and were scored for disease reaction (Infection Type) after 12 days of inoculation following the scale of Stakman et al. (1962).

Results and discussion

Among the 34 wheat lines used in the study, 19 lines and 2 checks (Agra Local and Kharchia Local) were negative, while 12 lines and the check RL6081were found to be positive for 2NS/2AS introgression by both the molecular markers VENTRIUP and URIC. The negative lines did not amplify any allele, i.e. having null allele, while positive check and 12 other bread wheat lines amplified an allele of 262 base pairs by molecular marker VENTRIUP (Fig. 1). The positive lines amplified an allele of the same size as reported by Helguera et al. 2003 in lines carrying 2NS/2AS translocation. Further analysis with CAPS marker URIC amplified an allele of 285 base pair in all the 34 lines (Fig. 2), but digestion with a restriction endonuclease, *mspII* produced fragments of 176 and 109 in negative checks Agra Local and Kharchia Local along with 19 lines. The positive check RL6081 and 12 test lines retained the 285bp fragment even after RE treatment (Fig. 3). Again, the amplification pattern and RE digestion for the marker URIC have followed the pattern reported for the presence and absence of 2NS/2AS translocation. The two molecular markers VENTRIUP and URIC identified the identical set of 12 bread wheat lines as positive for 2NS/2AS translocation. The marker analysis indicated that 12 test lines that include 7 released varieties (RV) and 5 lines in the advanced testing stage are carrying 2NS/2AS translocation (Table 3). The presence of translocation was further substantiated as 12 of the positive lines were found to be resistant in wheat blast screening by IIWBR, Karnal (AICRP, Report 2018-19; 2019-20). *A. ventricosa* translocation 2NS/2AS is known to confer resistance to blast in wheat

Table 1. The detail of lines along with their ecology of adaptation and stage of testing or release

S.No		Pedigree	Stage/Condition
1	Thatcher+*Lr37*	RL6081	Positive control
2	HD2733	ATTILA/3/TUI/CARC//CHEN/CHTO/4/ATTILA	RV-IR-TS-TAS-NEPZ
3	HD2888	C306/T.SPHAEROCOCCUM//HW2004	RV-RI-TS-TAS-NEPZ
4	HD2932	KAUZ/STAR//HD2643	RV-IR-LS-TAS-CZ
5	HD3086	DBW14/HD2733//HUW468	RV-IR-TS-TAS-NWPZ/NEPZ
6	HD3090	SFW/VAISHALI//UP2425	RV-IR-LS-TAS-PZ
7	HD3226	GRACKLE/HD2894	RV-IR-TS-TAS-NWPZ
8	HD2864	DL509-2/DL377-8	RV-IR-LS-TAS-CZ
9	HI1544	HINDI62/BOBWHITE/CPAN2099	RV-IR-TS-TAD-CZ
10	HI1612	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES	RV-RI-TS-TAS-NEPZ
11	HI1628	FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/PFAU/ WEAVER//BRAMBLING	RV-RI-TS-TAS-NWPZ
12	HI1633	GW322/PBW498	RV-IR-LS-TAS-PZ
13	HI1634	GW322/PBW498	RV-IR-LS-TAS-CZ
14	HI1637	GW366/K9465	AVT-IR-TS-TAD-CZ
15	HI8627	HD4672/PDW233	RV-RI-TS-TAD-CZ
16	HI8713	HD4672/PDW233	RV-IR-TS-TAD-CZ
17	HI8737	HI8177/HI8158//HI8498	RV-IR-TS-TAD-CZ
18	HI8759	HI8663/HI8498	RV-IR-TS-TAD-CZ
19	HI8805	IWP5070/HI8638//HI8663	RV-RI-TS-TAD-PZ
20	HI8823	HI8709/HD4676	AVT-RI-TS-TAD-CZ
21	Agra Local	Landrace	Negative control
22	Kharchia Local	Landrace	Negative control
23	HD2967	ALD/CUC//URES/HD2160M/HD2278	RV-IR-TS-TAS-NWPZ/NEPZ
24	HD3043	PJN/BOW//OPATA*2/3CROC_1/AE.SQ(224)//OPATA	RV-RI-TS-TAS-NWPZ
25	HD3059	KAUZ//ALTAR84/AOS/3/MILAN/KAUZ/4/HUITES	RV-IR-LS-TAS-NWPZ
26	HD3171	PBW343/HD2879	RV-RI-TS-TAS-NEPZ
27	HD3249	PBW343*2/KUKUNA//SRTU/3/PBW343*2/KHVAKI	RV-IR-TS-TAS-NEPZ
28	HD3293	HD2967/DBW46	RV-RI-TS-TAS-NEPZ
29	HD3334	DBW50/WR2502	AVT-IR-LS-TAS-NWPZ
30	HD3349	HD2932/HD3086	AVT-IR-TS-TAS-NWPZ
31	HD3368	HD2932/HD3086	AVT-RI-TS-TAS-NWPZ
32	HI1605	BOW/VEE/5/ND/VG9144//KAL//BB/3/YACO/4/CHIL/6/CASKOR/3/ CROC_1/ AE.SQ(224)//OPATA/7/PASTOR//MILAN/KAUZ/3/BAV92	RV-RI-TS-TAD-PZ
33	HI1653	NADI/COPIO//NADI	AVT-RI-TS-TAS- NWPZ
34	HI1654	SOKOLL/3/PASTOR//HXL7573/2*BAU/4/PANDION// FILIN/2*PASTOR/3/BERKUT	AVT-RI-TS-TAS- NWPZ

Table 2. Primer sequence of markers used to identify 2NS introgression

S.No	Marker	Primer Sequence
1	2AS-CAPS	
	URIC	5′- GGTCGCCCTGGCTTGCACCT -3′
	LN2	5′- TGCAGCTACAGCAGTATGTACACAAAA -3′
2	2NS Specific	
	VENTRIUP	5′- AGGGGCTACTGACCAAGGCT-3′
	LN2	5′- TGCAGCTACAGCAGTATGTACACAAAA -3′

Table 3. The Marker score of test lines along with checks for VENTRIUP and URIC

S.No	Genotype	VENTRIUP + LN2	URIC+ LN2	RD with mspII
1	Thatcher+*Lr37*	262bp	285bp	285bp
2	HD2733	-	285bp	176bp and 109 bp
3	HD2888	-	285bp	176bp and 109 bp
4	HD2932	-	285bp	176bp and 109 bp
5	HD3086	-	285bp	176bp and 109 bp
6	HD3090	-	285bp	176bp and 109 bp
7	HD3226	-	285bp	176bp and 109 bp
8	HD2864	-	285bp	176bp and 109 bp
9	HI1544	-	285bp	176bp and 109 bp
10	HI1612	-	285bp	176bp and 109 bp
11	HI1628	-	285bp	176bp and 109 bp
12	HI1633	-	285bp	176bp and 109 bp
13	HI1634	-	285bp	176bp and 109 bp
14	HI1637	-	285bp	176bp and 109 bp
15	HI8627	-	285bp	176bp and 109 bp
16	HI8713	-	285bp	176bp and 109 bp
17	HI8737	-	285bp	176bp and 109 bp
18	HI8759	-	285bp	176bp and 109 bp
19	HI8805	-	285bp	176bp and 109 bp
20	HI8823	-	285bp	176bp and 109 bp
21	Agra Local	-	285bp	176bp and 109 bp
22	Kharchia Local	-	285bp	176bp and 109 bp
23	HD2967	262bp	285bp	285bp
24	HD3043	262bp	285bp	285bp
25	HD3059	262bp	285bp	285bp
26	HD3171	262bp	285bp	285bp
27	HD3249	262bp	285bp	285bp
28	HD3293	262bp	285bp	285bp
29	HD3334	262bp	285bp	285bp
30	HD3349	262bp	285bp	285bp
31	HD3368	262bp	285bp	285bp
32	HI1605	262bp	285bp	285bp
33	HI1653	262bp	285bp	285bp
34	HI1654	262bp	285bp	285bp

(Gao et al. 2021). The molecular marker analysis and disease reaction to blast have confirmed the presence of 2NS/2AS translocation in 12 test lines. Among the negative test entries, 2 durum wheat lines HI8823 and HI8805 and 2 bread wheat lines HI1633 and HI1637 were found to be negative for 2NS/2AS translocation by marker analysis but they showed resistance to blast with a score of up to 10 in blast screening nursery of AICRP, Wheat (AICRP, Report 2018-19; 2019-20). It is likely due to the presence of some other gene(s) for blast resistance or because of escape in disease screening. He et al. (2020) reported QTLs for blast resistance with significant phenotypic variance on 7 different chromosome arms, including the chromosome arm 2AS. Hence, these lines, which were found negative for 2NS/2AS translocation but showed resistance to blast, needs further analysis. If these lines are confirmed as resistant to blast, they may provide an alternate source of resistance and could be utilized to map genes/QTLs responsible for blast resistance in wheat. The new QTLs/genes in combination of 2NS/2AS translocation combination can increase the durability and resistance spectrum of lines against wheat blast. Previously also the

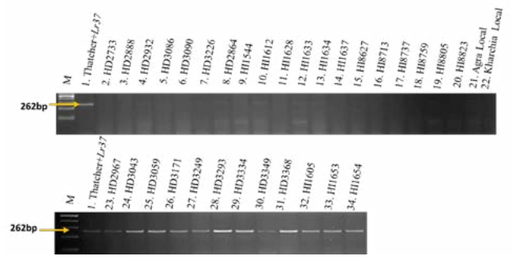

Fig. 1. Amplification with 2NS specific primer pair, VENTRIUP, and LN2. Here, M = 100 bp Marker, Sl. No. 1 = *Lr37* positive control; 2-22 = Genotypes lacking 2NS fragment; Sl. No. 23-34 = Genotypes with 2NS fragment present

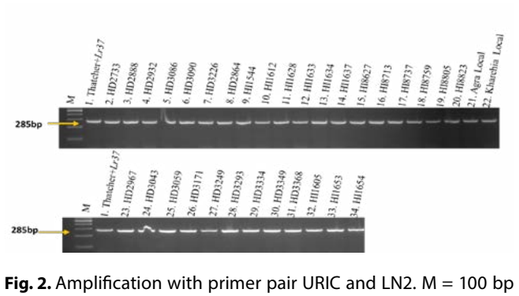

Fig. 2. Amplification with primer pair URIC and LN2. M = 100 bp Marker, Sl. No. 1 = *Lr37* positive control; Sl. No. 2-34 = all amplified with this primer pair

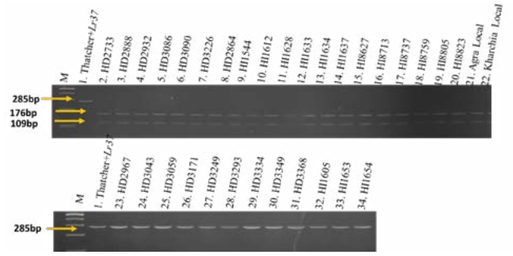

Fig. 3. Amplified PCR fragment from URIC and LN2 followed by *DpnII* digestion. M = 100 bp Marker, 1 = *Lr37* positive control; Sl. No. 2-22 = Genotypes having AA genome; Sl. No. 23-34 = Genotypes with NN translocation present

pyramiding of genes has been commonly used to increase the resistance of lines against diverse pathotypes (Singh 1 et al. 2017; Niharika et al. 2021). Therefore, the lines carrying a diverse resistance source against wheat blast need to be characterized and genes can be identified for further utilization in breeding programs. Further, the markers can be used to identify the lines carrying sources other than 2NS/2AS by removing the lines carrying this known translocation. Only the lines without this translocation can be sent for further screening against blast and the lines found to be resistant can be characterized as a novel source of resistance. Among the lines found to carry 2NS/2AS translocation, the lines HD3349 and HD3368 were resistant to leaf rust pathotype 77-5 and 77-9 at the seedling stage under controlled condition phenotyping in the glasshouse at New Delhi (Table 4). The observed leaf rust resistance of these lines could be due to additional *Lr* genes. Further, HD3349 was also found to be resistant to all the 23 pathotypes of leaf rust, 20 pathotypes of stem rust

Table 4. Seedling infection types of test lines and checks against leaf rust pathotype77-5 and 77-9

	Genotype/Pathotype	2020-21 IARI	
		77-5	77-9
1	Thatcher+*Lr37*	3	3
2	HD2733	3	3
3	HD2888	;	;
4	HD2932	3	3
5	HD3086	3	3
6	HD3090	;	;
7	HD3226	3	X+
8	HD2864	;	;
9	HI1544	;	;
10	HI1612	;1	X
11	HI1628	3	3
12	HI1633	;	;
13	HI1634	;	;
14	HI1637	;	;
15	HI8627	;1	X
16	HI8713	;1	X
17	HI8737	;1	;1
18	HI8759	;1	X
19	HI8805	;1	;1
20	HI8823	;	X+
21	Agra Local	3	3
22	Kharchia Local	3	3
23	HD2967	X+	X+
24	HD3043	3	3
25	HD3059	3	X+
26	HD3171	3	X+
27	HD3249	3	X-
28	HD3293	3	X+
29	HD3334	3	X
30	HD3349	;	;1+
31	HD3368	;	X
32	HI1605	;	X
33	HI1653	3	3
34	HI1654	3	X+

out of 23 and 14 pathotypes of yellow rust out of 16 used in the seedling screening of AVT entries by IIWBR, Karnal (Anonymous 2021). Further, the line HD3349 was developed by crossing HD2932/HD3086 but none of the parental lines carries 2NS/2AS translocation. Therefore, the likely mistake in labeling lines while handling breeding material or an outcross during handling of segregating material can be responsible for the presence of introgression. The natural outcrossing leading to the development of new variety has been reported and led to the development of NP114, which is a natural cross in the variety Federation (Pal 1966). Therefore, the present study has identified 12 genotypes with 2NS/2AS translocation. The lines are either released cultivar or a genotype under testing in AVT trials, which can be used to impart multiple biotic stress tolerance in the breeding program. Further, the molecular markers were robust to confirm the presence of 2NS/2AS translocation; hence they can be easily and efficiently utilized in the breeding program. All the lines found to carry translocation were resistant to blast, while some of the entries found to be resistant are not carrying translocation, and they need further evaluation.

Authors' contribution

Conceptualization of research (SKJ, V, MSS); Designing of the experiments (SKJ, V, PA); Contribution of experimental materials (JBS, SKJ, V); Execution of lab experiments and data collection (PA, NKS, RK, SKJ); Analysis of data and interpretation (SKJ, PA, V); Preparation of the manuscript (PA, SKJ, V, NM, MN, JBS, MSS).

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