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RESEARCH ARTICLE

Introgression and genetic mapping of leaf rust and stripe rust resistance in *Aegilops triuncialis*

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Abstract. The growing and cultivating resistant wheat crop varieties is important to meet the demands of the growing population and minimizing the yield losses due to foliar diseases. More important is the identification of novel resistance sources and transfer of resistance in ready to use form. In the current study, leaf rust (LR) and stripe rust (YR) resistant tetraploid nonprogenitors of wheat *Aegilops triuncialis* ($U^tU^tC^tC^t$) acc pau 3462 was crossed and backcrossed susceptible cultivar WL711(NN) by inducing homeologous pairing using CS *ph*^{1}. Recurrent parent type plants were selected in subsequent generation with resistance to LR and YR and BC_2F_7 introgression line ($2n$=42) named *ILtri* have been developed. To understand the nature and inheritance of LR and YR resistance genes and to map their genomic location, F_2 and $F_{2:3}$ mapping populations were developed by crossing *ILtri* with WL711(NN). In F_2 and $F_{2:3}$, the seedlings and adult plants segregated into 3R:1S and 1HR:2Seg:1HS ratios, respectively for both LR and YR, indicating inheritance of single dominant all stage resistance gene working against both the rusts. These genes were temporary designated as *Lrtri* and *Yrtri* and were inherited independently. Molecular mapping of 614 SSR markers mapped the *Lrtri* at a distance of 11.2 cM from SSR marker *Xwmc606*.

Keywords. wheat; all stage resistance; rust; nonprogenitor; simple sequence repeat; *Aegilops triuncialis*.

Introduction

Wheat, the third most important cereal, is the main sources of calories and protein for the human population across the world (Chaves *et al.* 2013; Igrejas and Branlard 2020). India, the world's third largest wheat producer with 99.70 Mt production constituting 13.64% of the total wheat production of the world. Any serious pest and/or pathogen infecting the crop is a threat to human food security. It has been reported that the current global investment in the field of wheat research is concentrating principally on breeding superior varieties and mapping / fine mapping the genomic regions for major biotic and abiotic stress resistance (Butler and Spencer 2010; Rajaram and Dubin 2019; Bharadwaj *et al.* 2020). But the strong backup of useful genes of various traits are required to tackle the future threats quickly (Shiferaw *et al.* 2013). Thus, well-planned prebreeding programmes are required which can generate ready to use gene(s)/introgression carrying lines to be utilized according to the challenges of particular area.

Among the available challenges of wheat, three rust diseases, namely stem or black (SR) *Puccinia graminis* f. sp. *tritici, Pgt*), leaf or brown rust (LR) (*Puccinia triticina, Pt*) and stripe or yellow rust (YR) (*Puccinia striiformis* f. sp. *tritici, Pst*) (Gill and Raupp 1987; Bai *et al.* 1994; Kolmer 1996; Line 2002; Marais *et al.* 2003; Wellings 2011; Aboukhaddour *et al.* 2020) impose major threats to yield. These are highly specialized biotrophic plant pathogenic fungi of Basidiomycota phylum which have been characterised into different physological races (Stakman and Piemeisel 1917). The genetic plasticity, constant evolution and easy dispersion of rust populations are global reasons of concern in ever growing wheat genetic improvement programmes. Estimation of losses in yield caused by wheat rusts

SA, conducted experiment, did all phenotyping, molecular mapping, manuscript writing. SK and PC, conceived and designed research, developed introgression lines. RS, helped in molecular mapping. GSD, SK and SA produced final draft of manuscript. AS helped material development and final selection of introgression lines. JK, screening against yellow rust, maintenance of different races of brown rust and yellow rust.

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began only in the 20th century (McIntosh 1995). Hence, the efforts have to be intensified towards breeding disease resistance for the existing virulences (Pannu *et al.* 2014). *Pst* pathogen is highly prevalent in temperate regions with cool and wet weather conditions (Chen *et al.* 2014). Approximately 88% of the world's wheat varieties are susceptible to *Pst* and global losses inflicted by the disease are nearly USD $1 billion annually (Wellings 2011; Beddow *et al.* 2015). Of the available *Pst* pathotypes in Punjab region, *Yr9* virulent pathotypes, 46S119 and 110S119 are found to be more prevalent, while the abundance of 78S84 (virulent on *Yr27*) is less (Singh *et al.* 2020). *Pt*, the causal agent of LR (Anikster *et al.* 1997; Bolton *et al.* 2008), is the most common and widely distributed wheat rust diseases of the three (Bolton *et al.* 2008; Huerta-Espino *et al.* 2011). While the grain losses due to LR display temporal and geographical variation, the economic significance of the disease is substantial (Kolmer 2005). *Pt* race 77, with its 13 pathotypes is the most prevalent and widespread wheat rust disease in India. Pathotype 77-5 of this race along with pathotype 104-2 had dominated for about 20 years (Bhardwaj 2011) while recently, pathotypes 77-9 along with 77-5 were found to be more prevalent (Prasad *et al.* 2017).

The perennial grasses of the Poaceae tribe Triticeae have broadened the genetic base of *Triticum aestivum* L. since the first hybrids were made during the early 1930s (Tsitsin 1962). Species belonging to the genus *Aegilops* L. are important sources of genetic material for generating pre-breeding material for expanding the genetic variability of cultivated bread wheat (Brink and Cooper 1940). It has been exploited for a wide range of traits including resistance to pests and diseases (Endo and Tsunewaki 1975; Endo 1978; Dhaliwal *et al.* 1991; Endo and Gill 1996; Liu *et al.* 2011; Ghazvini *et al.* 2012) and contribute >20% of the disease resistance gene deployed in wheat and may harbour many other, yet unidentified traits for wheat improvement (Aghaee-Sarbarzeh *et al.* 2001; Valkoun 2001; Kishii 2019)

Punjab Agricultural University has maintained a germplasm collection of >1500 wild species. *Ae. triuncialis* ($U^tU^tC^tC^t$) acc. pau 3462, a tetraploid, nonprogenitor species of wheat has been found to be resistant to LR and YR since last 15–20 years. Aim of the present study was to transfer the LR resistance (*Lrr)* and YR resistance (*Yrr)* genes into LR and YR susceptible hexaploid wheat variety WL711(NN) to generate stable introgression lines with minimum linkage drag. The nature and inheritance of transferred loci associated with these resistances were further analysed in the introgression line.

Material and methods

The plant materials used in this study was LR and YR resistance accession of *Ae. triuncialis* ($U^tU^tC^tC^t$) acc. pau 3462. Chinese spring (CS ph^1) stock (Chen *et al.* 1994) was used to induce homeologous pairing between U^tC^t genome of *Ae. triuncialis* with ABD genome of wheat. LR and YR susceptible cultivar (*T. aestivum*) WL711(NN) was used as recipient parent to transfer the LR and YR resistance genes to develop resistant introgression line. WL711 (S 308/Chris// Kalyansona) (Nagarajan and Singh 1997) was among the wheat derivatives developed by All India Coordinated Wheat Improvement Project (AICWIP) at Indian Agricultural Research Institute in 1965 (Ramadas *et al.* 2019) and was one of the prominent wheat varieties of Punjab state and northwestern plain zone. WL711(NN) is a near isogenic line (NIL) derived from *T. aestivum* WL711 reported to have *kr* alleles (crossability alleles) (Randhawa *et al.* 2018). Stable hexaploid introgression line *ILtri* derived by transferring LR and YR resistance gene from *Ae. triuncialis* was crossed with WL711(NN) to develop F_2, $F_{2:3}$ mapping population. The mapping population was then used to test the nature and inheritance of rust resistance and to identify genomic regions associated with resistance gene(s) using SSR markers.

Seedling screening of rust

The screening at seedling stage against *Pt* pathogens was done for F_2 and derived $F_{2:3}$ progenies along with parental lines. The seedling screening against *Pst* pathogen was done only in $F_{2:3}$ progenies as same F_2 seedlings cannot be tested both for LR and YR. Seedlings were sown by mid November along with the parental genotypes (*ILtri* and WL711(NN)) in plastic trays for screening against the most prevalent *Pt* pathotype 77-5. The first row of each tray was seeded by WL711 as susceptible check. For screening of $F_{2:3}$ progenies, seeds obtained from each of the F_2 plant were divided into two parts of 11 seed each and one set of was screened against *Pt* pathotype 77-5 and other set against the most prevalent *Pst* race 110S119. The seeded trays were maintained in different rust-free microclimate rooms for both stripe and brown rusts, maintained at 20°C. After a week, the seedlings were inoculated with the rust spores well mixed with the talcum powder, at a rate of 1 V of fresh urediniospores to 20 V of talcum powder according to Roelfs *et al.* (1992). The inoculum of LR pathotype 77–5 (=121R63–1) and YR pathotype 110S119 (=110E159) was provided by Regional Research Station, IIWBR, Flowerdale, Shimla, India. Inoculated seedlings were incubated at 9–12°C overnight on trays filled with water and covered with polythene hoods to provide 100% humidity. Trays were then shifted to different microclimates, respective for LR and YR screening. The LR and YR scores were recorded 14 days after inoculation using the 0–4 scale outlined in McIntosh (1995) by Stakman *et al.* (1962) https://naldc.nal.usda.gov/download/CAT10243018/PDF). The scale outlined incorporated response to rust (0, immune response; (;), hypersensitive flecks; 1, small uredia with necrosis; 2, moderate-size pustules with chlorosis; 3, moderate-to-large

sized uredia without necrosis or chlorosis; 4, large urediospores without any chlorosis or necrosis).

Adult plant screening

The F_2 plants and $F_{2:3}$ progenies in respective years along with parental genotypes were transplanted in field of School of Agricultural Biotechnology (SOAB), Punjab Agricultural University (PAU), Ludhiana after screening against LR and YR. The same F_2 plants were screened against LR and YR at adult plant stage. YR scoring was completed by mid of March and LR scoring by first week of April. Each rust was scored atleast thrice to confirm the respective score. In $F_{2:3}$, two different sets of progenies were sown, one for testing against LR and another screened against YR. In field, the experimental material was planted in 1.5 m rows with a row to row distance of 20 cm and screening was done at adult plant stage under field conditions. The experimental field area were also surrounded by a 30 cm wide row of susceptible spreader genotype BWL4444 (for LR), PBW343 (for YR) and WL711 (both for LR and YR). Recommended application of inorganic fertilizers (N, P, and K) provided essential nutrients. Artificial rust epidemic was created by spraying the experimental material with the mixture of uredinospores of known LR 77-5 (=121R63-1), 77-9 (=121R60-1), 104-2 (=21R55) and YR 78S84 (=78E16), 110S119 (=110E159) and local inoculum collected from farmer's field (table 1). The pathotype 77-9 and 104-2 in case of *Pt* and 78S84 in case of *Pst* were added artificially in natural inoculum collected from farmer's field to ensure the presence of all the prevalent and virulent races of this region. The experimental area was regularly irrigated to create congenial conditions for rust development. The scoring was done in accordance with the modified Cobb's scale, as developed by Peterson *et al.* (1948).

Chi-square (χ^2) analysis

To determine the nature and number of LR and YR resistance gene(s) in the mapping population, goodness of fit of the observed to the expected ratio of the phenotypic classes concerning the LR and YR severity and the response types were determined by χ^2 analysis for both the diseases according to Steel and Torrie (1960). The deviation of the observed experimental results of the response against both the rust pathogens by the mapping population and the parental lines, from the theoretically expected values was determined to check expected segregation ratio. The

Table 1. Avirulence/virulence pattern of LR and YR pathotypes used in the study.

Race	Avirulence formula	Virulence formula
77-5 (121R63-1)	Lr9, Lr18, Lr19, Lr24, Lr25, Lr28, Lr29, Lr32, Lr39, Lr42, Lr43, Lr45, Lr47	Lr1, Lr2a, Lr2c, Lr3, Lr10, Lr11, Lr12, Lr13, Lr14a, Lr14b, Lr14ab, Lr15, Lr16, Lr17, Lr20, Lr21a, Lr22b, Lr23, Lr26, Lr27+31, Lr30, Lr33, Lr34, Lr35, Lr36, Lr37, Lr38, Lr40, Lr44, Lr48, Lr49
104-2 (21R55)	Lr9, Lr10, Lr13, Lr15, Lr19, Lr20, Lr24, Lr25, Lr28, Lr29, Lr32, Lr36, Lr39, Lr40, Lr41, Lr42, Lr43, Lr45	Lr1, Lr2a, Lr2b Lr3, Lr11, Lr12, Lr14a, Lr14b, Lr14ab Lr16, Lr17a, Lr18, Lr21, Lr22a, Lr22b, Lr23, Lr26, Lr30, Lr33, Lr34, Lr35, Lr37, Lr38, Lr44, Lr48, Lr49
77-9 (121R60-1)	Lr2a, Lr2b, Lr2c, Lr9, Lr19, Lr24, Lr25, Lr28, Lr32, Lr39, Lr45	Lr1, Lr3, Lr10, Lr11, Lr12, Lr13, Lr14a, Lr14b, Lr14ab, Lr15, Lr16, Lr17a, Lr17b, Lr18, Lr20, Lr21, Lr22a, Lr22b, Lr23, Lr26, Lr27 + 31, Lr30, Lr33, Lr34, Lr35, Lr36, Lr37, Lr38, Lr42, Lr44, Lr46, Lr48, Lr49
78S84 (78E16)	Yr1, Yr 3b, Yr4b, Yr5, Yr10, Yr11, Yr14, Yr15, Yr17, Yr18, Yr24/26, Yr28, Yr29, YrSD	Yr2, Yr3a, Yr4A, Yr6, Yr7, Yr8, Yr9, Yr12, Yr19, YrSk, YrSu, Yr31
46S119 (46E159)	Yr1, Yr5, Yr10, Yr14, Yr15, Yr24, Yr26, Yr28, YrSp	Yr2, Yr3a, Yr3b, Yr4a, Yr4b, Yr6, Yr7, Yr8, Yr9, Yr11, Yr12, Yr 17, Yr19, Yr29, Yr31, Yrsk
110S119 (110E159)	Yr1, Yr5, Yr10, Yr15, Yr24, Yr26, Yr28, YrSp, Riebesel 147/51(Yr2,9,+)	Yr2, Yr3a, Yr3b, Yr4a, Yr4b, Yr6, Yr7, Yr8, Yr9, Yr11, Yr12, Yr14, Yr 17, Yr18, Yr19, Yr29, Yr31, Yrsk

calculated value of χ^2 is compared with the expected tabular value of χ^2 at (n–1) d.f. as follows:

$$\chi^2 = \Sigma(o - e)^2/e$$

where, n, group of classes under investigation; d.f., degrees of freedom, calculated as (n–1); o, plants obtained in a class; e, plants expected in a class.

Molecular marker analysis

DNA of F_2 single plants along with parental genotypes was extracted from leaf tissues of two-week-old seedlings using the modified CTAB method of Saghai-Maroof *et al.* (1984). DNA samples were quantified using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, USA), and working dilutions of 25 ng/μL were prepared for PCR assays. To identify the LR and stripe resistance associated SSR markers, bulked segregant analysis (BSA) was done (Michelmore *et al.* 1991). Equal amounts of normalized genomic DNA from 10 extremely resistant and 10 extremely susceptible individual plants were separately pooled to make resistant bulk (RB) and susceptible bulk (SB). LR and YR reaction of selected plants were confirmed from their derived F_3 progenies before making bulks so that only homozygous resistant and homozygous susceptible F_2 plants were selected. The SSR markers selected from equal interval of all 21 chromosomes of the A, B and D genomes of *T. aestivum* (Roder *et al.* 1995; Röder *et al.* 1998; Somers *et al.* 2004) were initially screened on the parental genotypes and respective bulks. The PCR assays were resolved on 6% poly acrylamide gel electrophoresis and the resolved amplicons were visualized under UV transilluminator. MapDisto v. 1.7.5. Beta 4 software was used for determining the linkage present between the genes and markers.

Results

Development of Ae. triuncialis specific introgression lines

For generation of the LR and YR resistant introgression lines, Chinese Spring (CS) *Ph1b* stock, carrying a suppressor for *Ph1* locus of wheat was crossed with *Ae. triuncialis* acc. pau 3462 to induce homoeologoues pairing. Pentaploid F_1s were crossed with rust susceptible wheat cultivar WL711(NN). The three way F_1s thus obtained were crossed and backcrossed with WL711(NN) to generate BC_2F_1 which was then selfed to BC_2F_2. Thereafter, the cycle of selfing continued upto BC_2F_7, selecting single plants, phenotypically similar to recurrent parent (for leaf, spike and seed morphology) and resistant to LR and YR. In BC_2F_7, the stable LR and YR resistant single plants with chromosome

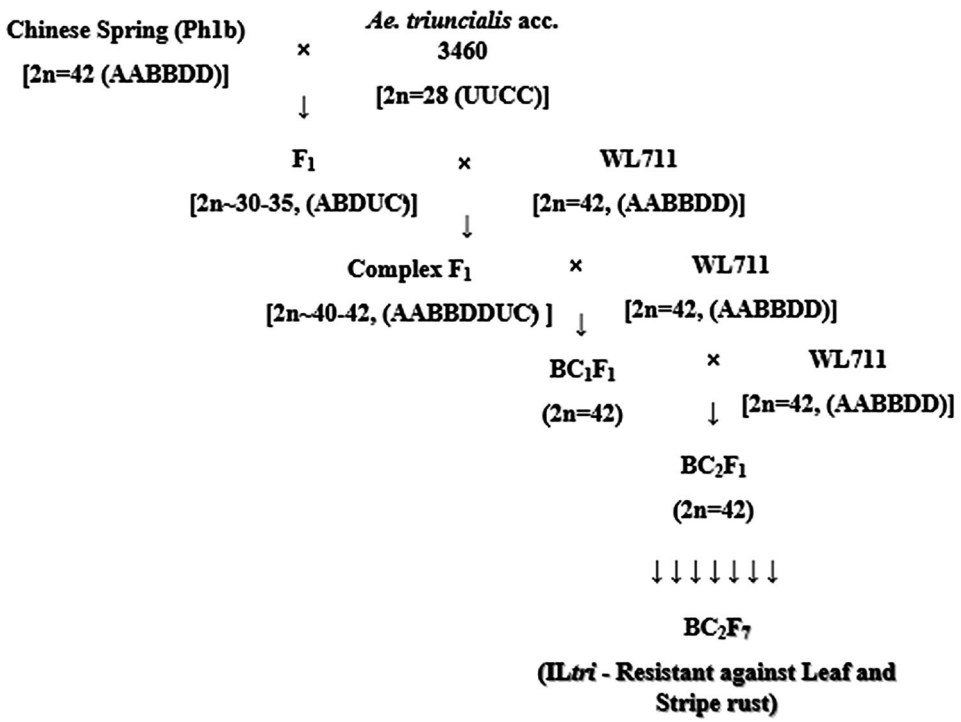

Figure 1. Schematic representation of generation of wheat–*Ae. triuncialis* introgression line *ILtri*.

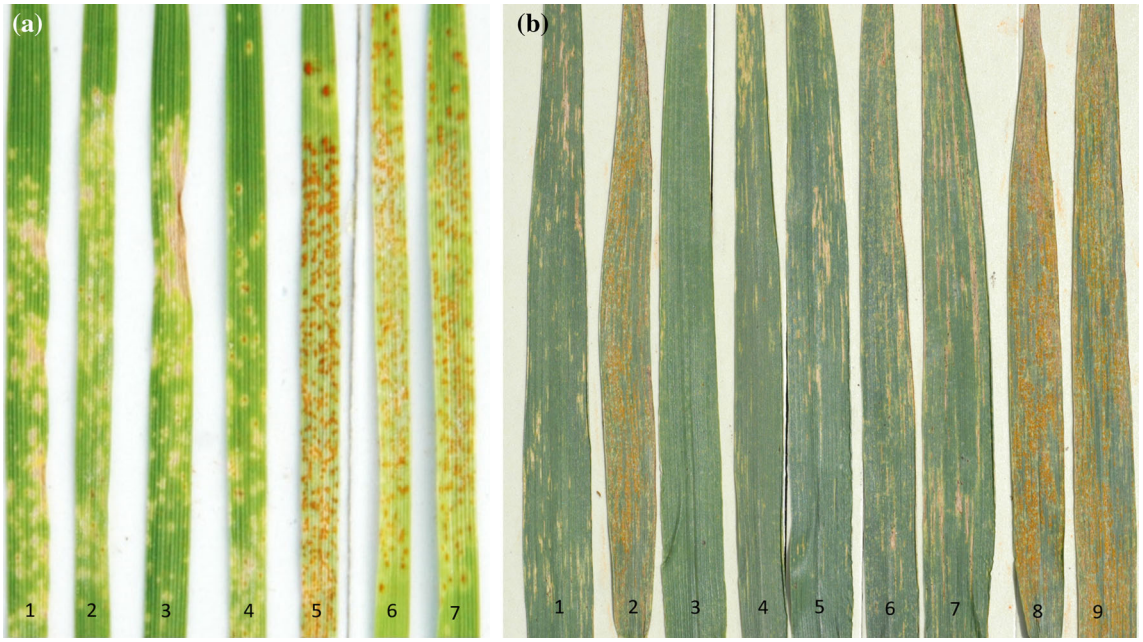

Figure 2. (a) LR reaction in F_2 generation at seedlings stage against pathotype 77-5 (1) *ILtri*; (2–4) resistant (infection type; to :1); (5–6) susceptible (infection type 3); (7) WL711(NN). (b) YR reaction in F_2 generation at adult plant stage against mixture of pathotypes (1) *ILtri*; (2) WL711(NN); (3–7) resistant (R to 10MR); (8–9) susceptible (60S to 80S).

Table 2. Segregation for LR and YR at seedling stage and adult plant stage in F_2 and $F_{2:3}$ generations from cross of *ILtri* x WL711(NN).

Pathotype	Generation	Total plants	Resistant plant		Susceptible plant	χ^2 (3:1)
Pt 77-5 at SS	F_2	230	181		49	1.68
Mixture of *Pt* races at APS						
Pst 110S119 at SS	F_2	220	181		39	2.33
Mixture of *pst* races at APS						
			HR	SEG		χ^2 (1:2:1)
Pt 77-5 at SS	$F_{2:3}$	220	65	109	46	3.23
mixture of *Pt* races at APS						
Pst 110S119 at SS	$F_{2:3}$	220	75	106	39	12.07
Mixture of *pst* races at APS						

SS, seedling stage; APS, adult plant stage.

number 2*n*=42 were selected. One of the progenies of single plant, named as *ILtri* was selected for further studies (figure 1).

Inheritance of rust resistance genes

LR resistance: At the seedling stage, *ILtri* showed infection type (IT) of;1, hence was resistant, WL711(NN) showed a susceptible IT of 3 against *Pt* pathotype 77–5 (figure 2a). Of the total 230 F_2 seedlings, 181 were resistant (R) and 49 were susceptible (S). The χ^2 value was calculated to be 1.68 (3R:1S) indicating that there is single dominant resistance gene effective in *ILtri* against *Pt* pathotype 77-5 at seedling stage (table 2). All the F_2 plants which were resistant for *Pt* at seedling stage remained resistant at the adult plant stage also and susceptible F_2 seedlings remained susceptible at adult plant stage against the mixture of different *Pt* pathotypes. Thus, the resistance conferred against *Pt* is all stage

Table 3. Two-way table representing the number of $F_{2:3}$ progenies with different reaction against LR and YR.

		LR			Total
YR		HR	Seg	HS	
	HR	38	26	11	75
	Seg	19	61	26	106
	HS	08	22	09	39
Total		65	109	46	220

Table 4. List of SSR markers polymorphic in resistant bulk and susceptible bulks with their positions according to map given by Somers *et al.* (2004).

SSR	Chromosome	Map position
Xbarc119	1AS	54
Xgwm778	1AL	–
Xwmc149	2AS	31
Xgwm165	4AS	2
Xgwm68	5BL	64
Xwmc357	5DL	82
Xwmc606	7BS	0
Xwmc323	7BS	1
Xgwm46	7BS	54
Xwmc9	7AL	72

resistance (ASR) or seedling resistance which become effective when the plant is at seedling stage and continue throughout the plant life cycle.

In the 220 $F_{2:3}$ progenies, there were 65 homozygous resistant (HR): 109 segregating (Seg): 46 homozygous susceptible (HS) progenies at the seedling stage (figure 2a; table 2), for which the χ^2 value of 3.23 showed a complete fit for 1 HR:2 Seg:1 HS, further confirming that the *Lrr* gene in *ILtri* was a single dominant gene . Also the segregation of HR/Seg/HS $F_{2:3}$ progenies at adult plant stage remained same as in seedling stage (table 2). Hence, single dominant ASR gene against LR, transferred from *Ae. triuncialis* to *ILtri* was temporarily designated as *Lrtri.*

YR resistance: At seedling stage, *ILtri* showed resistance IT score of 1 to 1^+ and WL711(NN) showed susceptible IT of 3 against *Pst* pathotype 110S119 . Of the 220 F_2 plants tested against mixture of *Pst* pathotypes at adult plant stage there were 181 resistant and 39 susceptible plants giving the best fit to single gene segregation ratio with a χ^2 value of 2.33 (figure 2b; table 2). All the 220 $F_{2:3}$ single plant progenies

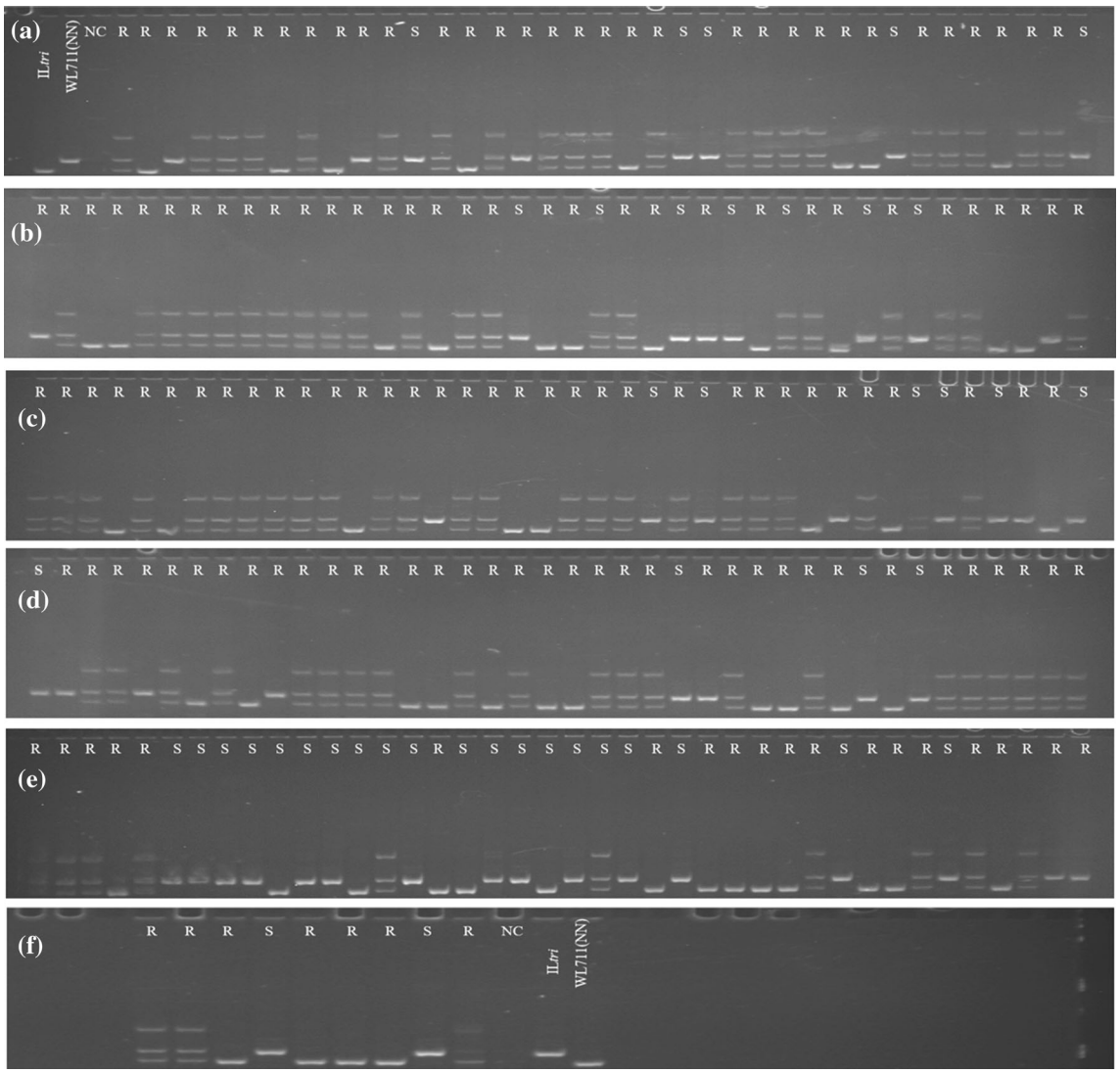

Figure 3. PCR amplification profile of resistant parent *ILtri*, susceptible parent WL711NN and 206 F_2 plants (R, resistant to LR; S, susceptible to LR; NC, negative control) with SSR marker *Xwmc606* on PAGE gel.

Table 5. List of LR resistance and YR resistance genes mapped on homoeologues 7A, 7B and 7D chromosomes.

Gene	Source	Chr	Marker	References
Leaf rust resistance genes (Lrr)				
Lr47	*Ae. speltoides*	7AS	CAPS/SSR	Dubcovsky *et al.* (1998)
Lr72	*T. turgidum*	7BS	SSR	Herrera-Foessel *et al.* (2014)
Lr43	*Ae. tauschii*	7DS	SSR	Hussien *et al.* (1997)
Lr29	*Ag. elongatum*	7DS	RAPD/SCAR	Tar *et al.* (2002)
Lr20	*T. aestivum*	7AL	RFLP, RAPD, STS	Neu *et al.* (2002)
Lr14a	*T. turgidum*	7BL	SSR	McIntosh *et al.* (1995)
Lr14b	*T. aestivum*	7BL		McIntosh *et al.* (1995); Sawhney *et al.* (1992)
Lr34	*T. aestivum*	7BL	RFLP	Kerber and Dyck (1969)
Lr68	*T. aestivum*	7BL	SSR	Herrera-Foessel *et al.* (2012)
LrBi16	*T. aestivum*	7BL	SSR, STS, SCAR	Zhang *et al.* (2015)
LrFun	*Fundulea 900*	7BL	SSR	Xing *et al.* (2014)
Lr19	*Ag. elongatum*	7DL	RFLP / STS/ RAPD	Gupta *et al.* (2006)
YR resistance genes (*Yrr*)				
Yr63	*AUS 27955*	7BS	SSR	Mcintosh *et al.* (2013)
Yr18	*T. aestivum*	7BL	SSR	Lagudah (2011)
Yr6	*T. aestivum*	7BL	SSR	Macer (1966)
Yr79	*PI 182103*	7BL	SSR	Qureshi *et al.* (2018)
Yr2	*T. aestivum*	7B	SSR	Lupton and Macer (1962)
Yr39	*Alpowa*	7BL	SSR	Rahmatov (2013)
Yr59	*PI 178759*	7BL	RGAP, SSR	Zhou *et al.* (2014)
Yr52	*PI 183527*	7BL	SSR	Ren *et al.* (2012)
YrC591	*C591*	7BL	SSR, STS	Xu *et al.* (2014)
YrZH84	*Zhou 8425B*	7BL	SSR	Li *et al.* (2006)
Yr67	*PI 189747*	7BL	AFLP, SSR	Li *et al.* (2009); Xu *et al.* (2014)
Yr33	*Batavia*	7DL	SSR	McIntosh *et al.* (2004)

were also screened for their response against *Pst* pathotype 110S119 at seedling stage. Of the 181 resistant F_2 plants there were 75 HR: 106 Seg progenies in $F_{2:3}$ while progenies of 39 susceptible F_2 plants were all HS in $F_{2:3}$ with χ^2 value of 12.07 for 1HR:2Seg:1HS (table 2). The $F_{2:3}$ progenies which were HR/Seg/HS at seedling stage remained HR/Seg/HS at adult plant stage also indicating that *Yrr* was also conferred by single dominant ASR genes just like *Lrr* gene and this gene was temporarily designated as *Yrtri*.

Linkage analysis of leaf and YR resistance genes

$F_{2:3}$ progenies from cross of *ILtri* with WL711(NN) were compared for their LR and YR cosegregation so as to detect any linkage between *Lrtri* and *Yrtri* genes (table 3). Of the 220 $F_{2:3}$ progenies, 38 (17.2%) were HR, nine (4.0%) were HS and 61 (27.7%) were Seg against both the *Pt* and *Pst* pathogens. Thus in total 49.1% (108) progenies the resistance genes *Lrtri* and *Yrtri* cosegregate. In rest of the 112 (50.9%) $F_{2:3}$ progenies *Lrtri* and *Yrtri* were showing independent assortment when compared for their LR and YR rust reaction, denying any close association between the two genes.

Chromosomal localization of Lrtri and Yrtri genes

A total of 614 SSR markers from 21 wheat chromosomes were amplified on the parental genotypes: *ILtri* and WL711(NN) and the RB and SB to determine the genomic regions associated with *Lrtri* and *Yrtri* transferred from *Ae. triuncialis* acc pau 3462. Of these, 196 SSRs were polymorphic while 418 were monomorphic between paretal lines *ILtri* and WL711(NN). Of these 196 SSRs, only nine SSRs amplified polymorphic fragments between RB and SB (table 4) with amplicon size of RB equals to *ILtri* and of SB equals to WL711(NN). All these nine SSRs were amplified on whole F_2 population to study any cosegregation of the marker with *Ae. triuncialis* specific *Lrtri* and *Yrtri* genes. Only one of these SSRs *Xwmc606* from short arm of chromosome 7B/7D was found to be partially linked to *Lrtri* gene flanking the *Lrr* gene at 11.2 cM (figure 3). *Yrr* gene, however, could not be mapped with available SSRs.

Discussion

The *Aegilops* species is a reservoir of novel resource of genes against various biotic and abiotic stresses (Kuraparthy *et al.* 2007a, b; Arrigo *et al.* 2011; Bansal *et al.* 2017; Niu *et al.* 2018; Narang *et al.* 2019; Kishii 2019). Since these species cannot be utilized directly, developing prebreeding/introgression material from these is like piling up ammunition for future emergencies so that as and when required the desired genes can be utilized quickly. In recent past, threats of stem rust pathotype, *Ug99* and wheat blast in Bangladesh could be handled effectively and quickly due to already

available prebreeding resistant material from *Ae. speltoides* (Mago *et al.* 2009, 2013) and *Ae. venticosa* (Cruz 2016; Velu *et al.* 2018; Mahmud 2019), respectively. Thus prepration in advance are required to safeguard the crop in fight against the ever evolving causes of biotic and abiotic stresses. Punjab Agricultural University has a collection of >1500 wild progenitor and nonprogenitor species and work on evaluation, transfer, identification and mapping of the different genes from these species has been carried from about last 20 years (Chhuneja *et al.* 2007; Kuraparthy *et al.* 2007a, b, Toor *et al.* 2016; Bansal *et al.* 2017; Kaur *et al.* 2018). *Ae. triuncialis*, a tetraploid, originated from hybridization of two diploids *Ae. umbellulata* and *Ae. markgrafii* (Greuter) Hammer (syn. *Ae. caudata* L.) (Murai and Tsunewaki 1986; Vanichanon *et al.* 2003) harbour useful resistance against cereal rust pathogens (Kamboj *et al.* 2020) and accession pau 3462 has been found to carry effective resistance against LR and YR from past many years.

ILtri have been developed in the present study using induced homoelogous pairing between UC genome of *Ae. triuncialis* acc pau 3462 and ABD genome of wheat. The series of selfing after three-way crossing with CS(*Ph1b*) and WL711(NN) yielded different recombination event products carrying introgression of variable sizes. Although the actual size of the introgression could be predicted with cumbersome molecular cytogenetic techniques. But in the current study visual selection for 5–6 generation for plants having morphological features of recurrent parent WL711(NN), alongwith resistance to LR and YR, increases the chance of eliminating large and deleterious introgressions while keeping small targeted ones only as any large introgression, substitutions or translocations will carry linkage drag. Further selection of plants with normal chromosome complement (2*n*=42) increase the chances of carrying forward the useful and smaller introgressions only . Till date about 20 *Lrr*, 11*Yrr* and 19 stem rust resistance (*Srr*) genes have been transferred from nonprogenitor wild wheats which constitute about 15–20% of the known genes only.

The tetraploid UC genome has contributed only a limited number of LR and YR resistance genes to the existing gene pool including *Lr58* against LR (Kuraparthy *et al.* 2007a, b), *Cre7* against cyst nematode (Andre 1998) *H30* against hessian fly (Gomez 2018). Ghazvini *et al.* (2012) reported the transfer of two stem resistance genes from *Ae. triuncialis* into wheat line Tr129. *ILtri* providing ASR to single races of *Pt* 77-5 and *Pst* 110S119 and to a broader spectrum of known and unknown pathotypes present in the field showed the wide potential of these resistance genes.

Lrtri and *Yrtri* genes showed independent assortment thus seems to be transferred as different alien fragments. F_2 mapping population from the selected *ILtri* have putatively mapped *Lrtri* genes on chromosome 7BS/7DS (http://wheat.pw.usda.gov; Somers *et al.* 2004), at a distance of 11.0 cM from SSR *Xwmc606*. None of the SSR in vicinity of *Xwmc606* position were found to be linked to *Lrtri* or *Yrtri* when tested in all three homeologous 7A, 7B and 7D. We were not able to further reduce the distance of 11.0 cM with the available SSRs. Frequency of SSRs towards the tip of short arm of chromosomes 7B and 7D is sparce, indicating need to use some high throughput marker technologies to reduce the distance between marker and resistance gene. Only nine of the total 614 SSRs tested were found to be polymorphic in bulks indicating the small number of *Ae. triuncialis* specific introgressions in WL711(NN). This is also supported by the fact that visual selection of stable recurrent parent type plants with full chromosome complement aids in selecting few cryptic alien introgression.

Most of the genes already known on 7BS/7DS are adult plant resistance genes (*Lr14a*, *Lr14b*, *LrBi16*, *Lr68*, *Lr34*, *Yr18*, *Yr39*, *Yr52*, *Yr59*, *Yr67*, *Yr79* and *YrC591*) (table 5). *Lr43*, an ASR gene on 7DS is mapped close to the centromere but is derived from *Ae. tauschii* (Hussein *et al.* 1997). Another ASR gene *Lr29* on 7DS chromosome has been transferred from *Agropyron elongatum* (Procunier *et al.* 1995; Tar *et al.* 2002) while *Lr43* gene from homeologue 7DS is transferred from *Ae. speltoides* (Dubcovsky *et al.* 1998). Therefore, the putatively mapped resistance genes *Lrtri* and *Yrtri* in *ILtri* are new resistance gene providing broad spectrum resistance and the *ILtri* is a useful prebreeding source for dual rust resistance.

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