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RESEARCH ARTICLE

Development and characterisation of leaf rust resistant *Triticum timopheevii* derived introgression lines in hexaploid wheat

Sneha Nymagoud, Sandhya Tyagi, Ajay Kumar Chandra, Priyanka Agarwal, M. Niranjana, Niharika Mallick, K. Raghunandan, Shailendra Kumar Jha, S. M. S. Tomar and Vinod*

Abstract

Tetraploid wheat, *Triticum timopheevii* (Zhuk.) (A^tA^tGG, $2n = 4x = 28$) is a well-known source of resistance against many pests and diseases. It has been practically utilized in various breeding programmes against leaf rust disease. The present study developed a set of 41 introgression lines (ILs) by crossing two *T. timopheevii* accessions *viz., T. timopheevii*-191 and *T. timopheevii*-235 with bread wheat genotype Chinese Spring. The F_1s were backcrossed thrice to susceptible cultivars, Agra Local and Kharchia Local, followed by selfing for five generations. All the ILs were screened for leaf rust resistance using leaf rust pathotype 77-5 at seedling stage in a glass house. Out of 41 ILs, 33 showed resistant reactions to leaf rust ('0;' to '13'). Two ILs, TTm Derivative-638 and TTm Derivative-793, showing high degree of resistance, were further tested against 19 different leaf rust pathotypes, produced "0;" to ";" type of infection type (IT). To characterize the genomic constitution of these two ILs and to detect the presence of introgression segment(s) of two wild *Triticum timopheevii* wheats, 35K Affymetrix Wheat Breeders' Axiom® array was used. SNP (Single nucleotide polymorphism) analysis confirmed *T. timopheevii* introgressions in two ILs and was used for graphical representation using software *GTM v*1.0. Genomic characterization revealed 30.59 and 20.30% of introgression from wild accessions, *T. timopheevi*-191 and *T. timopheevi*-235 into ILs TTm Derivative-638 and TTm Derivative-793, respectively. Genome-wise analysis in ILs TTm Derivative-638 and TTm Derivative-793 revealed maximum introgression in B genome (32.18) and A genome (22.75%), respectively. These ILs will help in widening the genetic base for leaf rust resistance in wheat.

Keywords: *Triticum timopheevii*, introgression lines, bread wheat, leaf rust

Introduction

Bread wheat (*Triticum aestivum* L.), an allohexaploid species (AABBDD, $2n=6x=42$), is one of the leading staple food crops worldwide and stands next only to rice (Igrejas and Branlard 2020). A number of biotic and abiotic stresses cause significant damage to wheat yield. Brown rust or leaf rust disease caused by *Puccinia triticina* Eriks., an obligate fungal pathogen, is one of the major biotic stresses that reduce grain yield and quality (Bolton et al. 2008; Kolmer 2013). The infection causes grain yield losses of more than 50% in susceptible cultivars (Hussein et al. 2005; Huerta-Espino et al. 2011; Terefe et al. 2011) and is characterized by reduced kernel weight and a lower number of kernels per spike (Bolton et al. 2008; Huerta-Espino et al. 2011; Tomar et al. 2014). The most adaptable method to reduce the impact of this widespread and devastating disease is to develop disease-resistant crop varieties (Dangl et al. 2013, Oliver 2014). Till date 82 *Lr* genes have been designated (Bariana et al. 2022). However, the breakdown of rust resistance genes by the ever-evolving pathogens has emphasized the importance of the search for new resistance genes for leaf rust (Ellis et al. 2014).

Wild and related species of wheat from primary, secondary and tertiary gene pool serve as a rich and novel source of diversity and carry high level of resistance against

Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi 110012, India

***Corresponding Author:** Vinod, Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India, E-Mail: vinod.genetics@gmail.com

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different diseases (King et al. 1997). Many of them have been utilized to create genetic variation for several agronomically important traits and resistance to biotic stresses. Species from primary, secondary and tertiary gene pools have been utilized to transfer rust resistance in wheat (Tomar et al. 2004; Gireesh et al. 2014; Niranjana et al. 2017; Singh et al. 2017; Nataraj et al. 2018; Kirti et al. 2020; Dinkar et al. 2020; Mallick et al. 2021, 2022a, 2022b; Raghunandan et al. 2022). Besides, wild relatives have been used to transfer some other useful traits such as protein quality, mineral content, salinity tolerance and physiological traits such as photosynthetic capacity (King et al. 1997; Reynolds et al. 2001; Li et al. 2013; Cruz et al. 2016; Sharma et al. 2018).

Triticum timopheevii Zhuk., a tetraploid wheat (2*n* =4*x*= 28, genome composition A^tA^tGG) is an excellent source of resistance against many pests and fungal diseases (Leonova et al. 2011). *T. timopheevii* has been exploited to transfer resistance against biotic stresses, like *Fusarium* head blight (Malihipour et al. 2016, 2017), powdery mildew (*Pm2*, *Pm6, Pm27,* and *Pm37*) (Allard and Shands 1954; Jorgensen and Jensen 1972; Peusha et al. 1995; Jarve et al. 2000; Perugini et al. 2008; Qin et al. 2011), black-point (Lehmensiek et al. 2004), Hessian fly, Septoria blotch, wheat curl mite and tan spot (Brown-Guedira et al. 1996). It has also been utilized in breeding for traits affecting grain quality such as milling yield and grain protein content (Lehmensiek et al. 2008), mineral content (Hu et al. 2017) and abiotic stresses, such as salt tolerance (Badridze et al. 2009; Yudina et al. 2016). *T. timopheevii* is also identified to be a great source for rust resistance and used as a donor source to improve resistance against rust diseases in wheat such as; leaf rust (*Lr18, Lr50, LrTt, LrTt2*, and *LrSelG12*) (Carpenter et al. 2018; Brown-Guedira et al. 2003; Leonova et al. 2004, 2010; Singh et al. 2017; Nataraj et al. 2018), and stem rust (*Sr36, Sr37, Sr40,* and *SrTt3*) (Allard and Shands 1954; McIntosh and Gyarfas 1971; Dyck 1992). Introgression breeding is an important way to explore novel genes and QTLs from wheat wild relatives (Devi et al. 2019). The development of interspecific hybrid-derived lines can broaden wheat's narrow gene pool. However, the direct transfer of the target genes from the related wild species into the genome of common wheat is complicated due to the genome incompatibility and cytological instability of early hybrid generations (Leonovaet al. 2011). Thus, it is a hypercritical task to develop stable introgression lines (ILs) via homologous/homoeologous recombination between the wild species such as *Triticum timopheevii* Zhuk. and common wheat cultivars/genotypes with stable resistance gene expression, while retaining common wheat characteristics to be used as a donor source. There is enough evidence that *Triticum urartu*, a diploid species with genome AA contributed A genome to both *T. timopheevii* and *T. turgidum* (Kilian et al. 2007) The B and G genomes of tetraploid wheat evolved from S genome of *Aegilops speltoides* Tausch. (Dvorak and Zhang 1990; Rodriguez et al. 2000). The B and G genomes share a greater homology than any other genomes in Triticeae (Gill and Chen, 1987). The pairing between A and A^t genomes is primarily homologous while that between B and G genome is homoeologous, thus, providing an opportunity to transfer genes from both A^t and G genomes of *T. timopheevii* (Gill and Chen 1987et al). Chinese Spring (CS) is a useful genotype in interspecific hybridization since it carries the crossability promoting alleles *kr1 and kr2,* making interspecific crosses easy. In contrast, the dominant alleles of the *Kr1,* and *Kr2 genes* reduce the crossability of hexaploid wheat with many alien species (Laurie and Bennett 1987).

The main task during the development of ILs between wild relatives and common wheat is to identify and characterize the introgressed segment from the wild donor (King et al. 2022). During the last three decades, different molecular markers played a major role in detecting the polymorphic content between the contrasting genotypes. Recently, the 35K Axiom@ single nucleotide polymorphisms (SNPs) array enables to handle of large-scale data to identify the SNP polymorphism (insertion/deletion) at even small segmental introgression level across the genome (Devi et al. 2019). This technology has been utilized in wheat to characterize the introgression of *Ambylopyrum muticum*, *Aegliops speltoides, Thinopyrum bessarabicum* and *Triticum urartu* (Grewal et al. 2018a,b; King et al. 2017, 2018). Graphical genotyping allows the graphical depiction of molecular data, which can assist in the identification of the extent of introgression from different parental and donor origin across all the chromosomes and genome. At ICAR-Indian Agricultural Research Institute, New Delhi, we are working to transfer useful genes from primary, secondary and tertiary gene pool into cultivated wheat. In the present communication, we report the data on the development of the *T. aestivum_T. timopheevii* introgression lines, evaluation of the lines for resistance to leaf rust disease at the seedling stage, and molecular characterization of two leaf rust resistant introgression lines using 35K Array.

Materials and methods

Plant material

In the present study, two *T. timopheevii* accessions (*T. timopheevii*-191 and *T. timopheevii*-235), Chinese Spring (CS), Agra Local (AL) and Kharchia Local (KL) and a set of 41 ILs derived from the two above mentioned *T. timopheevii* accessions were used. The ILs were developed at the Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi with the objective to transfer leaf rust resistance from *T. timopheevii* into common wheat.

Pathotypes and screening for leaf rust resistance

All the 41 Introgression Lines were screened for leaf rust

Table 1. Infection types on Agra Local, and 41 introgression lines (IL) of *T. timopheevi* against pathotype 77-5 of P. triticina in seedling stage at a mean temperature range of 20–28°C

S.No.	IL No.	Infection type	Pedigree of IL
CS/*T. timopheevi*-191/Agra Local BC3F5			
1.	TTmD-637	;	CS/*T. timopheevi*-191/Agra Local BC3F5-1
2.	TTmD-638	0;	CS/*T. timopheevi*-191/Agra Local BC3F5-2
3.	TTmD-639	0;	CS/*T. timopheevi*-191/Agra Local BC3F5-3
4.	TTmD-640	;	CS/*T. timopheevi*-191/Agra Local BC3F5-4
5.	TTmD-641	0;	CS/*T. timopheevi*-191/Agra Local BC3F5-5
6.	TTmD-643	0;	CS/*T. timopheevi*-191/Agra Local BC3F5-7
7.	TTmD-644	0;	CS/*T. timopheevi*-191/Agra Local BC3F5-8
CS/*T. timopheevi*-191/Kharchia Local BC3F5			
8.	TTmD-645	;11+	CS/*T. timopheevi*-191/Kharchia Local BC3F5-1
9.	TTmD-646	;11+	CS/*T. timopheevi*-191/Kharchia Local BC3F5-2
10.	TTmD-647	X	CS/*T. timopheevi*-191/Kharchia Local BC3F5-3
11.	TTmD-648	;1	CS/*T. timopheevi*-191/Kharchia Local BC3F5-4
12.	TTmD-649	;11+	CS/*T. timopheevi*-191/Kharchia Local BC3F5-5
13.	TTmD-650	;11+	CS/*T. timopheevi*-191/Kharchia Local BC3F5-6
14.	TTmD-651	X	CS/*T. timopheevi*-191/Kharchia Local BC3F5-7
15.	TTmD-652	;1	CS/*T. timopheevi*-191/Kharchia Local BC3F5-8
16.	TTmD-653	X	CS/*T. timopheevi*-191/Kharchia Local BC3F5-9
17.	TTmD-654	;11+3	CS/*T. timopheevi*-191/Kharchia Local BC3F5-10
18.	TTmD-655	X	CS/*T. timopheevi*-191/Kharchia Local BC3F5-11
19.	TTmD-656	X	CS/*T. timopheevi*-191/Kharchia Local BC3F5-12
20.	TTmD-657	3	CS/*T. timopheevi*-191/Kharchia Local BC3F5-13
21.	TTmD-658	X	CS/*T. timopheevi*-191/Kharchia Local BC3F5-14
22.	TTmD-659	;1	CS/*T. timopheevi*-191/Kharchia Local BC3F5-15
23.	TTmD-660	;1	CS/*T. timopheevi*-191/Kharchia Local BC3F5-16
24.	TTmD-661	;1	CS/*T. timopheevi*-191/Kharchia Local BC3F5-17
25.	TTmD-662	;1	CS/*T. timopheevi*-191/Kharchia Local BC3F5-18
26.	TTmD-663	;1	CS/*T. timopheevi*-191/Kharchia Local BC3F5-19
27.	TTmD-664	;1	CS/*T. timopheevi*-191/Kharchia Local BC3F5-20
28.	TTmD-665	;1	CS/*T. timopheevi*-191/Kharchia Local BC3F5-21
29.	TTmD-666	X	CS/*T. timopheevi*-191/Kharchia Local BC3F5-22
30.	TTmD-667	X	CS/*T. timopheevi*-191/Kharchia Local BC3F5-23
31.	TTmD-668	X	CS/*T. timopheevi*-191/Kharchia Local BC3F5-24
32.	TTmD-669	X	CS/*T. timopheevi*-191/Kharchia Local BC3F5-25
CS/*T. timopheevi*-235/Kharchia Local BC3F5			
33.	TTmD-793	;	CS/*T. timopheevi*-235/Kharchia Local BC3F5-1
34.	TTmD-794	3	CS/*T. timopheevi*-235/Kharchia Local BC3F5-2
35.	TTmD-795	;	CS/*T. timopheevi*-235/Kharchia Local BC3F5-3
36.	TTmD-796	;	CS/*T. timopheevi*-235/Kharchia Local BC3F5-4
37.	TTmD-798	3	CS/*T. timopheevi*-235/Kharchia Local BC3F5-6
38.	TTmD-799	3	CS/*T. timopheevi*-235/Kharchia Local BC3F5-7
39.	TTmD-800	3;	CS/*T. timopheevi*-235/Kharchia Local BC3F5-8

(Contd ....)

40.	TTmD-801	X	CS/*T. timopheevi*-235/Kharchia Local BC3F5-9
41.	TTmD-802	3	CS/*T. timopheevi*-235/Kharchia Local BC3F5-10
42.	AL	33+	
43.	Chinese Spring (CS)	33+	

Table 2. Infection types on Agra Local, *TTm*-793, *TTm*-638 along with the two accessions, *T. timopheevii*-235 and *T. timopheevii*-191 against 19 pathotypes of *P. triticina* in seedling stage at a mean temperature range of 20–28°C

S. No.	Pathotypes	235	TTm_793	191	TTm_638	AL
1.	77-7	;1-	;	;N	0;	33+
2.	77-2	0;	0;	0;	0;	33+
3.	12-3	0;	0;	0;	0;	33
4.	77-A	;1-	0;	0;	0;	3+
5.	10-6	;	0;	0;	0;	3+
6.	77-10	;	0;	;	;	33+
7.	77A-1	;	0;	0;	0;	33+
8.	77-5	;N	;	;N	0;	33+
9.	107-1	0;	0;	0;	0;	33+
10.	104-2	;N	;	;N	0;	3
11.	77-4	;	;	;	0;	33+
12.	12-5	;N	;	;N	0;	3
13.	77-6	;	;	;	;	33+
14.	77-9	;	;	0;	0;	3
15.	162A	;1--	0;	;1--	0;	3+
16.	104	;1-	0;	;	0;	3+
17.	77-8	;1--	;	;	0;	3
18.	104-4	;1--	;	;	0;	33+
19.	77-3	0;	0;	;	0;	33+

resistance against the most prevalent Indian pathotype 77-5 along with susceptible check Agra Local. Out of 41 ILs, two ILs TTm Derivative-638 (TTm-D638) and TTm Derivative-793 (TTmD-793) were tested against 19 Indian pathotypes of *Puccinia triticina* (77-7, 77-2, 12-3, 77-A, 10-6, 77-10, 77A-1, 77-5, 107-1, 104-2, 77-4, 12-5, 77-6, 77-9, 162A, 104, 77-8, 104-4, 77-3). The pure inoculum of different pathotypes was obtained from the Indian Institute of Wheat and Barley Research, Regional Station, Flowerdale, Shimla. The initial inoculum was multiplied and maintained on susceptible cultivar AL in a glass house at Division of Genetics, IARI, New Delhi. Disease inoculation was done by spraying 10 days old seedlings with suspension of uredeospores with a drop of Tween-20. Rust spores inoculated seedlings were incubated in a humid chamber for 48 hours. Later, the seedlings were shifted to glass house at temperatures ranging between 20°C and 30°C under ambient light and relative humidity conditions. Individual seedlings were scored for infection types (ITs) after 12 days of inoculation following 0–4 scale as described by Stakman et al. (1962).

Fig. 1. Infection types on Agra Local, original *T. timopheevii*-235 and *T. timopheevii*-191 and two ILs *TTm-793* and *TTm-638* against 15 pathotypes of *P. triticina* when tested at the seedling stage at a mean temperature range of 20–28°C

Fig. 2. Genome-wise analysis showing the relative proportion of the parental genome *T. Timopheevii*-191 in IL *TTm_638*

SNP genotyping and molecular analysis

Genomic DNA from parental genotypes (*T. timopheevii*-191 and *T. timopheevii*-235, Chinese Spring, Agra Local, and

Table 3. The chromosomal relative proportion of the parental genome of *T. Timopheevii*-191 in IL TTmD-638

S. No.	Chromosome	Polymorphic between 191 and 638	Polymorphic between 191 and AL	Polymorphic between 638 and AL	Region of 191
1	1A	17.25	45.15	51.88	27.89
2	1B	37.14	45.26	57.14	8.13
3	1D	24.28	47.39	57.97	23.10
4	2A	19.58	54.08	45.17	34.50
5	2B	12.31	50.53	48.39	38.21
6	2D	11.65	45.89	43.29	34.24
7	3A	6.62	44.45	45.45	37.83
8	3B	6.03	47.32	47.04	41.29
9	3D	6.66	44.94	46.05	38.28
10	4A	11.34	44.14	44.41	32.80
11	4B	7.98	37.68	39.26	29.71
12	4D	12.35	38.00	41.33	25.65
13	5A	29.61	40.27	52.08	10.66
14	5B	20.63	48.90	53.56	28.26
15	5D	29.79	40.40	50.38	10.60
16	6A	9.30	40.49	41.48	31.19
17	6B	9.39	47.97	48.26	38.58
18	6D	8.48	45.2	46.56	36.72
19	7A	7.31	45.36	45.47	38.04
20	7B	8.85	49.96	50.69	41.11
21	7D	9.80	45.41	45.41	35.61

Kharchia Local) along with the two selected ILs (TTmD-638 and TTmD-793) was isolated. Fresh leaf samples collected from 40 to 45 days old plants were crushed in liquid nitrogen with mortar and pestle. Isolation was done following CTAB method (Murray and Thompson 1980). Isolated purified DNA was quantified on 0.8% (w/v) agarose gel using Lambda Uncut DNA as standard and confirmed with NanoDropLite spectrophotometer (THERMO FISHER SCIENTIFIC INC., USA). DNA was diluted to the working stock concentration of 25 ng/µL and stored at -20°C. Diluted DNA of parental genotypes and two ILs was genotyped for SNP using Affymetrix 35K Wheat Breeders' Axiom® array (Allen et al. 2017) and this array consists of SNPs between different wheat genotypes, including *T. timopheevii* and other wild relatives of wheat.

SNP genotyping data obtained for parental genotypes along with the two ILs was filtered using Microsoft Excel software. SNPs were filtered out according to their chromosomal position, polymorphic and monomorphic content. The sorted data was analyzed using the software GTMv1.0 (Deblieck et al. 2020). GTM (GenoTypeMapper) is a software package that assists in the graphical depiction of molecular data, which can assist in the identification of the extent of introgression from different parental genotypes during the course of ILs development.

Results

Multi-pathotype screening

The 41 TTmDILs were screened against highly virulent pathotype 77-5 of leaf rust and showed infection types ranging from highly resistant ("0;") to susceptible (33+) types (Table 1). Out of 41, two ILs TTmD-638 and TTmD-793 expressed high degree of resistance with ITs ranging from '0;' to ';' were selected and further subjected for multi-pathotype screening along with the with two donor accessions *T. timopheevi*-191 and *T. timopheevi*-235 and susceptible genotype Agra Local. Multi-pathotype screening results showed that original donors *T. timopheevi*-191 and *T. timopheevi*-235 showed ITs "0;" to ";1—", two ILs TTmD-638 and TTmD-793 expressed ITs "0;" to ";'', and susceptible genotype Agra Local showed ITs 33^{+} to 3^{+}(Fig. 1, Table 2).

Analysis of Triticum timopheevii introgression

Analysis of SNP genotyping data showed that the introgression of genomic regions from *T. timopheevi* into two ILs TTmD-638 and TTmD-793. The extent of *T. timopheevi* introgression into ILs TTmD-638 and TTmD-793 was 30.59 and 20.30%, respectively.

In IL TTmD-638 around 30% parent genome (PG) was from original *T. timopheevii* accession *T. timopheevi*-191. Of 30% PG, largest insertion of *T. timopheevi*-191 genome

was observed in wheat genome B (32.18%), followed by genome A (30.59%) and genome D (29.17%) (Fig. 2). Within the genome B, the chromosome 3B revealed the most introgression (41.29%), while the chromosome 1B showed the least introgression (8.12%). Out of 7 chromosomes of group A, chromosome 7A (38.04%) showed largest extent of insertion from *T. timopheevi*-191, while chromosome 5A had the least percentage of introgression at 10.66%. Among the chromosomes of D genome, maximum introgression was observed for chromosome 3D (38.28%), while minimum introgression was observed for chromosome 5D (10.6%). The details of the parent genome introgression for all the 21 chromosomes are given in Table 3 and graphical representation is given in Fig. 3.

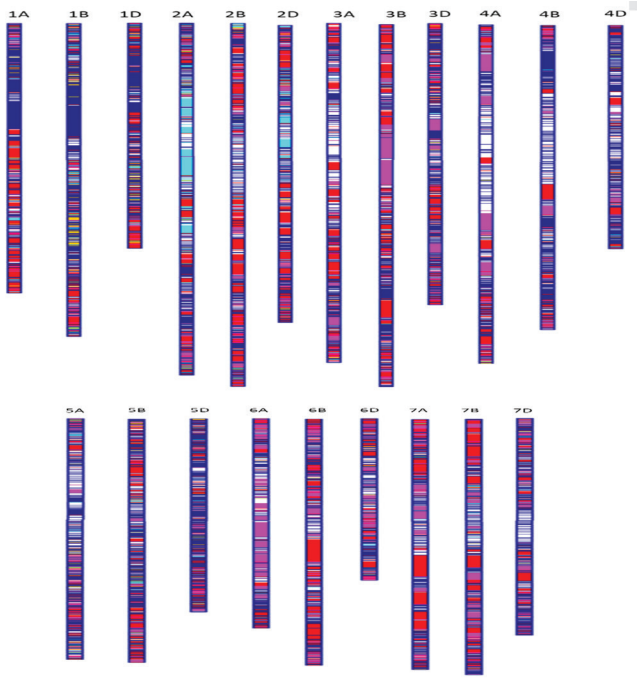

Fig. 3. Graphical representation of genomic constitution of *TTm*-638. Light blue colour –chromosomal region monomorphic between 191/AL; Red colour- chromosomal region carrying AL genome; Yellow colour – chromosomal region carrying CS genome; Pink colour - monomorphic between CS//AL; and blue colour – chromosomal region carrying 191 genomes

In comparison to IL TTmD-638, the degree of introgression observed was less in IL TTmD-793. Out of the three genomes of wheat, the genome A showed highest introgression (22.75%) from *T. timopheevi* accession *T. timopheevi*-235, followed by the genome B (21.18%), and the genome D (16.96%) (Fig. 4). Within the genome A, IL TTmD-793 introgression level ranged from 31.42 (1A) to 11.96% (4A). On the other hand, in the B genome, the chromosome 1B showed the most introgression (28.57%), whereas the 4B chromosome showed the least introgression (11.04%). Among the chromosomes of D genome, maximum

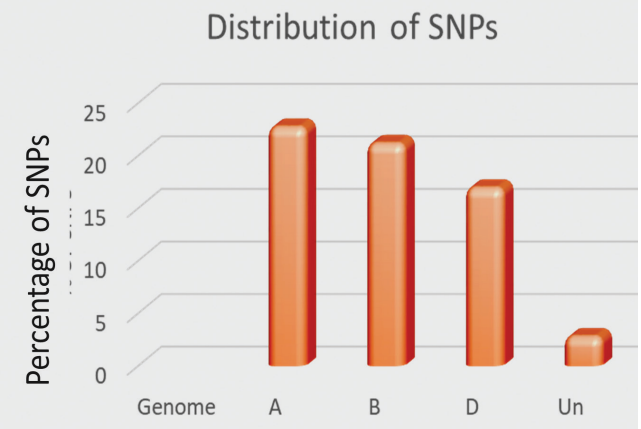

Fig. 4. Genome-wise analysis showing the relative proportion of the parental genome *T. Timopheevii* 235 in IL TTm-793

introgression was observed for chromosome 2D (24.56%), while minimum introgression was observed for chromosome 4D (11.6%) (Table 4, Fig. 5).

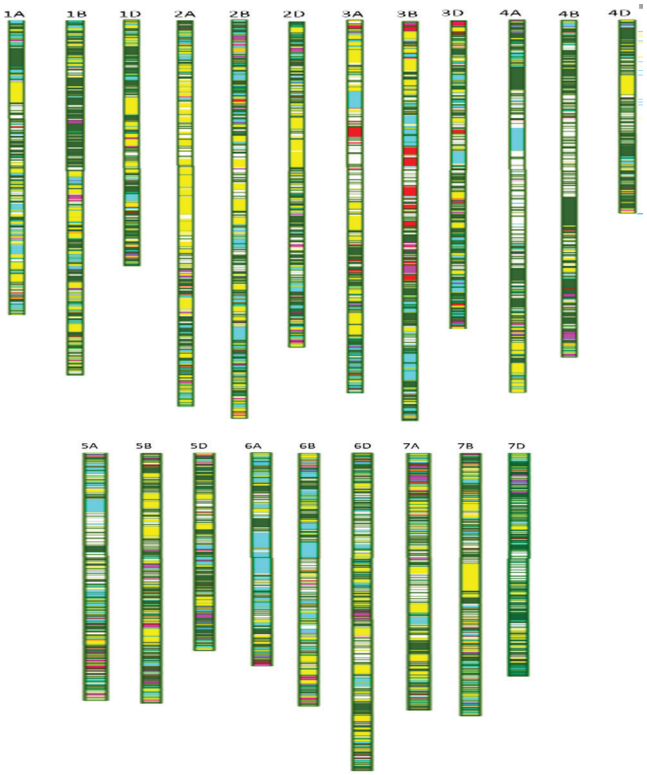

Fig. 5. Graphical representation of genomic constitution of TTmD-793. Light blue colour–monomorphic between 235//KL; Red colour– chromosomal region carrying– CS genome; Yellow colour – chromosomal region carrying– 235 genome; Pink colour-monomorphic between CS//KL; Dark Yellow– chromosomal region carrying– KL genome

Discussion

Wild relatives of wheat are valuable sources for novel genes for disease resistance. Introgression breeding is an

Table 4. The chromosomal relative proportion of the parental genome of *T. Timopheevii*-235 in IL *TTm*-793

S. No.	Chromosome	Polymorphic between 235 and 793	Polymorphic between 235 and KL	Polymorphic between 793 and KL	Region of 235 (%)
1.	1A	11.71	43.13	44.58	31.42
2.	1B	8.93	37.50	36.69	28.57
3.	1D	9.20	30.57	31.74	21.37
4.	2A	19.63	48.29	49.65	28.66
5.	2B	14.27	38.72	38.95	24.45
6.	2D	22.05	46.61	49.48	24.56
7.	3A	23.26	41.37	39.57	18.11
8.	3B	25.07	38.42	42.87	13.35
9.	3D	20.92	35.41	35.35	14.49
10.	4A	23.58	35.54	42.64	11.96
11.	4B	18.84	29.88	33.65	11.04
12.	4D	15.20	26.36	30.28	11.16
13.	5A	27.49	44.76	41.18	17.26
14.	5B	28.32	51.59	50.93	23.27
15.	5D	20.20	35.00	36.21	14.80
16.	6A	21.64	46.58	46.17	24.93
17.	6B	20.27	45.36	46.23	25.08
18.	6D	24.68	42.32	41.30	17.64
19.	7A	18.04	44.95	45.18	26.91
20.	7B	22.49	45.05	45.17	22.55
21.	7D	21.52	36.25	38.30	14.73

important way to exploit these novel genes and QTLs from wild wheat relatives (Devi et al. 2019). However, it is difficult to transfer resistance genes directly from wild relatives to any of the wheat cultivars because of genome incompatibility and cytological instability of the hybrids (Leonova et al. 2010). *Triticum timopheevii* is a tetraploid wheat, thought to have evolved from the same diploid progenitor species as *Triticum turgidum* and *Triticum aestivum*. There is convincing evidence that *Triticum urartu* contributed the A^t genome to *T. timopheevii* (Dvorak et al. 1993). Rodriguez et al. (2000), based on the studies on chromosome pairing suggested that the B genome of *T. aestivum* is closely related to the G genome of *T. timopheevii* and the S genome of *Ae. speltoides*. Meiotic analysis of F_1 hybrids between *T. timopheevii* and *T. aestivum* showed 70% pairing between A and A^t genomes and 30% pairing was observed between B and G genomes (Feldman 1966). A and B genomes are closely related to A^t and G genomes (Ogihara and Tsunewaki 1988; Dvorak et al. 1989, 1993). *T. timopheevi* belongs to the secondary gene pool and it can be crossed with *T. aestivum* cultivars. In the present study, initial cross with *T. timopheevii* was made using *T. aestivum* cultivar Chinese Spring. The presence of recessive crossability promoting alleles *kr1* and *kr2* (crossability) genes made the hybridization between wheat and *T. timopheevii* successful, whereas the dominant alleles of the *Krl* and *Kr2* genes reduce the crossability of hexaploid wheat with many alien species (Laurie and Bennett 1987). According to cytogenetic analysis gene transfer from *timopheevii* wheat to common wheat is possible by direct hybridization. The pairing of chromosomes during meiosis between A and A^t genomes is primarily homologous while pairing between B and G genomes is homoeologous (Gill and Chen 1987) allowing genes to transfer from both A^t and G genomes of *timopheevii* wheat. With the development of new genomic information and tools in *T. aestivum* and related species, utilization of the wide gene pool by molecular breeding is possible. Different molecular markers (AFLP, RAPD, STS, STMS, DAF, ESTs, SSR, SNP, miRNA-based SSRs *etc*.) have been utilized for breeding approaches, including MAS, QTL mapping, MTAs mapping using GWAS, characterization and diversity analysis studies (Gupta et al. 1999; Tyagi et al. 2021; Agarwal et al. 2021). Rapid detection and characterization of introgressions was a major bottleneck for the use of genetic variation from the wild relatives of wheat for crop improvement. However, in the present study the putative Wheat-*T. timopheevii* introgressions were discovered using an Axiom® SNP genotyping array.

Out of 41 ILs, 33 ILs showed resistant reaction ('0;' to '13') to leaf rust, and the rest were susceptible (Table1). The two *T. timopheevii* derived ILs TTmD-638 and TTmD-793 expressed

high degree of resistance to the broad spectrum of leaf rust pathogen at seedling stage (Table 2). These ILs will help in widening the genetic base of leaf rust resistance in wheat. SNP analysis confirmed *T. timopheevii* introgression in these lines. The introgression in TTmD-638 (30.59%) was higher than TTmD-793 (20.30%). Large numbers of wheat/wild relative introgressions were also observed in recent work on *T. militinae* (Nataraj et al. 2017), *Am. muticum* (King et al. 2017), *Ae. speltoides* (King et al. 2018), *T. urartu* (Grewal et al. 2018), and *T. timopheevii* (Devi et al. 2019). TTmD-793 showed much higher introgression of 22.75% in A genome. This is expected because A genome of wheat and A^t genome of *T. timopheevii*, having the same progenitor, *T. urartu* (A and A^t genomes) (Dvorak et al. 1993) and are homologous and show higher pairing during meiosis (Gill and Chen 1987).

The introgression in IL TTmD-638 interestingly showed higher introgression in B (32.18%) genome. The B and G genomes are homoeologous and may show sufficient pairing of chromosomes to enable introgression from G genome. Due to their common ancestors, an *Ae. speltoides*-like species, the G genome of *T. timopheevii* and the B genome of wheat exhibit significant similarities (Dvorak and Zhang 1990). The likelihood of recombination between two genomes increases with their degree of relatedness. D genome showed least introgression in both TTmD-638 with 29.17% and TTmD-793 with 16.96% introgression. Since D genome of wheat is contributed by *Triticum tauschii* (Coss.) Schmalh. (*Aegilops tauschii* Coss.), a diploid species with genome DD, which is not one of the progenitor species of *T. timopheevii*, preferential pairing is expected between A/A^t and B/G chromosomes, hence the least introgression in D genome. Jauhar et al. (1991) concluded, based on chromosome pairing in *ph1b*euhaploids of common wheat, that the A and D genomes are considerably related to each other than either is to B. On the basis of comparative genomics, Marcussen et al. (2014) demonstrated that A and B genomes gave rise to D genome through homoploid hybrid speciation. Nataraj et al. (2017) reported the introgression in the D genome (using SSR markers) of TMD7-5, TMD6-4 and TMD11-5 ILs with 2.8, 2.2 and 6.8%, respectively. Using D genome specific microsatellite markers Leonova et al. (2011) also reported that, four *T. timopheevii* introgression lines from Saratovskaya 29 revealed *T. timopheevii* fragments into D chromosomes *i.e*., 5D, 6D, and 7D chromosomes and one line derived from Tcelinnaya 20 showed *T. timopheevii* segments into D chromosomes *i.e.,* on 7D chromosome. Therefore, it is not surprising that *T. timopheevii* introgression were also located in the D genome. The two *T. aestivum – T. timopheevii* introgression lines TTmD-638 and TTmD-793 provide new sources for leaf rust resistance which may be used in wheat improvement programs. Further, these two ILs can be used to investigate and map genes for leaf rust resistance.

Authors' contribution

Conceptualization of research (V, SKJ, NM); Designing of the experiments (SN, SKJ, V); Contribution of experimental materials (V); Execution of field/lab experiments and data collection (SN, RK, ST, AKC, PA); Analysis of data and interpretation (SN, ST, PA, SKJ, V); Preparation of the manuscript (SN, ST, AKC, PA, NM, NM, SKJ, SMST, V).

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