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Evaluation of *Aegilops tauschii* Coss. germplasm for agro-morphological traits and genetic diversity using SSR loci

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Abstract

A set of 85 *Aegilops tauschii* accessions were evaluated for morphological traits, disease resistance, high molecular weight glutenin subunits and genetic diversity using D genome specific SSR markers. Screening for leaf rust, stripe rust and Karnal bunt (KB) resistance over four years identified *Ae. tauschii ssp strangulata* as a potential source for new leaf rust and stripe rust resistance genes and *Ae. tauschii ssp tauschii* as a source for KB resistance. The *Ae. tauschii* germplasm also showed a lot of variability for high molecular weight glutenin profile showing 16 combinations of slow (1Dx) and fast (1Dy) mobility subunits. A total of 51 D-genome specific simple sequence repeat (SSR) markers amplified a total of 241 alleles in 85 accessions of *Ae. tauschii* and two bread wheat cultivars. The number of alleles per locus varied from 2 to 11 with an average of 4.75. Polymorphism Information Content (PIC) values for all the 51 primer pairs ranged from 0.02 to 0.88 with an average of 0.62. A phylogenetic tree generated, based on Dice dissimilarity matrix and cluster analysis, led to the clustering of the *Ae tauschii* accessions into two major groups. The cluster I comprised primarily the *strangulata* accessions whereas the cluster II comprised of accessions belonging to both the subspecies indicating some gene flow from ssp *strangulata* to *tauschii*. Genetically diverse *Ae. tauschii* accessions with disease resistance and better agronomic and quality traits are being used for the introgression of desirable traits to bread wheat for commercial exploitation.

Key words: *Aegilops tauschii,* disease resistance, D genome, genetic diversity, SSR

Introduction

Aegilops tauschii Coss., a diploid self-pollinating goatgrass species has contributed the D genome to common wheat (*Triticum aestivum* L.) [1, 2]. Hybridization of *Ae. tauschii* ssp. *strangulata* (DD) with tetraploid wheat, *T. turgidum* L. (AABB) about 7000 years ago led to the development of hexaploid wheat *Triticum spelta* (L) Thell (AABBDD) from which common wheat evolved [3]. The D genome of *Ae. tauschii* has much greater genetic diversity compared to the D genome of *T. aestivum* as observed for high and low molecular weight glutenin subunits (HMW GS) [4], gliadins [5] and AFLP and SSR markers [3, 6-9]. The hybridization events that led to the evolution of bread wheat are thought to have involved only a few accessions of *Ae. tauschii.* In fact *Ae. tauschii*, represents more than 90% of the total genetic variability present in the D-genome [10]. Much of the genetic diversity in *Ae. tauschii* gene pool thus remained unutilized and may not be represented in the *T. aestivum* gene pool [11]. The *Ae. tauschii* germplasm may be harboring novel alleles and genes for biotic and abiotic stress tolerances, productivity and quality traits.

Ae. tauschii has been a valuable source of genes for resistance to diseases, nematodes, insects and quality traits [12]. A number of reports have indicated the presence of variation for useful physiological traits such as resistance to low temperature [13] tolerance to salt [14], good performance in low-yielding drought-stressed environments [15], heat tolerance [16] and quality traits such as grain micronutrient concentrations [17].

Ae. tauschii has been a subject of intensive genetic studies because of its importance as a potential donor of desirable genes for the improvement of cultivated

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wheats. CIMMYT recognized the potential of *Ae. tauschii* germplasm in broadening the gene pool of wheat and developed 1,000 synthetics from 460 *Ae. tauschii* accessions [18]. These synthetics were later backcrossed to many CIMMYT and global elite breeding lines to introduce new variation for various morphological and agronomic traits [19, 20], resistance to biotic stresses [21, 22] and abiotic stresses [23]. The synthetics were also shown to be very diverse at the molecular level, and genetically distinct from cultivated wheats [24].

Based on spikelet morphology, *Ae. tauschii* has been divided into two subspecies, *tauschii* and *strangulata* [25, 26]. Subspecies *strangulata* has been proposed as the D genome donor of wheat [27]. The two typical forms, ssp. *tauschii* and ssp. *strangulata* are connected by a continuous range of intermediate forms [28]. Some studies have even shown the difficulty to distinguish the two subspecies of *Ae. tauschii* based on molecular markers and suggest high gene flow between these subspecies.

The Punjab Agricultural University, Ludhiana, India, has been maintaining an active collection of ~250 *Ae. tauschii* accessions. These accessions have been found to carry resistance genes for various biotic stresses including leaf rust, stripe rust, powdery mildew and Karnal bunt. A number of accessions have been reported to carry genes for high grain Fe and Zn content [17] and heat tolerance [16]. It would be highly desirable to exploit this variation to improve wheat germplasm for resistance to biotic stresses, tolerance for abiotic stresses and productivity traits. However, to effectively utilize this germplasm it is essential to assess the genetic variability and diversity in these two subspecies. The present investigation was, therefore, undertaken to characterize a random sample from *Ae. tauschii* germplasm for morphological traits, disease resistance, high molecular weight glutenin subunits and genetic diversity so that genetically diverse accessions with traits of economic importance could be used in the wheat germplasm enhancement.

Material and methods

The *Ae. tauschii* germplasm, consisting of 85 *Ae. tauschii* accessions was used for agro-morphological, disease reaction and molecular characterization. These accessions originated from Afghanistan (3), Azerbaijan (8), Georgia (1), Iran (12), Israel (16), Kyrgyzstan (1), Tajikistan (2), Turkmenistan (7), Uzbekistan (3), USSR (10) and some with unknown origin (22) and were introduced from Wheat Genetic and Genomics Resource Center, Kansas State University; University of Missouri; CIMMYT; ICARDA; IPK, Gestersleben and collected from Israel. Two bread wheat cultivars PBW343 and WH542 were also included in the set for comparison. PBW343 was selected as it is the most widely grown cultivar of North India and is being used as recurrent parent in most of the germplasm enhancement activities. WH542 is another well-adapted genotype in the NWPZ of India. All the accessions were grown in the field in 1.0 m rows with row-to-row distance of 70 cm in the experimental fields of Punjab Agricultural University, Ludhiana (30°52'N, 75°56'E), India. All the accessions flowered between 2nd week of March to 1st week of April under normal day length conditions.

Evaluation of morphological traits

Data for each of the accessions were recorded for growth habit (erect/semi erect/spreading), leaf colour, days to flowering, spike morphology and grain characteristics. The spikes were categorized into cylindrical (long, narrow, tapering towards tip) or moniliform (curved rachis segment, longer and narrower than adjacent spikelet). The weight of 100 grains was recorded from the harvest of 2008, 2009 and 2010 and average grain weight was calculated. For determining grain colour, grains were immersed in 5% NaOH solution for 2 h and recorded as amber versus red.

Disease screening

All the accessions were screened for leaf rust and stripe rust resistance under artificial epiphytotic conditions for four years *viz*., 2004-05, 2006-07, 2007-08 and 2008-09. Infector rows of susceptible cultivar Agra Local were planted all around the experimental plot and sprayed with a mixture of urediniospores of *Puccinia triticina* (109R31-1, 121R63-1 and 21R5) and *Puccinia striiformis* (46S102, 47S103, 46S119 and 78S84). The stripe rust pathotype 78S84 was included for screening during years 2007-08 and 2008-09 only. At the adult plant stage, data were recorded according to the modified Cobb's scale [29] which included disease severity (percent leaf area affected) and infection type; 0 = immune; R = resistant, MR = moderately resistant; MS = moderately susceptible; S = susceptible. Karnal bunt screening was done as per Chhuneja *et al.* [30].

SDS-PAGE for high molecular weight glutenin subunits

The composition of HMW glutenin subunits of the *Ae. tauschii* accessions was determined by polyacrylamide

gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE) using 10 per cent acrylamide according to the method of Smith and Payne [31] with a few modifications. Gels were stained in a solution of 0.25% Commassie Blue R250 in a mixture of methanol: acetic acid: water (4:1:4) and destained in methanol: acetic acid: water (5:1:5) overnight.

Marker analysis

DNA from at least five plants from each accession was isolated following CTAB method [32]. A set of 51 SSR markers spanning all the seven linkage groups were selected for diversity analysis. Polymerase chain reaction (PCR) was performed in a reaction volume of 20µl containing 50-100 ng template DNA, 0.2 mM of each dNTPs, 1X PCR reaction buffer (10 mM Tris HCl + 50 mM KCl + 0.01% w/v gelatin, pH 8.3), 1.5 mM $MgCl_2$, 0.25 mM each forward and reverse primer and one unit of *Taq* DNA polymerase. PCR was performed in thermocycler (Eppendorf) with initial denaturation for 2 min at 94^{o}C, followed by 35 cycles of 1 min at 94^{o}C, 1 min at 55-60^{o}C (depending upon the primer), 1 min at 72^{o}C and a final 7 min extension at 72^{o}C. Amplification products were fractionated on 2.5% agarose gel and visualized in Gel Documentation System (UVP).

Statistical analysis

For each primer pair, the total number of alleles, number of polymorphic alleles and polymorphism information content (PIC) were calculated for all the accessions. The bands were scored in binary format as presence (1) and absence (0) to measure genetic diversity and genetic distances between different accessions. The software package, DARwin5 [33] was used for estimation of genetic diversity of the *Ae. tauschii* accessions which was assessed by clustering analysis in which a Dice similarity matrix was generated using SSR data and the dendrogram was constructed using unweighted neighbour joining method. Bootstrap analysis was performed to test the robustness of the tree with 1000 bootstraps.

Results and discussion

Variation for agro-morphological traits

Ae. tauschii accessions exhibited variation for growth habit, spike morphology, leaf colour, days to flowering and grain colour and weight. The spike morphology was recorded to identify the accessions belonging to ssp *tauschii* and ssp. *strangulata.* Fifty one accessions had moniliform spike with a curved rachis segment which was longer and narrower than the adjacent spikelets, characteristic of ssp. *strangulata*, while 34 accessions had cylindrical spike typical of ssp. *tauschii* (Table 1). Seventy accessions showed spreading growth habit while 15 had semi-erect growth habit (data not given). Some of these accessions were very vigorous with apparently higher biomass. Days to 50% flowering, after seeding, ranged from 157 –177 days (data not given). Based on visual observations on leaf colour, 31 accessions had dark green leaves, 34 accessions had green leaves, 15 accessions had pale green leaves (Table 1). All the selected accessions had red grain colour. The 100-grain weight varied from 0.52 g to 2.07 g in different accessions with accession pau14236 showing the lowest and pau14330 has the highest grain weight, indicating about 3.5 fold variation in grain size within *Ae. tauschii* germplasm. Accessions of ssp. *strangulata*, in general, had bolder grains (average 100-grain weight of 1.37 g) than those of ssp. *tauschii* (0.97 g) . *Per se* the grain weight of *Ae. tauschii* is much less than the cultivated wheat but several fold variability existed within the *Ae. tauschii*. Cluster I with majority of the accessions of ssp. *strangulata* had higher average 100-grain weight (1.35 g) as compared with cluster II (1.10 g) which comprises of both the subspecies. Some of these accessions with high grain weight can be used for introgressing this useful variability into cultivated wheats.

Screening for disease resistance

Screening for leaf rust and stripe rust resistance identified 40 accessions as resistant/moderately resistant to both the rusts, 11 as resistant to leaf rust but susceptible to stripe rust; 3 as susceptible to leaf rust but resistant to stripe rust while 31 accessions were recorded susceptible to both the rusts (Table 1). Out of 40 leaf rust and stripe rust resistant accessions, 36 accessions belonged to ssp. *strangulata* and only 4 accessions to ssp. *tauschii*. Out of 11 accessions that had leaf rust resistance only, 7 belong to ssp. *strangulata* while all the rust susceptible accessions, except six, belong to ssp. *tauschii*. The leaf rust resistance genes *Lr21, Lr22a, Lr32, Lr39, and Lr42* have been introgressed from *Ae. tauschii* to bread wheat and some of these have been exploited commercially [34]. *Lr21* and *Lr39* have been introgressed from *Ae. tauschii* ssp. *tauschii* and *Lr22a* and *Lr42* from ssp. *strangulata* [34,10]. However, only one of the catalogued stripe rust resistance gene *Yr28* has been derived from *Ae. tauschii*. The leaf rust and stripe rust resistant accessions identified in the present investigation might carry some additional genes which can be transferred bread wheat through bridging species.

Over three years of screening under artificial inoculations, 20 accessions were identified to be resistant to KB and six accessions were moderately resistant (Table 1), details of which were presented elsewhere [30]. Unlike rusts, out of 20 KB resistant accessions, 16 belonged to ssp. *tauschii* and four to ssp. *strangulata.* For Karnal bunt, although a number of sources of resistance have been identified, the development of resistant cultivars has been slow because of difficulties encountered in combining minor genes and screening for Karnal bunt in early segregation generations. Since *Ae. tauschii* ssp. *tauschii* has been identified to have variation for KB resistance, so any future prebreeding programmes for transfer of KB resistance from *Ae. tauschii* should be focused on ssp. *tauschii* using *T. durum* as bridging species.

Characterization for high molecular weight glutenin subunits

Ae. tauschii is expected to be a useful genetic material for improving the baking quality of bread wheat as considerable variations in seed storage protein composition has been reported in *Ae. tauschii* [4,5]. Seventy two accessions of *Ae. tauschii* were analyzed for high molecular weight (HMW) glutenin subunits through SDS-PAGE alongwith PBW343 and WH542. Comparative analysis was made for the subunits coded by D genomes of bread wheat and *Ae. tauschii* at *Glu-D1* locus. The HMW profile of all the accessions studied during the present investigation is summarized in Table 2. Sixteen types of HMW glutenin subunit combinations were observed which were characterized by a pair of slower and faster mobility subunits with former numbered from 2.1 to 5 and latter from 10.4 to T_3 in the order of increasing mobility [35]. Both the cultivated wheat varieties possessed subunit pair 5 and 10 at *Glu-D1* locus. Among *Ae. tauschii* accessions, four slower mobility (1Dx) and eight faster mobility (1Dy) HMW subunits were observed. HMW glutenin subunit pair 1Dx2+1Dy12 was the most frequent followed by 1Dx5+1Dy10, 1Dx2+1Dy10, 1Dx2.1+1Dy12. Out of 25 accessions having 1Dx2+1Dy12 combination, 19 belonged to ssp. *strangulata* and six to ssp. *tauschii*. Contrary to this, out of nine accessions with 1Dx5+1Dy10 pair, six belong to ssp. *tauschii* and three to ssp. *strangulata*.

A number of novel HMW subunits have been identified and characterized from different accessions of *Ae. tauschii* in previous investigations [35-37]. Whether the subunits identified in the present investigation are similar to earlier reported subunits will be established after molecular characterization of the new subunits using gene specific primers. Effect of the new subunits of HMW glutenins on viscoelastic properties of dough would be studied by developing near isogenic lines (NILs) in the hexaploid wheat

Diversity analysis using SSR markers

A summary of the number of the alleles, major allele frequencies and PIC values for all the 51 SSR markers used is presented in Table 3. Amplification profile of 51 SSR markers was recorded with each band representing a different allele, with a particular primer pair. A total of 241 alleles were identified across 87 genotypes, including 85 *Ae. tauschii* accessions and two bread wheat cultivars. The number of alleles per primer varied from 2 to 11 with an average of 4.75 alleles per locus (Table 3). Polymorphism Information Content (PIC), a measure of allelic diversity at a locus, was estimated for all the SSR marker loci. The values ranged from 0.02 (*Xcfd156*) to 0.88 (*Xwmc331*) with an average PIC value of 0.62 for all the 51 primer pairs (Table 3). PIC values of 31 primer pairs was higher than the mean value.

A dice similarity matrix was generated based on the polymorphic SSR data which was then used to construct phylogenetic tree for deciphering the genetic divergence of the *Ae. tauschii* accessions. Based on the Dice similarity matrix, an average dissimilarity coefficient of 0.54 was recorded with the maximum dissimilarity coefficient of 0.78 between accessions pau14195 and pau3757; pau13757 and pau3750; pau14331 and pau3751 and minimum of 0.06 between accessions pau3760 and pau5514. Thus the dendrogram generated from the dissimilarity matrix and cluster analysis using Darwin5 (5.0.158) indicated high level of genetic diversity among the *Ae. tauschii* accessions studied. Cluster analysis led to the classification of the *Ae tauschii* accessions into two major groups (Fig. 1) with 42 and 43 accessions each. The cluster I comprised mainly of the accessions belonging to subspecies *strangulata* (36/42). The cluster II comprised of 28 accessions belonging to ssp. *tauschii* and 15 to ssp. *strangulata*. The wheat cultivars PBW343 and WH542 grouped with the cluster II (Fig. 1). Cluster II was observed to be more heterogeneous with 10 different sub clusters and three individual accessions. Cluster I encompassed seven sub clusters and one individual accession. Clustering of *Ae. tauschii* accessions, however, did not correspond with origins of different accessions.

Table 1. Characterization of the selected *Ae. tauschii* accessions for agro-morphological traits and resistance to rusts and Karnal bunt

S. No.	Cultivar/ accession	Origin[I]	Spike type	Leaf colour[$]	Average 100GW[@]	Leaf rust reaction[#]	Stripe rustreaction[#]	KB*
1	PBW343	-	Square	G	3.9	S	S	MS
2	WH542	-	Square	G	3.3	S	S	S
3	894	USSR	moniliform	DG	1.07	R	R	-
4	3472	Afghanistan	cylindrical	G	0.87	S	S	R
5	3738	Iran	cylindrical	DG	-	MR	R	-
6	3743	Iran	moniliform	PG	0.97	R	S	-
7	3750	Iran	moniliform	DG	1.10	R	R	S
8	3751	Iran	moniliform	DG	1.17	MR	MR	-
9	3753	U	cylindrical	G	1.06	S	S	R
10	3757	Iran	moniliform	DG	1.48	R	R	MR
11	3758	Iran	moniliform	DG	1.52	R	MR	S
12	3759	Iran	cylindrical	PG	1.14	S	S	S
13	3760	U	moniliform	G	1.36	R	MR	S
14	3761	U	moniliform	G	1.63	R	MR	S
15	3769	U	moniliform	DG	1.54	R	MR	S
16	3786	U	moniliform	DG	1.28	R	MR	S
17	3805	Iran	moniliform	DG	1.52	R	MR	-
18	3822	U	moniliform	DG	1.06	S	S	-
19	3823	U	moniliform	DG	0.84	MR	R	S
20	3826	U	cylindrical	G	0.91	S	S	R
21	5514	USSR	moniliform	DG	0.91	S	S	R
22	9385	USSR	cylindrical	G	0.92	S	S	S
23	9785	USSR	cylindrical	G	0.68	S	S	-
24	9787	USSR	moniliform	DG	1.62	S	R	-
25	9790	USSR	moniliform	DG	1.13	R	S	MR
26	9791	USSR	moniliform	PG	1.21	S	S	S
27	9793	USSR	moniliform	DG	1.49	R	MR	S
28	9794	USSR	moniliform	DG	1.53	R	R	-
29	9795	USSR	cylindrical	G	1.19	S	S	S
30	9796	Iran	moniliform	DG	1.51	R	S	S
31	9799	Iran	moniliform	DG	1.33	R	R	-
32	9804	Iran	moniliform	DG	1.39	R	R	S
33	9807	Iran	moniliform	DG	1.64	R	R	S
34	9809	U	moniliform	DG	1.58	MR	MR	S
35	9810	U	moniliform	G	1.60	R	S	S
36	9821	U	moniliform	DG	0.99	R	MR	S
37	9824	U	cylindrical	PG	1.14	S	S	MR
38	9826	U	moniliform	DG	1.41	R	R	S
39	9827	U	cylindrical	PG	0.87	S	S	S
40	9830	U	cylindrical	PG	0.71	S	S	-
41	13757	Uzbekistan	cylindrical	PG	0.77	S	S	S
42	13764	Azerbaijan	cylindrical	PG	1.46	S	S	S
43	14091	Turkmenistan	moniliform	G	1.25	S	S	MR
44	14102	Azerbaijan	moniliform	G	1.26	MR	MR	R

45	14106	Azerbaijan	cylindrical	G	1.27	R	S	R
46	14109	Turkmenistan	moniliform	G	1.13	R	R	S
47	14122	Turkmenistan	cylindrical	-	1.51	R	R	S
48	14159	Turkmenistan	moniliform	DG	1.38	S	MR	MR
49	14163	Turkmenistan	cylindrical	G	1.31	R	S	-
50	14164	Kirgizstan	cylindrical	G	-	R	MR	S
51	14165	Azerbaijan	moniliform	G	1.27	R	R	-
52	14171	Azerbaijan	moniliform	G	1.42	R	R	S
53	14173	Azerbaijan	moniliform	G	1.34	MR	S	S
54	14175	Azerbaijan	moniliform	G	1.62	R	MR	S
55	14180	Afghanistan	moniliform	DG	1.13	S	S	R
56	14183	Afghanistan	cylindrical	DG	0.79	R	S	R
57	14189	Turkmenistan	cylindrical	G	0.61	S	S	S
58	14192	Uzbekistan	cylindrical	G	0.85	MR	S	R
59	14195	Uzbekistan	cylindrical	-	1.23	R	R	R
60	14197	U	cylindrical	PG	0.83	S	S	MR
61	14198	Turkmenistan	cylindrical	G	0.85	S	S	R
62	14199	Azerbaijan	cylindrical	PG	0.95	S	S	R
63	14219	Tajikistan	cylindrical	G	1.39	S	R	R
64	14233	Georgia	cylindrical	-	-	S	S	R
65	14236	Tajikistan	cylindrical	G	0.52	S	S	R
66	14240	U	moniliform	DG	1.30	R	S	R
67	14245	U	cylindrical	-	-	S	S	R
68	14251	Israel	cylindrical	PG	0.71	S	S	-
69	14318	Israel	cylindrical	PG	-	S	S	-
70	14323	Israel	moniliform	DG	1.59	MR	R	S
71	14330	Israel	moniliform	G	2.07	R	R	S
72	14331	Israel	moniliform	G	1.87	R	R	S
73	14333	Israel	moniliform	G	1.63	R	R	S
74	14334	Israel	moniliform	DG	-	R	R	S
75	14335	Israel	moniliform	G	1.64	R	R	S
76	14336	Israel	moniliform	G	1.90	R	R	S
77	14337	Israel	moniliform	G	1.66	R	R	S
78	14339	Israel	moniliform	DG	1.51	R	R	S
79	14342	Israel	moniliform	G	0.72	S	S	S
80	14344	Israel	moniliform	G	1.59	R	R	S
81	14350	Israel	cylindrical	-	-	S	S	R
82	14351	Israel	cylindrical	PG	0.67	S	S	R
83	14354	Israel	cylindrical	PG	0.83	S	S	R
84	14361	U	moniliform	G	1.42	R	R	S
85	14576	U	moniliform	G	1.39	R	S	-
86	14577	U	moniliform	DG	-	MR	MR	S
87	14578	U	cylindrical	PG	1.09	S	S	-

!-Unknown origin;
$DG-dark green leaves; G-green leaves; PG-Pale green leaves;
@Average grain weight based on the harvest from year 2008, 2009 and 2010;
#Consensus data based on disease reaction over four years 2005, 2007, 2008 and 2009;
*consensus data based on three years. Detailed data available in Chhuneja *et al.* 2008.

Table 2. HMW glutenin subunit profiling of the *Ae. tauschii* accessions

HMW profile	No. of accessions	Accessions
(1Dx+1Dy)		
5+10	2	PBW343, WH542
5+10	9	9827, 13757, 13764, 14109, 14197, 14198, 14199, 14577, 14330
5+10.1	3	3472, 14236, 14251
5+ T1	1	14189
5.1+T2	1	9821
5+12	2	14192, 14159
5.1+12	1	9787
2+12	25	3738, 3758, 3760, 3761, 3769, 3805, 3826, 5514, 9385, 9785, 9791, 9796, 9804 ,9807, 9809, 14171, 14183, 14318, 14334, 14335, 14336, 14337, 14339, 14342, 14344
2+10	8	894, 3759, 14164, 14351, 14354, 14361, 14576, 14578
2+T2	4	3751, 3786, 3822, 14323
2+10.3	2	9795, 14106
2.1+10.3	1	14163
2.1+10	2	9824, 9826
2.1+12	6	3757, 3823, 9793, 9810, 14173, 14333
2.1+T3	1	14180
2.1+T2	1	14165,14175
2.1+10.4	1	14195

In the previous investigations it has been reported that although it is possible to classify *Ae. tauschii* germplasm into ssp. *strangulata* and *tauschii* based on spike morphology but morphological classification may not exactly correspond with the grouping based on molecular marker data [10, 3]. Two typical forms of ssp. *tauschii* and *strangulata* have been reported to be connected by continuous range of intermediate forms [28]. Some reports have also shown the difficulty to distinguish the two subspecies based on molecular markers and suggested high gene flow between two subspecies [3, 38]. Low genetic distance and therefore high gene flow between two subspecies has also been reported [8]. In the present study also, cluster II contained mixture of both the subspecies indicating some gene flow between these subspecies.

Association of the cluster analysis with disease data indicated that 29 accessions of cluster I were resistant to both leaf rust and stripe rust and six accessions were resistant to leaf rust only, while only nine accessions of cluster II were resistant to both the rusts. Seven accessions that grouped in cluster II were resistant to leaf rust only and one accession was resistant to stripe rust only. Situation was reverse for KB since the KB resistance was more prevalent in the accessions grouped in cluster II with 21 accessions from cluster II and only 5 from cluster I showing high to moderate level of resistance for KB.

The present investigation has depicted a high level of genetic variation in the *Ae. tauschii* germplasm for agronomical traits, HMW glutenin subunits, resistance to biotic stresses and diversity at molecular level. *Ae. tauschii* accessions with multiple disease resistance and better quality and agronomic traits have been identified. *Ae. tauschii* ssp. *strangulata* and ssp. *tauschii* have been identified as a potential sources of resistance for rusts and KB, respectively. Genetically diverse *Ae. tauschii* accessions with better agronomic, quality and disease resistance traits are being used to introgress desirable traits to bread wheat for germplasm enhancement.

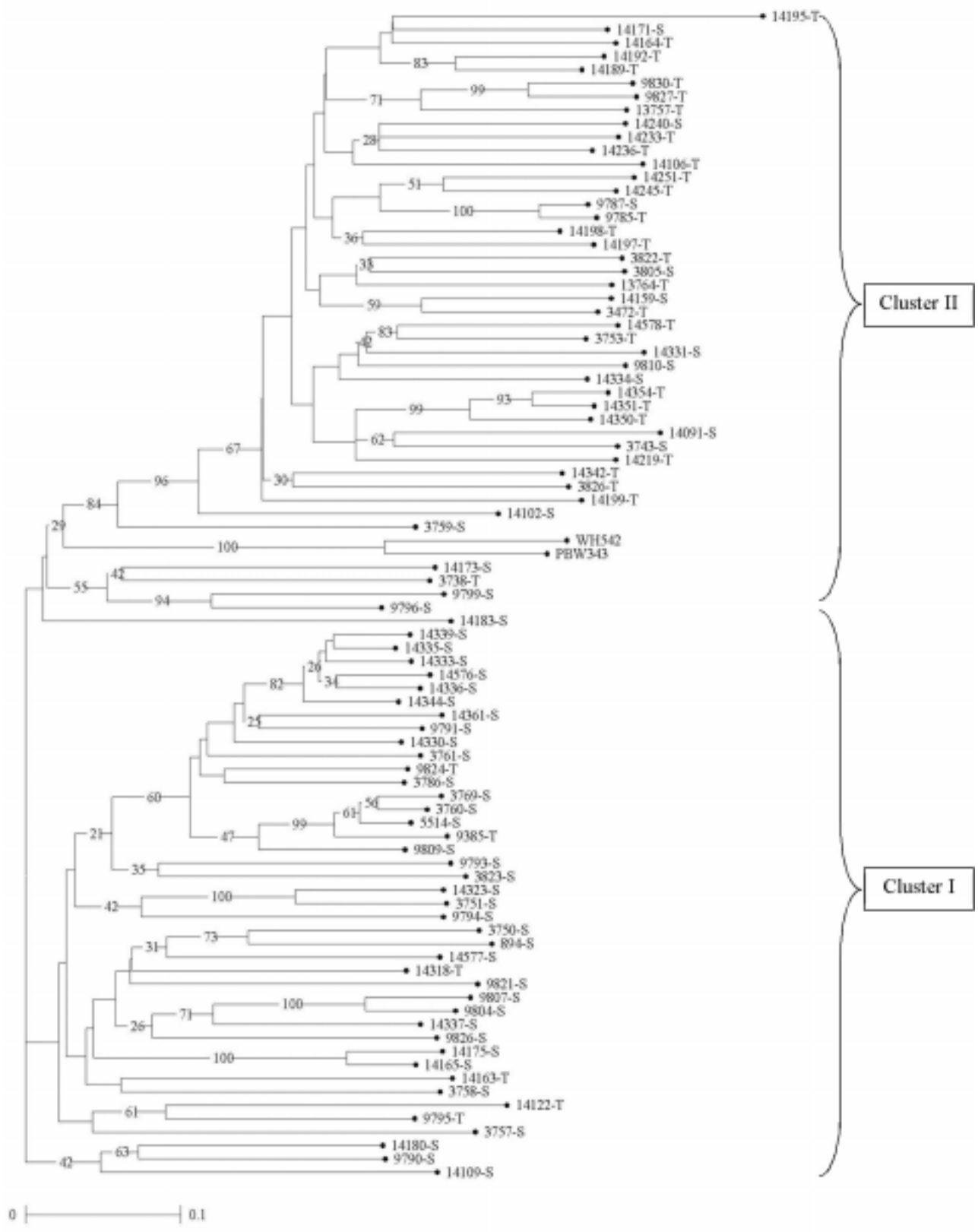

Fig. 1. Phylogenetic tree generated using unweighted neighbor joining method from dice similarity matrix with computer software DARwin5. *Ae. tauschii* accessions belonging to the subspecies *strangulata* and *tauschii*, has been indicated by adding 'S' and 'T', respectively to the accession numbers.

Table 3. Chromosomal (Chr) location, number of alleles and PIC values of the SSR primers used for diversity analysis of *Ae. tauschii* accessions

S.No.	SSR marker	Chr location	Alleles	Major allele frequency	PIC value
1	Xwmc93	1D	3	0.53	0.51
2	Xcfd15	1D	5	0.68	0.73
3	Xcfd282	1D	6	0.37	0.76
4	Xcfd59	1D	5	0.38	0.69
5	Xcfd63	1D	3	0.52	0.54
6	Xgwm106	1D	2	0.62	0.47
7	Xwmc429	1D	4	0.34	0.70
8	Xgwm122	2D	3	0.63	0.51
9	Xcfd116	2D	2	0.53	0.5
10	Xcfd160	2D	2	0.52	0.5
11	Xcfd17	2D	3	0.43	0.65
12	Xcfd233	2D	5	0.33	0.76
13	Xcfd56	2D	6	0.34	0.76
14	Xwmc111	2D	8	0.17	0.87
15	Xwmc112	2D	6	0.27	0.81
16	Xwmc181	2D	4	0.97	0.07
17	Xcfd152	3D	3	0.39	0.44
18	Xcfd35	3D	6	0.37	0.78
19	Xcfd55	3D	3	0.48	0.59
20	Xcfd64	3D	2	0.87	0.23
21	Xcfd71	3D	5	0.34	0.73
22	Xgdm72	3D	5	0.29	0.77
23	Xgwm383	3D	7	0.24	0.82
24	Xwmc492	3D	3	0.53	0.55
25	Xcfa2174	4D	2	0.97	0.07
26	Xcfd84	4D	4	0.41	0.68
27	Xwmc285	4D	5	0.29	0.75
28	Xwmc331	4D	10	0.19	0.88
29	Xwmc74	4D	8	0.35	0.78
30	Xwmc89	4D	6	0.48	0.64
31	Xcfd156	5D	2	0.99	0.02
32	Xcfd18	5D	3	0.41	0.64
33	Xcfd266	5D	8	0.44	0.71
34	Xcfd29	5D	7	0.30	0.80
35	Xcfd7	5D	11	0.35	0.87
36	Xcfd8	5D	3	0.41	0.63
37	xcfd81	5D	4	0.49	0.68
38	Xgwm272	5D	5	0.33	0.76
39	Xbarc173	6D	9	0.22	0.86
40	Xbarc204	6D	6	0.23	0.81
41	Xcfd287	6D	4	0.43	0.69
42	Xcfd33	6D	3	0.48	0.62
43	Xcfd42	6D	5	0.33	0.73
44	Xcfd49	6D	4	0.58	0.60
45	Xcfd60	6D	4	0.51	0.58
46	Xcfd80	6D	5	0.37	0.66
47	Xcfa2040	7D	2	0.56	0.49
48	Xcfd14	7D	6	0.40	0.76
49	Xcfd25	7D	5	0.81	0.34
50	Xcfd31	7D	5	0.61	0.57
51	Xcfd41	7D	4	0.79	0.35

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