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Research Article

Association between agronomic traits and molecular markers with take-all disease severity in bread wheat *Triticum aestivum*

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Abstract: Identifying resistant genotypes is necessary to control wheat take-all disease *Gaeumannomyces graminis* var. *tritici*. In this study, 30 bread wheat genotypes were evaluated under greenhouse and field conditions. The genotypes were evaluated with fifteen molecular markers (SSR and specific primers for translocation wheat-rye). The genotypes were divided into four groups based on disease severity (the greenhouse) and agronomic traits (the field). Chi-square results showed the interactions for these groupings. The correlation between disease severity and agronomic traits indicated that plant resistance is strongly dependent on plant yield. Based on cluster analysis for molecular data (based on simple matching similarity coefficient and UPGMA method), genotypes were separated into resistant and susceptible ones. The correlation between disease severity and amplified loci showed that disease resistance is interactive with xbarc232, xbarc124, and gpw95001 markers. Resistance to take-all disease is probably associated with the interaction of several genes. These results add significant information to our knowledge of the chromosomal location of genes for the take-all disease.

Keywords: chi-square, cluster analysis, disease severity, resistance gene, take-all

Introduction

Bread wheat *Triticum aestivum* L. is the most important crop globally, and its yield is affected by several diseases. High yield, disease resistance, and abiotic stresses tolerance are essential for wheat breeding. Breeding for disease resistance is necessary. The take-all disease is caused by the fungus *Gaeumannomyces graminis* var. *tritici* and is one of the most important root diseases of winter wheat in all cropping areas around the world (Ramanauskienė *et al.*, 2019). *Gaeumannomyces graminis* is a soil-borne pathogen, and soil-borne diseases are hard to control due to their broad host ranges and difficulties in targeting the pathogen population in the soil with chemicals (McMillan *et al.*, 2018). Genetic diversity in wheat genotypes can be beneficial in identifying and grouping resistant and susceptible genotypes in response to disease. Assessing genetic diversity is a crucial goal in plant breeding. Genetic diversity among and within close cultivars of closely related crops is an effective solution for plant breeding programs to identify resistant cultivars to biotic and abiotic stresses (Gupta *et al.*, 2003). Molecular markers that identify differences between genotypes and cultivars at the genome level help assess genetic diversity and resistance to disease. Genetic diversity in plant resistance to disease is essential

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in plant disease control and management. Before evaluating genetic diversity related to disease resistance, it is necessary to identify cultivars that are resistant and susceptible to the disease by phenotypic methods and evaluated by agronomic traits. Afterward, polymorphisms between genotypes are measured with molecular markers, and differences at the molecular level are determined (Gibson and Bishop, 2005). Molecular markers such as restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), sequence-tagged sites (STS), and microsatellites (also termed SSRs) provide alternative tools for accurately identifying the resistance gene of interest in a specific chromosome. They can be used to tag genomic regions, clone the gene of interest through map-based cloning, near-isogenic line development, and resistance genes pyramiding into single cultivars by marker-assisted selection. SSR markers are the most common and widely used molecular markers because of the advantages associated with co-dominance, high repeatability, high levels of polymorphism, and chromosome specificity (Röder *et al.,* 1998). Moreover, they have gained considerable importance in plant genetics and breeding. They are commonly used for gene mapping in wheat. SSR markers have also been successfully used in resistance to stripe rust and powdery mildew and resistance gene mapping (Järve *et al.*, 2000; Liu *et al.*, 2002; Silvar *et al.*, 2011; Masoudi *et al.*, 2017; Fatemi-fard *et al.,* 2018). In an investigation of genetic diversity of resistance to bacterial blight in wheat using 12 ISSR markers, wheat cultivars were divided into four groups (resistant to very sensitive). The results of this study show the importance of the high efficiency of ISSR markers in identifying the source of disease resistance (Fatemi-fard *et al.,* 2018). In research to evaluate genetic diversity and analyze the association of morphophonological traits and powdery mildew in wheat germplasm, 60 wheat genotypes were evaluated with ISSR, IPBS, and IRAP primers. Cluster analysis showed that powdery mildew caused the lines to be grouped in three groups of sensitivity, resistance, and median (Masoudi *et al.,* 2017). In research using SSR markers in 10 wheat genotypes to investigate genetic diversity based on rust resistance genes. The results showed that SSR markers could distinguish and characterize wheat genotypes (Sehgal *et al.,* 2012).

Studies have shown that resistance is controlled by several genes in the case of wheat take-all disease (Kim *et al.,* 2003; McMillan *et al.,* 2018; Saberi Riseh *et al.,* 2021). The derivatives *Triticum durum-Haynaldia villosa* amphiploid were analyzed for resistance to take-all disease using the cytological method and PCR-based molecular analyses with RAPD markers. The results showed that primer S1230 amplified a specific fragment in *H. villosa* and its derivatives (Huang *et al.,* 2007). Another research by reverse transcription-quantitative PCR and RNA-seq on *Ggt*-inoculated wheat roots and healthy control root samples revealed 3973 differentially expressed genes in *Ggt*-inoculated wheat roots (Zhang *et al.,* 2020).

An effective way to identify sources of take-all disease resistance is to use molecular markers in cultivars that are phenotypically resistant to the disease. To date, many studies have investigated resistance to take-all disease in cultivars and wild ancestors of bread wheat. Previous research screened about 1000 wheat bread genotypes for resistance to take-all disease in the greenhouse and agronomic traits in the field and selected some resistant genotypes. This research evaluated some bread wheat (resistant and susceptible) with SSR to identify molecular markers associated with take-all disease response in bread wheat.

Materials and Methods

Fungus resource and preparation of inoculum

In research, several *Gaeumannomyces graminis* var. *tritici* isolates were isolated from the roots of take-all infected wheat plants from different provinces of Iran, including Esfahan, Markazi, Mazandaran, Tehran, East Azerbaijan, Western Azerbaijan, Ardabil, Qazvin, and Golestan. Pathogenicity tests indicated that all isolates

were pathogenic on wheat, and one of them, 'T-41' collected from Mazandaran, has had strong pathogenicity (Sadeghi *et al.,* 2010, 2012). Therefore, T-41 isolate was selected for our research. The selective medium for fungal culturing was potato dextrose agar (PDA) containing streptomycin (0.03 g in 1000 ml PDA). The fungus was purified once every 20 days; the border grew in a Petri-dish, and the fully developed fungus was stored in the refrigerator at 4 °C. Because of a high colonization rate and the uniformity of propagules, millet was chosen for inoculums preparation. A mixture of 100 g of cooked millet seed and 100 grams of wet sand was poured into a flask and autoclaved twice at 120 °C for 20 min. For fungus propagation, a few discs of mycelia, one centimeter in diameter, from the edge of the growing colonies were inoculated into each flask and incubated at 20-25 °C for 15 days. The flasks were then removed and incubated for 15 days at 20-28 °C in a laboratory environment under natural and fluorescent light. The flasks were shaken several times for aeration. They were then refrigerated until the time of use (Gholizadeh-Vazvani *et al.,* 2017).

Plant materials

About 1000 genotypes of bread wheat *Triticum aestivum* L. (collected and received from different locations of Iran and other countries) were planted in one line in the field at Vali-e-Asr University of Rafsanjan. A single plant was selected from each line then these genotypes were screened for resistance and susceptibility to take-all (T-41 isolate) in the greenhouse (Gholizadeh-Vazvani *et al.,* 2017, 2015).Thirty genotypes of this germplasm (including VRU1553, VRU1622, VRU1528, VRU134, VRU2056, VRU1879, VRU452, VRU462, VRU2109, VRU2156, VRU2182, VRU1530, VRU8033, VRU1474, VRU565, VRU414, VRU2161-1, VRU2149, VRU905, VRU3787, VRU9058, VRU1557, VRU2002, VRU9019, VRU510, VRU591, VRU690, VRU8039, VRU705, and VRU1554) were selected and used in this research.

Greenhouse experiments

A suitable sieved soil (EC = 1.2-2 dsm^{-1}, pH = 7.5-8) was autoclaved at 121 °C for one hour. Seeds were disinfected in a 1% sodium hypochlorite solution for one minute and then planted in pots containing 800 g of soil in the greenhouse. Inoculation was performed 14 days after planting. Four grams of inoculum were dumped close to the plant's crown and covered with sand. Greenhouse temperature ranged from 20-25 °C. Seven weeks after inoculation, the percentage of the blackened crown was measured and recorded. Infection levels based on the percentage of necrosis in the roots and crowns were scored on a scale of 0 to 5 (Ownley *et al.,* 2003). Disease severity (DS) was calculated according to the formula (Sum of scores in each pot/5 × number of plants).

0 = roots and crowns without necrotic spots: 1 = roots with one or more necrotic spots and crowns without symptoms: 2 = roots with continuous necrotic spots (more than 25% and less than 50% necrosis of roots) and crowns without symptoms: 3 = more than 50% necrosis of the roots and blackened crowns: 4 = roots approximately black with 75% blackened crowns: 5 = blackened and dried roots and crowns.

Field experiments

The above 30 genotypes were planted, and trials were conducted during two growing seasons (2018-2019 and 2019-2020) in the farm at Vali-e-Asr University of Rafsanjan. Each genotype was planted in a row. Three individual plants were selected from each row, and five agronomic traits, including plant height, main spike length, the weight of seeds per spike, the number of seeds per spike, the number of spikelets, and 1000 kernel weight, were determined. Field experiments were repeated twice, and data averages were used in this study. The data were analyzed in a completely randomized design with three replications.

DNA extraction

Genomic DNA was extracted from young leaves using the standard CTAB method proposed by Doyle and Doyle (1990) with

minor alterations. Add 1 µl of RNAase and complete the volume up to 51 µl with sterile ddH_2O. The purity and quantity of the isolated DNA were evaluated by determining the spectrophotometric absorbance of the samples at 230, 260, and 280 nm using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific). The isolated genomic DNA (5 µl) was loaded on 0.8% agarose gel stained with DNA-safe stain dye (0.025 $\mu l \cdot mL^{-1}$) to check DNA quality. Samples were subjected to electrophoresis in TBE1X buffer for 1 hour at 80 V. The gels were photographed under a Gel Documentation system.

The molecular markers used in this study

The SSR technique was used to identify promising resistant loci with a total of 12 pairs of markers (Dena Zist Company, Mashhad, Iran). Also, in this study, three rye specific primers (RyeR3/F3, PAW161, and O-SEC5'-A/O-SEC3'-R, (Dena Zist company, Mashhad, Iran)) were applied to investigate the distribution of 1RS arms in 30 genotypes with the positive control (Rasad cultivar) and negative control (Chinese Spring) to verify the results. The chromosomal location, annealing temperature, and primer sequences to SSR markers and specific Rye primers are shown in Table 1.

Table 1 Primers sequence for SSR marker and specific Rye primers.

Marker	Sequence (5′→3′)	chromosomal location	Reference
xbarc244	F:GCGAAGAATTAATAAACAAGGTACATGATA	7D	Deng *et al.,* 2017
	R:CCGTCTCAAATTTACACCGCTATATG		
gpw95001	F:TTCTTCTCCCCTCCAACCTT	4D	Nguyen *et al.,* 2011
	R:GTGTCTTTGTGGATGCTTATGATCTCCC		
xbarc056	F:GCGGGAATTTACGGGAAGTCAAGAA	5A	Lin *et al.,* 2006
	R:GCGAGTGGTTCAAATTTATGTCTGT		
xbarc232	F:CGCATCCAACCATCCCCACCCAACA	5A, 5B, 5D	Alam *et al.,* 2013
	R:CGCAGTAGATCCACCACCCCGCCAGA		
xbarc144	F:GCGTTTTAGGTGGACGACATAGATAGA	5D	Miranda *et al.,* 2006
	R:GCGCCACGGGCATTTCTCATAC		
gpw95024	F:AATTCAGTCCACACGCCC	6B	Båga *et al.,* 2007
	R:GTGTCTTAGCAGACATCAGAGCGGG		
xbarc246	F:GCGTAATGTCCCCTTCCTTCTGGT	4A	McCartney *et al.,* 2016
	R:GCGATATTCTAGGCTTGTGCGACTTGAG		
xbarc124	F:TGCACCCCTTCCAAATCT	2A	Alam *et al.,* 2013
	R:TGCGAGTCGTGTGGTTGT		
Xbarc3	F: TTCCCTGTGTCTTTCTAATTTTTTTT	6A	Lowe *et al.*, 2011
	R: GCGAACTCCCGAACATTTTTAT		
Xbarc253	F: GGGAAGACACGACACGACTC	7A	Rehman Arif *et al.,* 2020
	R: TCGTAAGATTACCTCGGATGAAGAA		
Xbarc249	F: GCGTGTGTAAGATCGGACCAAAGAGAG	-	-
	R: GTGGCAGAATATTTGATCAGTAGTT		
Xbarc233	F: GCGTCCTAGTTCTCAAATTGCCCCGTCA	4A	Song *et al.,* 2005
	R: CGCTTTCCTCCTCGCCCTTCCAC		
RyeR3/F3	F:GATCGCCTCTTTTGCCAAGA	Translocation wheat-rye	Weng *et al.,* 2007
	R:TCACTGATCACAAGAGCTTG		
O-SEC5/A	F:CTATTAGTTCGAAAAGCTTATGA	1BL.1RS	Yediay *et al.,* 2010
	R:GCATATGACTCAAATTATTTTTT		
PAW161	F:TGAGGGCCCAGACGGCCCTTTTTG	Translocation wheat-rye	Tabibzadeh *et al.,* 2013
	R:TTATCGCAATTACAACTCAAATTT		

PCR amplification reaction for SSR markers and specific primers

PCR was performed with 10 µl reaction mixtures including 5 µl master mix (10X assay buffer, $MgCl_2$, dNTPs 10 mm total, 5 $U \cdot \mu l^{-1}$ Taq-DNA polymerase) (Dena Zist company, Mashhad, Iran), 3 µl sterile ddH_2O, and 3 µl genomic DNA (30 $ng \cdot \mu l^{-1}$) for SSR markers and 1 µl genomic DNA (30 $ng \cdot \mu l^{-1}$) for specific primers. The PCR products were electrophoresed on 2.5% (for SSR markers) and 1.5% (for specific primers) agarose gels in 1X tris borate EDTA buffer stained with DNA safe stain dye (0.025 $\mu l \cdot ml^{-1}$) by horizontal gel electrophoresis at 110 V for 3 h, then band profiles were visualized under ultraviolet light and photographed with gel documentation. The size of the amplified products was evaluated by comparing them with a 50 and 100 base pair as the molecular size standard (Dena Zist Company, Mashhad, Iran). The PCR conditions shown in Table 2.

Statistical analysis

Molecular data were scored as presence (1) or absence (0) of amplified bands (Nei and Li 1979). Polymorphism was determined using polymorphism information content (PIC) values, resolving power (Rp), and effective multiple ratios (Powell *et al.*, 1996). The genetic similarity and dissimilarity coefficient among the wheat genotypes was calculated using a simple matching coefficient, and clustering analysis was done with the NTSYS-pc 2.20 software (Rohlf, 1993). The data matrix was then subjected to analysis of molecular variance (AMOVA) to partition the genetic variation between and within the populations using GenALEx software with 999 permutations (Peakall and Smouse, 2006). Principal coordinate analysis (PCoA) and scores for the first and second components were also plotted. Simple correspondence analysis in Minitab software was used to investigate the relationship between molecular markers, agronomic traits, and disease severity using correlation and $\chi 2$ statistics.

Results

Disease severity and agronomic traits

Differences ($P \leq 0.001$) were observed on the disease severity between different genotypes in the analysis of variance (Additional file 1). The highest values of disease severity were observed in genotypes: VRU705, VRU1554 (100%), VRU690, VRU591, VRU510, VRU8039 (93%), VRU9019 (88%), VRU2002 (86%), VRU1557 (78%), and VRU9058 (70%) that are considered (Susceptible group). Intermediate values were recorded for "VRU3787 (50%), VRU905 (47%), VRU2149 (45%), VRU2161-1 (43%), VRU414 (37%), and VRU565 (35%)" that are named (Moderately susceptible group), and for genotypes "VRU1474, VRU8033 (23%) VRU1530, VRU2182, VRU2156 (20%) and VRU2109 (13%)" (Moderately resistant group). While the genotypes VRU134, VRU1528, VRU1622, VRU1553, and VRU2056 did not show any visible disease symptom, so they did not present disease value. The genotypes VRU462, VRU452, and VRU1879 with 11%, 11%, and 7% disease severity were named (Resistant group) (Table 3). Single trait ANOVA (Additional file 2) and mean comparison showed that plant height ranged from 33-103.33 cm. The genotype VRU705 showed minimum plant height (33 cm), whereas genotypes VRU2156 (103.331 cm) and VRU452 (102.66 cm) showed maximum plant height. Data showed that spike length ranged from 7-15 cm. The genotype VRU905 showed minimum spike length (7cm), whereas genotype VRU452 and VRU2156 with 15 cm and 14 cm showed maximum spike length. Spikelet numbers ranged from 11.6 (VRU1554) to 23.6 (VRU452). The results showed that the number of seeds per spike ranged from 21.33 (for VRU705 and VRU565) to 75 (for VRU1622). Seed weight per panicle ranged from 0.8 gr (VRU705) to 2.96 (VRU1622). Chlorophyll index ranged from 36.33 for VRU690 to 60.33 for VRU8033. Statistical differences between other genotypes are shown in Table 3.

Table 2 The PCR conditions for markers in this research.

Markers	PCR conditions
SSR	94 °C: 3 min, 35 cycles 94 °C: 30 s for denaturation, 50-65 °C: 30 s for annealing, 72 °C: 40 s for extension and 72 °C: 5 min for the final extension
PAW161	95 °C: 3 min, 30 cycles 94 °C: 45 s for denaturation, 60 °C: 1 min for annealing, 72 °C: 90 s for extension and 72 °C: 5 min for the final extension
O-SEC5A	95 °C: 5 min, 35 cycles 94 °C: 1 min for denaturation, 50 °C: 1 min for annealing, 72°C: 3 min for extension and 72 °C: 10 min for the final extension
RyeR3/F3	94 °C: 3 min, 35 cycles 94 °C: 1 min for denaturation, 55 °C: 1 min for annealing, 72 °C: 3 min for extension and 72 °C: 7 min for the final extension

Table 3 Mean comparisons of disease severity and agronomic traits between genotypes.

Genotypes	Disease severity[1]	Plant height (cm)[2]	Spad[2]	Spike length (cm)[2]	Spikelet number[2]	Number of seeds per spike[2]	Seed weight per panicle (g)[2]
VRU134	0 l	68.00g-i	59.66a	11.00e-i	21.33a-c	60.67b	2.18b-d
VRU1474	0.253 i	71.00g	58.67a	13.00bd	19.00a-f	54.67bc	1.68e-g
VRU1528	0 l	95.33ab	48.70a-f	9.00j-m	17.00b-f	24.33mn	1.28h-k
VRU1530	0.206 i	84.33cd	54.33a-d	10.66f-j	20.67a-d	43.00e-g	1.92de
VRU1553	0 l	82.33c-e	58.33ab	12.66b-e	18.67b-f	35.33h-k	1.54f-i
VRU1554	1.000 a	80.66d-f	48.00a-f	7.66l-n	11.67h	23.33mn	1.55f-h
VRU1557	0.780 c	82.33c-e	43.60b-f	10.66f-j	14.67f-h	40.30e-j	2.22b-d
VRU1622	0 l	81.00def	41.00d-f	12.66b-e	21.67ab	75.00a	2.96a
VRU1879	0.070 kl	60.66h-k	51.60a-e	7.66l-n	15.33e-h	42.00e-h	1.99c-e
VRU2002	0.860 b	68.33gh	46.00a-f	10.66f-j	15.33e-h	38.30f-j	1.914de
VRU2056	0 l	71.33g	41.00d-f	7.33mn	13.00gh	33.33j-l	1.21i-l
VRU2109	0.133 jk	73.33fg	57.00a-c	11.33d-f	15.33e-h	23.66mn	1.03k-m
VRU2149	0.450 ef	80.66d-f	46.60a-f	11.33d-h	18.67b-f	37.00g-k	1.71e-g
VRU2156	0.200 ij	103.33a	55.33a-d	14.00ab	20.66a-d	52.00cd	2.47b
VRU2161	0.430 fg	89.00bcd	46.67a-f	10.67f-j	16.66c-g	46.00de	1.92e-d
VRU2182	0.203 ij	70.66g	47.67a-f	11.67d-g	20.00a-e	46.33de	1.74ef
VRU3787	0.506 e	53.33kl	41.33d-f	8.33k-n	12.66gh	38.33f-j	0.90lm
VRU414	0.370 gh	54.00kl	54.33a-d	12.00c-f	14.66f-h	41.00e-i	1.77ef
VRU452	0.083 k	101.66a	59.33a	15.00a	23.66a	54.66bc	2.81a
VRU462	0.110 k	84.33cd	53.67a-d	7.33mn	12.67gh	38.00f-j	1.35h-k
VRU510	0.916 b	54.33kl	41.30d-f	7.33mn	12.67gh	25.66mn	0.93lm
VRU565	0.356 h	75.33e-g	52.67a-e	9.00j-m	12.67gh	21.33n	1.21j-l
VRU591	0.933 ab	58.66jk	42.33c-f	10.00g-k	15.33e-h	40.66e-j	1.10j-m
VRU690	0.933 ab	47.00lm	36.33f	9.33i-l	12.67gh	38.00f-j	1.17j-l
VRU705	1.000 a	37.00n	42.33c-f	11.00e-i	13.67gh	21.33n	0.80m
VRU8033	0.236 i	85.33cd	60.33a	13.67ac	20.67a-d	43.66e-g	2.21b-d
VRU8039	0.933 ab	66.66g-j	58.33ab	9.67h-k	16.33d-h	29.66k-m	1.4g-j
VRU9019	0.880 b	42.66mn	38.33e-f	8.66k-n	15.00f-h	26.00l-n	1.07j-m
VRU905	0.470 ef	90.00bc	41.67d-f	7.00n	13.33gh	34.33i-k	1.39g-j
VRU9058	0.703 d	59.33i-k	50.00a-f	9.67h-k	15.33e-h	44.66d-f	2.26bc

[1]: in greenhouse, [2]: in the field, [1, 2]: Means followed by the same letters are not significantly different (LSD test, $P < 0.05$).

Cluster analysis based on agronomic traits

Multivariate analysis was done to understand the extent of the similarities and differences among the studied genotypes based on the total studied traits. In cluster analyses, all seven agronomic traits of 30 genotypes were used and grouped into four different categories with a dendrogram slice in 6.73 of Euclidean distance using Ward's method (Fig. 1). Cluster profiles and the average traits of each cluster are shown in Table 4. A high degree of genetic divergence was observed. Cluster I formed the biggest cluster (eight genotypes) and also clustered II (eight genotypes), III (eight genotypes), and IV (six genotypes). In cluster analysis, clusters I and II exhibited less similarity (more variance) than other members of the respective clusters. The members of clusters I and III had high genetic diversity. Maximum distance from the centroid was observed in cluster I (3.12), and this cluster had the highest mean performance for the number of seeds per spike, spikelets per spike, spike length, plant height SPAD, 1000 kernel weight, and seed weight per panicle.

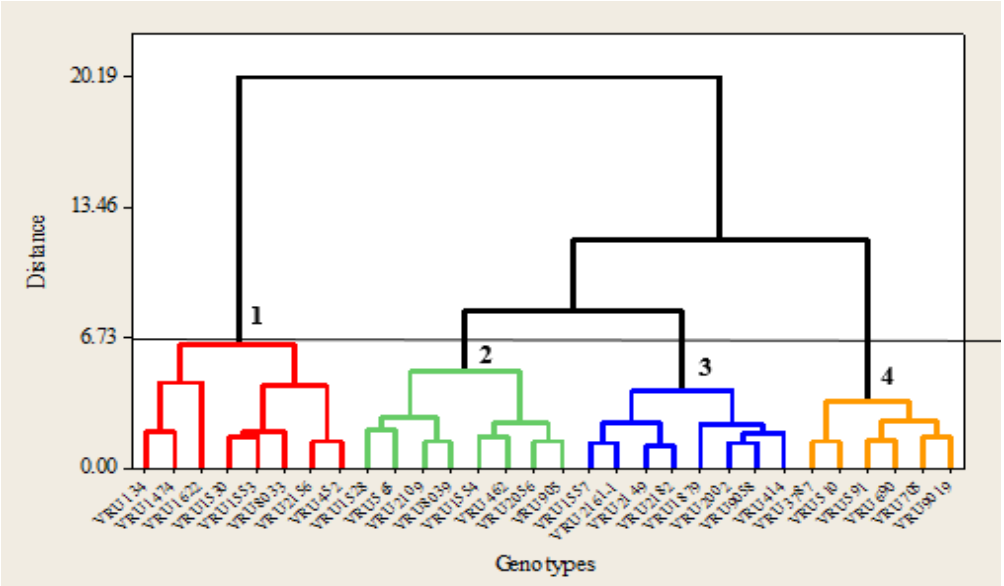

Figure 1 Cluster analysis with Ward linkage grouping and Euclidian distance for classifying genotypes based on agronomic traits.

Association analysis

Pearson correlation and simple corresponding analysis through contingency table were conducted to study the relationship between agronomic traits and disease response (Tables 5 and 6).

Correlations between agronomic traits and disease severity are shown in Table 5. A significant ($p \leq 0.05, 0.01$) and positive correlations were demonstrated among the various attributes. Overall, 28 associations with their correlation coefficients were generated from the 8 quantitative traits. At 5% and 1% probability levels, 17 associations were positively correlated, and six were negatively correlated. All correlations between agronomic traits were positive and significant. Negative correlations were observed between disease severity and agronomic traits. Plant performance should also be considered in wheat breeding programs for disease resistance. Plant yield, along with disease resistance, is an important trait. An inverse relationship was observed between disease level and grain yield. As the severity of the disease increased, performance-related traits decreased (Table 5). The results showed that interactions with related traits to yield probably led to resistance to take-all disease.

Table 4 Profiling of the cluster and the average trait of each cluster.

Entries	Entries	Cluster I	Cluster II	Cluster III	Cluster IV
Profiling of the clusters	Within cluster sum of squares	36.5534	19.0093	14.2831	10.6477
	Average distance from centroid	2.0559	1.5277	1.3065	1.3125
	Maximum distance from centroid	3.1273	1.8148	1.7424	1.5743
Traits	Height	84.6249	79.6249	70.6250	48.8333
	SPAD (chlorophyll index)	55.8750	50.1250	48.3334	40.3333
	Spike length	12.8334	8.5417	10.5417	9.1111
	Spikelet number	20.7917	14.0000	16.3333	13.6667
	Number of seeds per spike	52.3750	28.5000	41.9583	31.6667
	Seed weight per panicle	2.2268	1.3075	1.9453	1.0004
	1000-kernel weight	43.1205	39.4523	41.0320	32.8868

Table 5 Correlation between disease severity and agronomic traits.

Traits	DS	H	SPAD	LS	SP	NS	WS	W1000
DS	1							
H	-0.559**	1						
SPAD	-0.503**	0.443*	1					
LS	-0.319ns	0.342ns	0.527**	1				
SP	-0.548**	0.498**	0.536**	0.810**	1			
NS	-0.448*	0.296ns	0.215*	0.557**	0.709**	1		
WS	-0.403*	0.546**	0.379*	0.613**	0.730**	0.804**	1	
W1000	-0.395*	0.628**	0.400*	0.496**	0.524**	0.135ns	0.531**	1

*, ** and ns: Significant at 0.05 and 0.01 level of probability, and not significant, respectively.
DS: disease severity, H: plant height, SPAD: chlorophyll index, LS: Spike length, SP: number of seed per spike, WS: seed weight per panicle, W1000: 1000-kernel weight**.**

Table 6 The result of the chi-square test for two groups.

Group	Disease severity			
	Susceptible	Moderately susceptible	Moderately resistant	Resistant
1	0 $\chi^2 = 2.667$	0 $\chi^2 = 1.600$	4 $\chi^2 = 3.600$	4 $\chi^2 = 1.633$
2	2 $\chi^2 = 0.167$	2 $\chi^2 = 0.100$	1 $\chi^2 = 0.225$	3 $\chi^2 = 0.352$
3	3 $\chi^2 = 0.042$	3 $\chi^2 = 1.225$	1 $\chi^2 = 0.225$	1 $\chi^2 = 0.602$
4	5 $\chi^2 = 4.500$	1 $\chi^2 = 0.033$	0 $\chi^2 = 1.200$	0 $\chi^2 = 1.600$

χ^2 total = 19.771* > χ^2 table = 16.919, df = 9, p-value = 0.019**.**

Association between the bilateral grouping of individuals based on two criteria (agronomic traits and disease response), was studied by contingency table and chi-square test. For this purpose, we must determine if the two grouping criteria are independent (grouping based on agronomic traits and grouping based on disease response) or no. As stated earlier in the classification based on the disease severity, the genotypes are divided into four groups (resistant, moderately resistant, susceptible, and moderately susceptible). In cluster analysis based on agronomic traits, four clusters were obtained. According to the results of simple correspondence analysis, we reject H0 that says the two groupings criteria are independent because calculated $\chi 2$ (19.771) is significant at 0.019 level of probability (Table 6). The two groupings criteria are not independent and are related to each other.

The relationship among wheat genotypes using SSR markers

No amplified DNA bands were observed in 4 SSR markers (xbarc3, xbarc253, xbarc249, and xbarc233). Among SSR molecular markers, eight markers showed polymorphism (Table 7). Polymorphism information content (PIC) values ranged from 0.087 to 0.50, with an average value of 0.30. The amplified bands per primer ranged from 30 (in xbarc56 and xbarc144) to 201 (in xbarc246), with an average of 10 to 20 bands per primer. The percentage of polymorphic bands across the SSR markers ranged from 33.33% (in gpw95001) to 100% (xbarc244, xbarc232, xbarc144, and xbarc56), with an average of 85.04% polymorphism. The primers xbarc56 (0.42 for diversity index and 0.67 for resolving power), xbarc232 (0.43 for diversity index and 0.53 for resolving power), xbarc 144 (0.22 for

diversity index and 0.53 for resolving power), and gpw95024 (0.3 for diversity index and 0.53 for resolving power) exhibited the highest values. In contrast, gpw95001 (0.02 for diversity index and 0.02 for resolving power) and xbarc 246 (0.15 for diversity index and 0.21 for resolving power) showed the lowest values (Table 7).

A total of 739 scored bands were generated using the SSR markers, similarity matrix based on simple matching and cluster analysis by UPGMA method. These genotypes were grouped into five major clusters at similarity 0.75 (Fig. 2). This grouping shows that the informative alleles associated with take-all disease resistance can properly differentiate genotypes based on phenotype. In addition to showing allelic diversity, these primers showed beneficial results in diversity and dependence of markers on the resistance and susceptibility of genotypes.

Table 7 Number of total bands, number of amplified loci, the average of band in locus, number of polymorphic loci, percent of polymorphic loci, average of diversity index and resolving power for different SSR markers**.**

Marker	Total number of bands	Number of amplified loci	Number of polymorphic loci	The average of band in locus	Percent of polymorphic loci	Average of diversity index	Average of resolving power
gpw95001	61	3	1	20	33	0.02	0.02
gpw95024	123	8	6	15	75	0.30	0.53
xbarc232	102	9	9	11	100	0.43	0.53
xbarc56	30	3	3	10	100	0.42	0.67
xbarc144	30	2	2	15	100	0.36	0.47
xbarc244	42	4	4	10	100	0.22	0.53
xbarc246	201	10	8	20	80	0.15	0.21
xbarc124	150	12	11	12	92	0.26	0.37

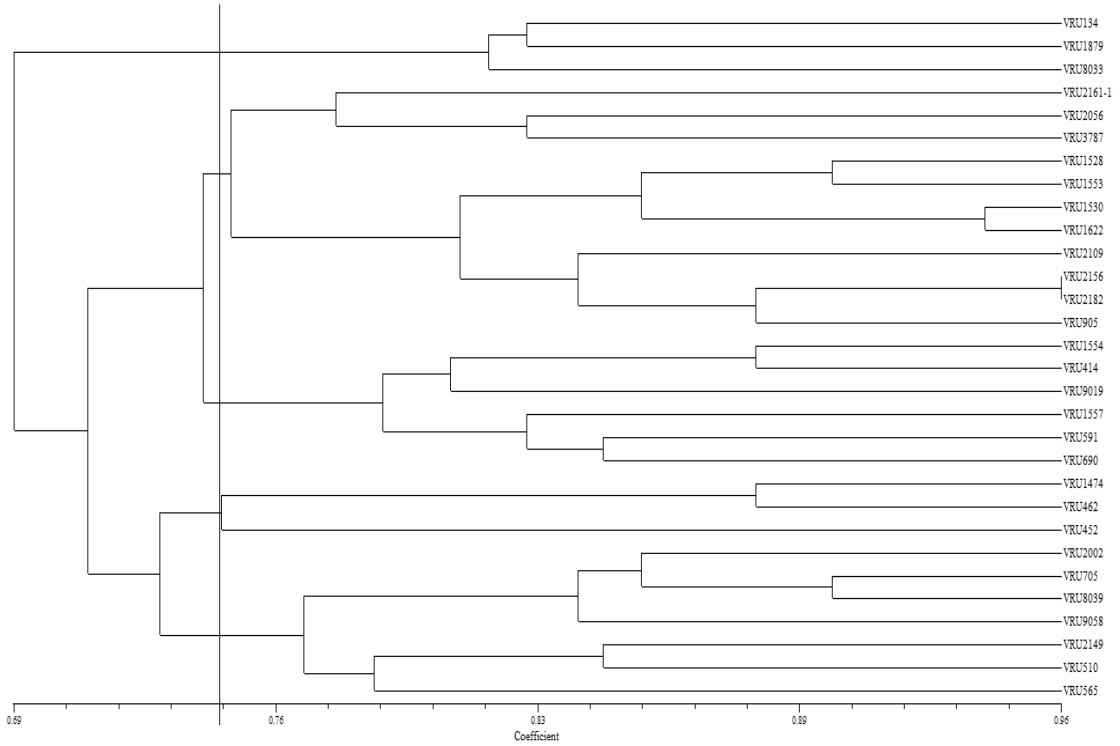

Figure 2 Dendrogram of cluster analysis based on simple matching similarity coefficient and UPGMA method.

Correlation among characters (disease severity and amplified loci)

According to the grouping of genotypes based on SSR markers, resistant genotypes were separated from susceptible genotypes. Accordingly, a good result has been obtained by correlating disease severity and the 51 loci of SSR markers. Only 14 loci of SSR markers showed a significant relationship (At a 0.1, 0.05, and 0.01 probability level) with the disease severity (Table 8). A positive correlation was found between disease severity and xbarc232-1, xbarc232-8, xbarc124-3, xbarc124-10, xbarc124-12, xbarc246-8, xbarc244-2, gpw95024-5, and gpw95024-8 loci (r = 0.575, 0.320, 0.430, 0.601, 0.318, 0.316, 0.383, 0.324, and 0.320 receptivity), indicating that presence of these markers will increase disease severity or increase susceptibility to disease. Negative correlations were found between xbarc232-3, xbarc232-5, xbarc124-2, xbarc124-6, and gpw95024-2 markers and disease severity (-0.434, -0.419, -0.320, -0.420, and -0.403, respectively). These markers indicate resistance to take-all or reduce the disease severity.

Moreover, a negative correlation was found between xbarc232-1 and xbarc232-3 (-0.437*), xbarc232-3 and xbarc124-10 (-0.582*), and gpw95024-1 and xbarc244-2 (-0.392*). It seems that the presence of locus xbarc232-3 (resistance locus) prevents the presence of loci xbarc232-1 and xbarc124-1 (susceptible loci). Gpw95024-2 locus (resistance locus) prevents the presence of the xbarc244-2 locus (susceptible locus) (Table 8). These loci in the identification of genetic loci are probably associated with susceptibility or resistance and may serve to modulate take-all disease in wheat. Figs 3-a, 3-b, and 3-c show agarose gel 2.5% of amplified products using xbarc232, xbarc124, and xbarc246 markers.

Stepwise multiple regression

Stepwise regression was performed on all significant markers using the MINITAB14 to determine the minimum SSR associated with disease resistance. Stepwise regression of SSR molecular markers was performed to identify suitable markers interacting with disease severity. Loci with the lowest p-value in correlations (Table 8) were chosen and then added in a stepwise regression to select the best set of markers associated with the take-all disease. Six loci accounting for 67.38% of the phenotypic variation for disease severity were detected (Table 9).

Table 8 Correlation between disease severity (DS) and amplified loci.

Loci	DS	xbarc232-1	xbarc232-3	xbarc232-5	xbarc232-8	xbarc124-2	xbarc124-3	xbarc124-6	xbarc124-10	xbarc124-12	xbarc246-8	xbarc244-2	gpw95024-2	gpw95024-5	gpw95024-8
DS	1														
xbarc232-1	0.575**	1													
xbarc232-3	-0.434*	-0.437*	1												
xbarc232-5	-0.419*	-0.161^{ns}	0.145^{ns}	1											
xbarc232-8	0.320*	0.086^{ns}	-0.235^{ns}	-0.132^{ns}	1										
xbarc124-2	-0.320*	-0.343*	0.294^{ns}	0.043^{ns}	-0.053^{ns}	1									
xbarc124-3	0.430*	0.375*	-0.641**	-0.066^{ns}	0.081^{ns}	-0.650***	1								
xbarc124-6	-0.420*	-0.223^{ns}	0.075^{ns}	0.230^{ns}	-0.385*	0.650***	-0.318*	1							
xbarc124-10	0.601**	0.426*	-0.582**	-0.270^{ns}	0.308*	-0.171^{ns}	0.428*	-0.099^{ns}	1						
xbarc124-12	0.318*	0.101^{ns}	-0.375*	-0.145^{ns}	0.067^{ns}	0.196^{ns}	0.264^{ns}	0.302^{ns}	0.400*	1					
xbarc246-8	0.316*	0.036^{ns}	-0.134^{ns}	-0.408*	-0.036^{ns}	-0.105^{ns}	-0.141^{ns}	-0.161^{ns}	0.175^{ns}	0.134^{ns}	1				
xbarc244-2	0.383*	0.449*	-0.196^{ns}	0.043^{ns}	0.145^{ns}	-0.154^{ns}	0.237^{ns}	-0.237^{ns}	0.043^{ns}	-0.049^{ns}	-0.105^{ns}	1			
gpw95024-2	-0.403*	-0.067^{ns}	0.167^{ns}	-0.218^{ns}	-0.336*	0^{ns}	-0.151^{ns}	0.302^{ns}	-0.218^{ns}	0^{ns}	0^{ns}	-0.392*	1		
gpw95024-5	0.324*	0.381*	-0.167^{ns}	-0.267^{ns}	0.291^{ns}	-0.131^{ns}	-0.050^{ns}	-0.201^{ns}	0.218^{ns}	0.167^{ns}	0.356*	0.196^{ns}	-0.111^{ns}	1	
gpw95024-8	0.320*	0.396*	-0.301^{ns}	0.029^{ns}	0.144^{ns}	-0.367*	0.262^{ns}	-0.413*	0.175^{ns}	-0.033^{ns}	0.286^{ns}	0.419*	-0.267^{ns}	0.356*	1

*, ** and ^{ns}: Significant at 0.05 and 0.01 level of probability, and no significant, receptivity.

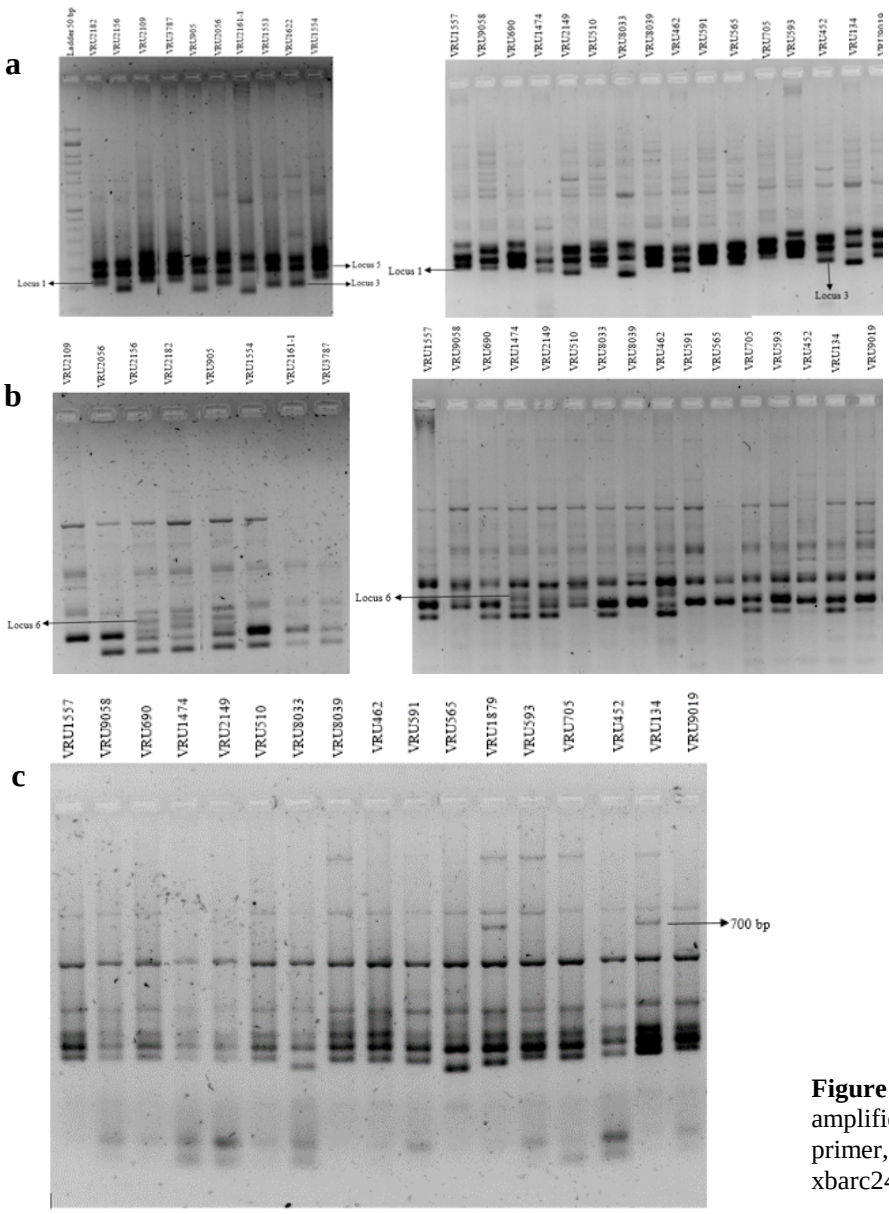

Figure 3 Agarose gel 2.5% of amplified products by (a) xbarc232 primer, (b) xbarc124 primer, (c) xbarc246 primer.

Table 9 Stepwise multiple regression analysis between disease severity as a dependent variable and 14 SSR marker loci as independent variables.

Step	locus	Constant	x_1	x_2	x_3	x_4	x_5	x_6	R-Sq (adj)
1	xbarc124-10 (x_1)	0.105	0.47^{**}						33.80
2	xbarc124-6 (x_2)	0.204	0.44^{**}	-0.29^{*}					45.46
3	xbarc232-1 (x_3)	0.162	0.34^{**}	-0.25^{*}	0.232^{*}				52.45
4	Xbarc124-12 (x_4)	0.048	0.24^{*}	-0.33^{**}	0.234^{*}	0.25			57.23
5	gpw95024-2 (x_5)	0.135	0.20	-0.27^{*}	0.250^{*}	0.25^{*}	-0.159^{ns}		60.60
6	xbarc232-5 (x_6)	0.392	0.14^{ns}	-0.18^{ns}	0.255^{**}	0.21^{ns}	-0.242^{*}	-0.235^{*}	67.38

Disease severity = $0.14x_1 - 0.18x_2 + 0.255x_3 + 0.21x_4 - 0.242x_5 - 0.235x_6$.

*, ** and ^{ns}: Significant at 0.05 and 0.01 level of probability, and not significant, receptivity.

Molecular markers (gpw95024 and xbarc232) are associated with resistance to take-all in bread wheat. Gpw95024 SSR marker and locus 5 xbarc232 SSR marker located on chromosome 6B and 5B were significantly associated with resistance to take-all and fit into a stepwise regression. Locus 1 in xbarc232 marker is interaction with susceptible to take-all disease. Probably this locus is an indirect interaction effect through other loci.

This genetic analysis revealed that multiple loci control the trait, and there are distinct genetic sources of resistance to take-all disease in different genotypes. Generally, five primer pairs and 14 loci (xbarc232, xbarc124, xbarc244, xbarc246, and gpw95024) were polymorphic between the resistant and susceptible genotypes. Xbarc232 had detected loci on chromosome arms 5AL, 5BL, and 5DL; xbarc124 had detected loci on chromosome arm 2A; gpw95024 had loci detected on chromosome arm 6B, and xbarc246 had detected loci on chromosome arm 4A.

Clustering of wheat genotypes based on 14 selected marker loci

To investigate the relationship between the diversity of molecular markers and the disease severity, cluster analysis was performed based on these 14 markers. In the complete cluster analysis based on genetic similarity using the Jaccard coefficient and UPGMA method, 30 wheat genotypes were grouped into four genetic clusters (Fig. 4).

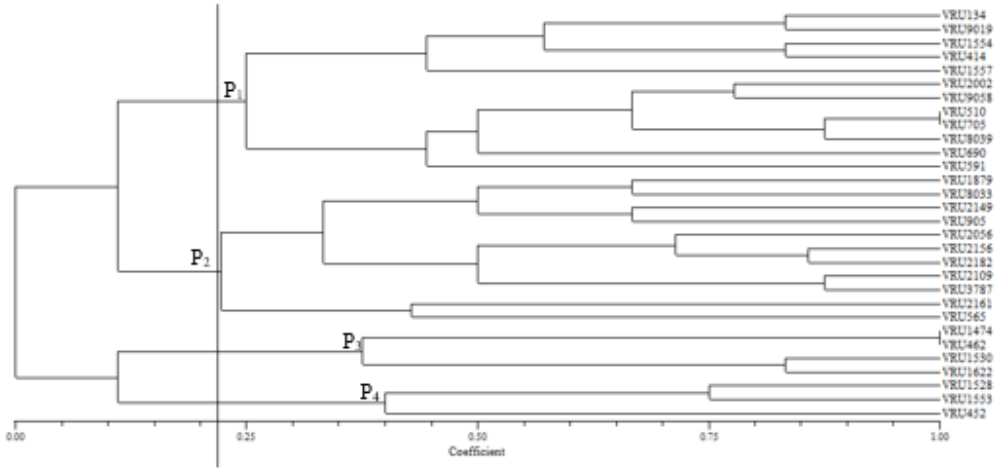

Figure 4 Dendrogram of genotypes based on 14 loci selected from the result of correlation, with Jaccard coefficient and complete method.

AMOVA (analysis of molecular variance) was performed in model-based populations (Table 10). AMOVA has justified and confirmed our cutting location to produce four clusters so that the genetic difference between the clusters was significant at the 0.01 level. The genetic distance obtained between the four populations of bread wheat varied from 0.148 to 0.445 (Table 11). PCoA, showed that the two extracted components together accounted for 93% of the variance in the data matrix and separated the four clusters well (Fig. 5).

Corresponding analysis

To show the relationship between disease response and genetic diversity of genotypes derived from molecular markers, the association between the bilateral grouping of individuals based on two criteria (molecular genetic diversity in cluster analysis and disease response), a simple corresponding analysis was conducted (MINITAB software) using contingency table and chi-square test (Table 12). The results showed that the p-value of the table is less than $p < 0.001$, the null hypothesis is

rejected, and the alternate hypothesis is accepted. So there is a significant relationship at the level of 0.001 between these two groups, and they aren't independent. Therefore these 14 markers used in cluster analysis are most likely related to the disease response of genotypes and explain the most variability in disease severity.

Specific primers

Only six genotypes (4 resistance and 2 susceptible) showed translocation wheat-rye based on specific primers. The 1450 bp amplified fragment (in RYER3/F3 primer) was sequenced in the VRU1879 resistance genotype. The result of the blast showed that the genotype VRU1879 sequence revealed high sequence similarity with *T. aestivum* (Falat) and Rye species (retrotransposon gypsy-like) (additional file 3). These results were not reported in this research because resistance to take-all disease was not demonstrated with these primers.

Table 10 Analysis of molecular variance (AMOVA) for 14 loci SSR markers for 4 clusters.

Source	df	SS	MS	Est. Var.	%	F
Among Pops	3	29.126	9.709	1.171	40%	4.64**
Within Pops	26	46.008	1.770	1.770	60%	
Total	29	75.133		2.941	100%	

**: Significant at 0.01 level.

Table 11 Pairwise population matrix of Nei genetic distance.

Population	P1	P2	P3	P4
P1	0.000			
P2	0.148	0.000		
P3	0.421	0.334	0.000	
P4	0.445	0.230	0.377	0.000

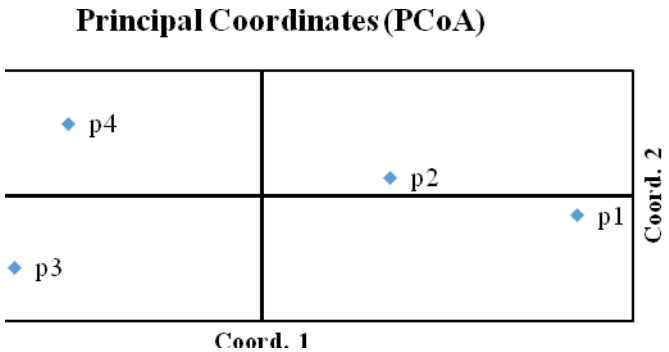

Figure 5 Scatter plot of 4 populations based on first and second components of principal coordinate analysis using SSR data. **P_1:** VRU591, VRU690, VRU8039, VRU705, VRU510, VRU9058, VRU2002, VRU1557, VRU414, VRU1554, VRU9019, VRU134; **P_2:** VRU565, VRU2161, VRU3787, VRU2109, VRU2182, VRU2156, VRU2056, VRU905, VRU2149, VRU8033, VRU1879; **P_3:** VRU1474, VRU462, VRU1530, VRU1622; **P_4:** VRU1528, VRU1553, VRU452.

Table 12 Cluster analysis of 14 loci for two grouping criteria.

Group	Disease severity			
	Susceptible	Moderately Susceptible	Moderately Resistant	Resistant
1	10	1	0	1
	$\chi^2 = 9$	$\chi^2 = 0.817$	$\chi^2 = 2.4$	$\chi^2 = 1.513$
2	0	5	4	2
	$\chi^2 = 3.667$	$\chi^2 = 3.564$	$\chi^2 = 1.473$	$\chi^2 = 0.297$
3	0	0	2	2
	$\chi^2 = 1.333$	$\chi^2 = 0.8$	$\chi^2 = 1.8$	$\chi^2 = 0.817$
4	0	0	0	3
	$\chi^2 = 1$	$\chi^2 = 0.6$	$\chi^2 = 0.6$	$\chi^2 = 6.050$

χ^2 total = 35.729*** > χ^2 table = 21.66, df = 9, p-value = 0.001.

Discussion

Genetic diversity in plant resistance to disease is essential in plants' disease control and management. Before evaluating genetic diversity related to disease resistance, it is necessary to identify cultivars that are resistant and susceptible to the disease by phenotypic methods and evaluated by agronomic traits. The difference between the cultivars in disease severity is due to potential differences between the pathogen's penetration and development. It can also be attributed to differences in the root system of the plant. When a pathogen attacks a plant, a set of biochemical reactions are activated in the plant, including pathogenic-related proteins and associated proteins with cell wall thickness; the expression of this group of proteins causes resistance to take-all disease (Gholizadeh-Vazvani *et al.,* 2015, 2016; Saberi Riseh *et al.,* 2021). According to the functional annotation of genes, two protein genes can improve the systematic resistance of plant roots to take-all disease (Sheng-Sheng *et al.,* 2020). In other research, in the wheat root, transcriptional responses against *Gaeumannomyces graminis* var. *tritici* determined that 75 DEGs are involved in cell wall reorganization, and 23 DEGs are pathogenesis-related proteins; these genes may play roles in wheat resistance against *Ggt* (Zhang *et al.,* 2020). Genotypes that performed better could be used in wheat breeding programs. As in one study, several wheat lines in field and greenhouse conditions were examined for rust disease. Comparing the mean of traits for different lines identified several lines as resistant and sensitive. The correlation coefficient of traits with disease severity was also significant. Based on these assessments, these lines were suggested to be used more in wheat breeding programs (Afshari, 2012).

In one research, cluster analysis was done on agronomic traits using Ward's method, 35 bread wheat genotypes were classified into four groups (Babaei-Zarch et al., 2013). In cluster analyses, all seven agronomic traits of 30 genotypes were used and grouped into four different categories with a dendrogram slice in 6.73 of Euclidean distance using Ward's method. The members of cluster I had a lower disease severity (12%) compared to other clusters II, III, and IV (37%, 48%, and 85%, respectively). In other reports, genotypes with different degrees of resistance to leaf rust disease (susceptible, partially resistant, and resistant) were evaluated to determine the role of plant resistance in the adult stage in reducing yield loss. These genotypes were selected to determine the effect of leaf rust infection on grain yield using 1000-kernel weight (Draz *et al.,* 2015).

According to our results, agronomic traits (measured in the field) are influenced by disease severity. The genes that control plant yield (increase in spikelet number, 1000-seed weight, height) may be associated with the gene for resistance to take-all disease in wheat. Probably resistance to take-all disease is the result of genes interaction. Gene interaction includes the different phenotypic ratios. Previous research showed that the amount of Fe in seed grain in infected treatments with *Gaeumannomyces graminis* var. tritici under field conditions was significantly increased compared to the control.

Genotypes VRU485, VRU1528, VRU501, VRU8031, and VRU585 were better than the others considering the traits mentioned above in the infected treatment.

The disease index measured in the greenhouse had a negative correlation with plant height, seed weight per spike, flag leaf area, 100-kernel weight, and amount of grain iron under infected conditions in the field, indicating that greenhouse evaluations can be used to select genotypes tolerant to take-all disease (Gholizadeh-Vazvani et al., 2016). Having a sound knowledge of the relationship between plant agronomic traits and resistance to disease can effectively be applied in a breeding program. Resistant genotypes are the most economical and effective means of reducing yield loss caused by disease. However, breeding genotypes for disease resistance is a continuous process, and plant breeders need to

add new effective sources to their breeding materials (Draz *et al.,* 2015). Spike properties influence grain yield, and the spikelet number plays a very important role in the wheat grain yield (Sabaghnia *et al.,* 2015). Genetic variability for agronomic traits in response to biotic and abiotic stress is necessary for plant germplasm to adapt to the environment.

Statical methods and multivariate analysis can assist wheat breeders in selecting plants for Help to identify traits related to increased grain yield (Arain et al., 2018). The interaction between biotic and abiotic stress resistance and selection for plant productivity, high yield, and quality is very important (Summers and Brown, 2013). Any new disease resistance needs to be selected within a genetic background that meets current requirements for yield, quality, agronomy, and resistance to other diseases and stresses (Summers and Brown, 2013).

We showed that resistant and susceptible genotypes are genetically different, and the correlation between disease severity and markers is negative. The negative correlation between disease severity of take-all and agronomic traits obtained in this study indicates that susceptible genotypes genetically have a low yield, and resistance to take-all disease is probably associated with functional genes. Another research showed that resistance to the *septoria tritici* was correlated with some of the agronomic traits measured in an experimental field. The number of seeds per spike and 1000-grain weight was inversely correlated with the disease(Kidane *et al.,* 2017). In another study, markers related to resistance to *Sclerotnia Scleritiorum* in sunflower (*Helianthus annuus* L.) were identified using association analysis. In this study (Paknia *et al.,* 2018), contamination progress, 100 seeds weight, plant yield was investigated. Molecular analysis was performed with 30 pairs of SSR markers. Findings identified 5 resistant lines. Three markers were identified with resistance to this disease (Paknia *et al.,* 2018).

The correlation between molecular markers and important traits such as disease resistance and qualitative and quantitative traits was examined in marker selection. This method will help identify plants carrying the target genes simultaneously without exposure to pathogens in early generations. The selected markers from the stepwise regression explain the most phenotypic variation, similar to the variation explained by all markers considered together for each trait (Mamidi *et al.,* 2014, 2015).

Stepwise regression allows the selection of markers from major QTL and makes it easy to choose a subset of markers to use in marker-assisted selection (Mamidi *et al.,* 2014, 2015). When the loci were tested by regression analysis with disease severity, three primer pairs, xbarc232-3 and xbarc124-6, were located in resistance genotypes. In research, genetic diversity concerning Fusarium head blight (FHB) resistance was investigated among 295 winter wheat cultivars and advanced breeding lines using 47 wheat SSR markers. A total of 404 SSR alleles were detected. The number of alleles per locus ranged from 2 to 21. Association analysis between SSR markers and the FHB disease traits detected markers significantly associated with FHB resistance that these results will assist in the selection of parental lines to increase the efficiency of breeding efforts for FHB resistance (Zwart *et al.,* 2008). The number of resistance alleles in the detected QTL-tagging SNPs was significantly correlated with both adult plant resistance and seedling resistance against powdery mildew in wheat (-0.816 and 0.810, respectively); wheat accessions with more favorable alleles showed stronger powdery mildew resistance (Simeone *et al.,* 2020). Xbarc246 could amplify a specific fragment of 700 bp for VRU134 and VRU1879 (resistance genotype).

The SSR markers used in this research were placed on chromosome arms 5BL, 6B, 4A, and 2A, suggesting resistance genes might be located on the long arm of these chromosomes. In research on identifying genetic loci with resistance to take-all disease, with 384 pairs of simple sequence repeats, it was determined that four markers (xwmc388, xbarc369, xwmc565, and xbarc197) successfully produced specific

bands in resistance accession. Using the Chinese spring reference genome sequence, 62 genes were found on wheat chromosome 2A (Sheng-Sheng *et al.*, 2020). The xbarc232 marker was for spot blotch resistance (Mishra *et al.*, 2014), and the xbarc124 marker was for Fusarium head blight (Wang *et al.*, 2015; Dai *et al.*, 2017). The genetic model analysis showed that take-all resistance was controlled by two major genes with additive, dominant, and epistasis effects (Dashti *et al.*, 2018; Sheng-Sheng *et al.*, 2020). Exploiting genetically diverse genotypes to identify genes for improving crop performance is needed to ensure global food security (Bhatta *et al.*, 2019).

Conclusion

One primary goal of this research was to identify bread wheat genotypes accessions possessing resistance to take-all disease and study the association of SSR markers and agronomic traits with disease severity. A significant association between take-all disease severity and SSR markers was found in this germplasm. The selection of these marker loci leads to identifying QTL regions in the wheat genome. These results add significant information to our knowledge of the chromosomal location of genes for the take-all disease. Therefore agronomic traits and SSR markers were associated with resistance QTLs to take-all. According to these results, genes for take-all resistance probably have been located on B chromosome.

The best way to identify and isolate cultivars with different levels of disease tolerance is to study the differences in DNA levels and to identify markers that are consistent with trait control genes. An important statistical technique can assist wheat breeders in selecting crop plants for increased grain yield. In this research, the chi-square test and correlations between disease severity and agronomic traits were used to show that resistance to take-all disease is interactive with agronomic traits. Consecutive advances in yield in new cultivars are required to feed an ever-increasing population. The most important interactive markers with resistance to take-all disease were xbarc232, xbarc124, and gpw95024. These markers are probably linked to take-all disease. Moreover, most resistant genotypes (VRU1622, VRU1528, VRU8033, VRU1553, VRU2056, VRU2182, VRU1879, and VRU134) with different SSR markers are polymorphic. In this study, we identified loci associated with resistance to take-all disease. The next steps are to sequence these loci, identify the control genes, identify the proteins encoding these loci, and finally identify the location of these loci on the chromosomes in the RIL population. According to the results, genes are involved in resistance to take-all disease and are likely to lead to resistance by direct or indirect effects.

Declarations

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Conflicts of interest: The authors declare no conflict of interest.
Availability of data and material: The authors declare these data are not in other papers, and they agree to publish these data in this paper.
Author contribution: The authors declare to contribute to this article.
Ethics approval: All author agrees to have participated in this article.
Consent to participate: The authors declare consent for publication of this article.
Consent to publish: The authors declare consent for publication of this article.

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ارتباط بین صفات زراعی و نشانگرهای مولکولی با شدت بیماری پاخوره در گندم نان *Triticum aestivum*

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چکیده: شناسایی ژنوتیپ‌های مقاوم برای کنترل بیماری پاخوره *Gaeumannomyces graminis* var. *tritici* ضروری می‌باشد. در این مطالعه ۳۰ ژنوتیپ گندم نان در شرایط مزرعه و گلخانه ارزیابی شدند. ژنوتیپ‌ها با ۱۵ مارکر مولکولی (SSR و پرایمرهای اختصاصی برای شناسایی جابه‌جایی کروموزومی گندم-چاودار) ارزیابی شدند. براساس میانگین شدت بیماری در گلخانه و میانگین صفات زراعی در مزرعه، ژنوتیپ‌ها به ۴ گروه تقسیم شدند و تجزیه کای مربع رابطه بین این دو شاخص را تأیید نمود. هم‌بستگی بین شدت بیماری و صفات زراعی نشان داد که مقاومت گیاه به‌شدت به‌عملکرد گیاه وابسته است. براساس تجزیه خوشه‌ای براساس مارکرهای مولکولی، ژنوتیپ‌ها به گروه‌های مقاوم و حساس تقسیم‌بندی شدند و هم‌بستگی بین شدت بیماری و لوکوس‌های تکثیرشده نشان داد که مقاومت به بیماری با مارکرهای xbarc232، xbarc144 و gpw9001 در ارتباط می‌باشد. با توجه به نتایج می‌توان احتمال داد که مقاومت به بیماری پاخوره با چندین ژن در ارتباط است. نتایج این مطالعه اطلاعات مهمی را به دانش ما در رابطه با مکان کروموزومی ژن‌ها به مقاومت به بیماری پاخوره می‌افزاید.

واژگان کلیدی: کای‌مربع، تجزیه خوشه‌ای، شدت بیماری، ژن مقاومت، پاخوره