applied sciences

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Genome-Wide Association Study Identifies Two Loci for Stripe Rust Resistance in a Durum Wheat Panel from Iran

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Abstract: Stripe rust (*Puccinia striiformis* f. sp. *tritici* (*Pst*)) is one of the most devastating fungal diseases of durum wheat (*Triticum turgidum* L. var. *durum* Desf.). Races of *Pst* with new virulence combinations are emerging more regularly on wheat-growing continents, which challenges wheat breeding for resistance. This study aimed to identify and characterize resistance to *Pst* races based on a genome-wide association study. GWAS is an approach to analyze the associations between a genome-wide set of single-nucleotide polymorphisms (SNPs) and target phenotypic traits. A total of 139 durum wheat accessions from Iran were evaluated at the seedling stage against isolates *Pstv-37* and *Pstv-40* of *Pst* and then genotyped using a 15K SNP chip. In total, 230 significant associations were identified across 14 chromosomes, of which 30 were associated with resistance to both isolates. Furthermore, 17 durum wheat landraces showed an immune response against both *Pst* isolates. The SNP markers and resistant accessions identified in this study may be useful in programs breeding durum wheat for stripe rust resistance.

Keywords: durum wheat; stripe rust; *Pstv-37* and *Pstv-40* isolates; GWAS; SNP markers

Citation: Mehrabi, A.A.; Steffenson, B.J.; Pour-Aboughadareh, A.; Matny, O.; Rahmatov, M. Genome-Wide Association Study Identifies Two Loci for Stripe Rust Resistance in a Durum Wheat Panel from Iran. *Appl. Sci.* **2022**, *12*, 4963. https://doi.org/10.3390/app12104963

Academic Editor: Alexios Polidoros

Received: 6 March 2022
Accepted: 25 March 2022
Published: 13 May 2022

1. Introduction

Based on estimation by UN-FAO, the global demand for agricultural products will increase by 50% by 2050 [1]. Meeting this challenge will require significant improvement in the rates of genetic gain in grain yield for cereal crops such as wheat, rice, barley, and maize, as well as the development of new cultivars that adapted to different environments and their stresses. Undoubtedly, wheat is one of the key cereals that supplies the main portion of food demand worldwide.

Durum wheat (*Triticum turgidum* L. var. *durum* Desf.), with a genomic constitution of AABB, is an economically important tetraploid cereal crop widely cultivated in the Mediterranean basin with a yearly production average of 40 million tonnes [2]. According to the International Grains Council [3], durum wheat comprises 5% of total wheat production over a cultivation area of 16 million hectares worldwide [3]. Bread and durum wheat together account for about 20% of calories and protein consumed by humans and are therefore important components of the diet [4,5]. In durum wheat's primary cultivation area in the Mediterranean basin, crop productivity is strongly affected by various environmental stresses, such as drought, salinity, heat, etc. Moreover, recent changes in climate have exacerbated these negative effects on durum wheat production. Biotic stresses can also reduce the productivity of durum wheat in this region [6]. Among the many biotic stresses affecting the crop, stripe rust or yellow rust (causal agent: *Puccinia striiformis* Westend. f.

sp. *tritici* Erikss. (*Pst*)) can be particularly devastating. When the plants are attacked by this disease, their leaves sustain severe damage, disrupting energy capture and contributing to premature senescence. Losses in grain yield due to stripe rust can be as high as 70% during epidemics [7–9].

In many countries, resistance to stripe rust is one of the most important priorities in wheat breeding programs. Resistance to stripe rust of wheat is usually classified into two categories: (i) seedling resistance (also called all-stage resistance or ASR) and (ii) adult plant resistance (APR) [10]. Seedling resistance confers a higher resistance level than APR, but is race-specific and therefore easily overcome by new virulence types arising in pathogen populations [11]. In contrast, APR is best recognized in mature plants, is partial in its effect, non-race-specific, and more durable. Over 84 *Yr* (yellow rust) resistance genes have described in wheat, of which only 12 have been identified in durum wheat [12,13]. Tetraploid wheats such as *T. dicoccoides*, and *T. turgidum* possess a high level of genetic diversity for agronomically important traits and may be useful as sources of stripe rust resistance genes [14]. Indeed, several important *Yr* genes have been described in durum wheat, including *Yr15* (*T. dicoccoides*), *Yr26* (*T. turgidum*), *Yr35*, and *Yr36* (*T. dicoccoides*), etc. [13]. Over the last few decades, many other genes and quantitative trait loci (QTL) for stripe rust resistance have been identified and are being used in wheat breeding programs [15]. These resistances are critical for ensuring reliable production of durum wheat under the threat of future stripe rust epidemics.

Genome-wide association study (GWAS) is a genomic tool for detecting associations between target traits and genetic variants based on linkage disequilibrium (LD) in natural populations. Obtaining information on the genetic architecture of traits in a short period of time and at a lower cost are the main advantages for using GWAS in breeding programs [15]. This genetic approach has been successfully used in many studies of human, animal, and plant research. In many crops, GWAS has been widely applied for identifying QTLs for agronomic traits such as grain yield and its related components, biochemical activities, root system architecture, physiological features as well as tolerance/resistance to abiotic and biotic stresses [16–23]. GWAS has been used more extensively in wheat than almost any other crop. One of the possible reasons for this may be the availability of a reference genome sequence facilitated by the International Wheat Genome Sequencing Consortium (IWGSC), which has greatly facilitated the detection of loci associated with target traits [24]. Additionally, the availability of SNP chips with a wide range of markers (from 9K to 820K) has greatly facilitated high throughput genotyping and the construction of high-resolution maps for targeting genes underpinning both qualitative and quantitative traits in wheat [25]. In this study, we employed GWAS to identify loci for stripe rust resistance in a highly diverse and unique durum wheat panel using the Illumina iSelect 15K wheat array.

2. Materials and Methods

2.1. Genetic Materials

One hundred and twenty-three durum wheat accessions from Iran were used in this investigation. These accessions were mostly landraces selected for the panel based on their diverse agro-morphological traits from previous studies. Additionally, sixteen other accessions from the former Yugoslavia, Afghanistan, Portugal, Bulgaria, Argentina, Australia, and Iraq also were included in the study. Additional passport information is presented in Table 1.

Table 1. Passport and disease reaction data of durum wheat landraces used in a genome-wide association study of stripe rust resistance.

No.	Gene Bank Code	Region	*Pstv-37*	*Pstv-40*	No.	Gene Bank Code	Region	*Pstv-37*	*Pstv-40*
1	IUGB-00650	Iran, Moghan	MS	MR	71	IUGB-00720	Iran, Unknown	MS	MS
2	IUGB-00651	Iran, Gorgan	R	R	72	IUGB-00721	Iran, Unknown	S	S
3	IUGB-00652	Iran, Mianeh	MS	MR	73	IUGB-00722	Iran, Unknown	R	R
4	IUGB-00653	Iran, Shirvan	S	S	74	IUGB-00723	Iran, Unknown	MS	MR
5	IUGB-00654	Iran, Unknown	MS	S	75	IUGB-00724	Iran, Unknown	S	S
6	IUGB-00655	Iran, Unknown	S	MR	76	IUGB-00726	Iran, Unknown	S	S
7	IUGB-00656	Iran, Unknown	S	MS	77	IUGB-00727	Iran, Unknown	R	R
8	IUGB-00657	Iran, Mashhad	S	S	78	IUGB-00728	Iran, Bam	R	MS
9	IUGB-00658	Iran, Mashhad	S	S	79	IUGB-00729	Iraq	R	R
10	IUGB-00659	Iran, Mashhad	MS	R	80	IUGB-00730	Iran, Shoushtar	MS	MS
11	IUGB-00660	Iran, Mashhad	MS	MS	81	IUGB-00731	Iran, Kermanshah	R	S
12	IUGB-00661	Iran, Mashhad	MS	MR	82	IUGB-00732	Iran, Kermanshah	MS	S
13	IUGB-00662	Iran, Mashhad	S	MS	83	IUGB-00733	Iran, Golpaygan	S	S
14	IUGB-00663	Iran, Mashhad	S	MS	84	IUGB-00734	Iran, Unknown	S	MS
15	IUGB-00664	Iran, Mashhad	S	MS	85	IUGB-00735	Iran, Unknown	S	MS
16	IUGB-00665	Iran, Mashhad	MR	MR	86	IUGB-00736	Iran, Unknown	MS	MS
17	IUGB-00666	Iran, Mashhad	S	MS	87	IUGB-00737	Iran, Unknown	MR	R
18	IUGB-00667	Iran, Galuran	S	MS	88	IUGB-00738	Iran, Unknown	MS	R
19	IUGB-00668	Iran, Galuran	S	S	89	IUGB-00740	Iran, Mashhad	S	S
20	IUGB-00669	Iran, Kabkali	S	MS	90	IUGB-00741	Iran, Mashhad	MR	MS
21	IUGB-00670	Iran, Shetaban	S	S	91	IUGB-00742	Iran, Mashhad	MR	R
22	IUGB-00671	Iran, Zigh Abad	S	MS	92	IUGB-00743	Iran, Mashhad	S	MS
23	IUGB-00672	Iran, Mahidasht	MR	R	93	IUGB-00744	Iran, Mashhad	S	MS
24	IUGB-00673	Iran, Unknown	MR	MR	94	IUGB-00745	Iran, Mashhad	MS	MS
25	IUGB-00674	Iran, Songhor	R	R	95	IUGB-00746	Iran, Mashhad	S	S
26	IUGB-00675	Iran, Kangavar	R	R	96	IUGB-00747	Iran, Mashhad	MS	MS
27	IUGB-00676	Iran, Aleshtar	MS	MR	97	IUGB-00750	Iran, Mashhad	R	R
28	IUGB-00677	Iran, Azna	S	MS	98	IUGB-00751	Iran, Mashhad	R	R
29	IUGB-00678	Iran, Delfan	R	R	99	IUGB-00752	Iran, Mashhad	R	R
30	IUGB-00679	Iran, Mehran	MR	MR	100	IUGB-00753	Iran, Mashhad	MR	MR
31	IUGB-00680	Iran, Shebab	MS	S	101	IUGB-00754	Iran, Mashhad	R	R
32	IUGB-00681	Yugoslavia	MS	MR	102	IUGB-00755	Iran, Unknown	MS	MS
33	IUGB-00682	Afghanistan	S	S	103	IUGB-00757	Iran, Unknown	MS	MS
34	IUGB-00683	Iran, Dehgolan	S	MS	104	IUGB-00758	Iran, Unknown	MS	MS
35	IUGB-00684	Iran, Marivan	S	S	105	IUGB-00759	Iran, Lorestan	MS	MS
36	IUGB-00685	Portugal	R	R	106	IUGB-00760	Iran, Lorestan	R	R
37	IUGB-00686	Afghanistan	S	MS	107	IUGB-00761	Iran, Unknown	S	MS
38	IUGB-00687	Bulgaria	S	S	108	IUGB-00762	Iran, Paveh	MS	MS
39	IUGB-00688	Argentina	R	R	109	IUGB-00763	Iran, Kermanshah	MR	MR
40	IUGB-00689	Australia	MS	MS	110	IUGB-00764	Iran, Unknown	MR	MS
41	IUGB-00690	Bulgaria	S	MS	111	IUGB-00765	Iran, Kermanshah	MS	R
42	IUGB-00691	Iran, Lorestan	MS	MS	112	IUGB-00766	Iran, Kermanshah	MS	MS
43	IUGB-00693	Iran, Dezful	MR	MR	113	IUGB-00767	Iran, Kermanshah	MS	MS
44	IUGB-00694	Iran, Lorestan	MS	MS	114	IUGB-00768	Iran, Kermanshah	S	MS
45	IUGB-00695	Iran, Lorestan	MS	R	115	IUGB-00769	Iran, Kermanshah	MS	MS
46	IUGB-00696	Iran, Lorestan	S	S	116	IUGB-00770	Iran, Gachsaran	MR	MS
47	IUGB-00697	Iran, Lorestan	S	S	117	IUGB-00771	Iran, Kermanshah	MS	MS
48	IUGB-00698	Iran, Lorestan	R	R	118	IUGB-00772	Iran, Hamadan	MS	S
49	IUGB-00699	Iran, Lorestan	R	R	119	IUGB-00773	Iran, Eizeh	S	R
50	IUGB-00700	Iran, Lorestan	S	MS	120	IUGB-00774	Iran, Eizeh	S	S
51	IUGB-00701	Iran, Lorestan	S	MS	121	IUGB-00775	Iran, Dezful	S	S
52	IUGB-00702	Iran, Lorestan	MS	MS	122	IUGB-00776	Iran, Dezful	MS	MR
53	IUGB-00703	Iran, Lorestan	MS	MS	123	IUGB-00777	Iran, Ardebil	MS	MR
54	IUGB-00704	Iran, Kermanshah	S	S	124	IUGB-00778	Iran, Ardebil	S	MS
55	IUGB-00705	Iran, Kermanshah	R	R	125	IUGB-00779	Iran, Ardebil	MS	MS
C56	IUGB-00706	Iran, Lorestan	MR	MR	126	IUGB-00780	Iran, Ahar	MS	MS
57	IUGB-00707	Iran, Lorestan	MR	MR	127	IUGB-00781	Iran, Ahar	MS	S
58	IUGB-00708	Iran, Lorestan	MS	MS	128	IUGB-00782	Iran, Lorestan	S	MS
59	IUGB-00709	Iran, Lorestan	MS	MS	129	IUGB-00783	Iran, Lorestan	MS	MR

Table 1. *Cont.*

No.	Gene Bank Code	Region	*Pstv-37*	*Pstv-40*	No.	Gene Bank Code	Region	*Pstv-37*	*Pstv-40*
60	IUGB-00710	Iran, Lorestan	MS	S	130	IUGB-00784	Iran, East Azarbayjan	MS	MS
61	IUGB-00711	Iran, Unknown	MS	S	131	IUGB-00785	Iran, Lorestan	MS	MS
62	IUGB-00712	Iran, Lorestan	MS	MS	132	IUGB-00786	Italy	MR	MR
63	IUGB-00713	Iran, Unknown	MS	MS	133	IUGB-00787	Italy	R	MR
64	IUGB-00714	Iran, Unknown	S	S	134	IUGB-00788	Italy	MR	MR
65	IUGB-00715	Iran, Unknown	MS	MS	135	IUGB-00790	Italy	S	MS
66	IUGB-00716	Iran, Unknown	MS	MR	136	IUGB-00791	Italy	MR	R
67	IUGB-00717	Iran, Lorestan	MS	MS	137	IUGB-00792	Italy	R	R
68	IUGB-00718	Iran, Unknown	MS	S	138	IUGB-00793	Italy	MS	S
69	IUGB-00718	Iran, Unknown	MS	S	139	IUGB-00945	Iran, Dareh Shahr	MS	MR
70	IUGB-00719	Iran, Unknown	S	S					

IUGB, Ilam University Gene Bank; General stripe rust phenotyping classes were as follows: R = Resistant; MR = Moderately Resistant; MS = Moderately Susceptible; and S = Susceptible.

2.2. Disease Evaluations

Seeds of each durum accession were planted and grown in cone racks of 98 cones per rack in a temperature-controlled greenhouse at 22 ± 2 °C. Three seeds were planted in each cone per accession and replicated three times. Seven to 10 days after planting, the primary leaves of seedlings were inoculated with a suspension of *Pst* urediniospores in a lightweight mineral oil. The panel was tested with *Pst* races *Pstv-37* and *Pstv-40* and the reaction of genotypes were categorized in four classes (R, MR, MS & S). Descriptive statistic of panel responses are illustrated in Figure 1. Immediately after inoculation, the oil carrier was allowed to fully evaporate from the plants. Then, they were placed overnight in a dew chamber at 10 °C for 24 h in the dark. After the infection period, plants were kept in growth chamber with a diurnal temperature regime of 20 ± 2 °C for 18 h in the light and 18 ± 2 °C for 6 h in the dark. The assessment of stripe rust infection types (IT) was based on the standard 0 to 9 scale described by Line et al. (1992) [26]. ITs of 0 to 6 were considered indicative of a resistant response and 7 to 9 as a susceptible response.

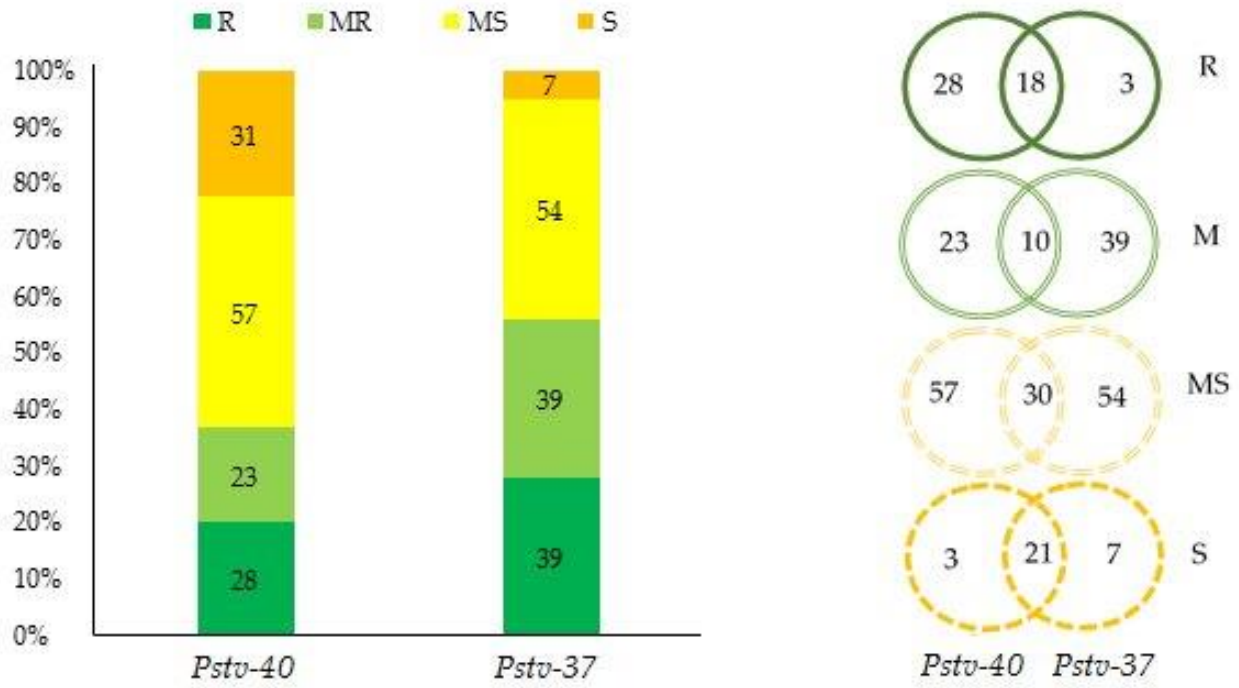


Figure 1. Summary of the frequency distribution for stripe rust reaction in 139 durum wheat accessions to two races of *Pst* at the seedling stage.

2.3. DNA Isolation and Genotyping Assay

The total genomic DNA was extracted from fresh, young leaves of each accession following the method of Doyle and Doyle [27]. The quality of DNA was determined using electrophoresis on a 1% agarose gel. All accessions were genotyped using the wheat 15K Illumina SNP chip [28]. Markers with minor allele frequency (MAF) less than 5% and missing values of more than 10% were removed from subsequent analysis. A total of 6280 SNPs were scored and used in the final association analysis.

2.4. GWAS Analysis

Analysis of population structure in the panel was performed using STRUCTURE software ver. 2.3.4 [29] and generated the Q matrix. This analysis was done with a total of 100,000 MCMC (Markov Chain Monte Carlo) iterations and a burn-in-length of 100,000 for each K. For each K value, 10 independent runs were carried out. The relative kinship (K) matrix was estimated using TASSEL software ver 5.2.32 [30]. Linkage disequilibrium (LD) was calculated using 6280 SNPs with known map positions across the 14 durum wheat chromosomes. Squared allele frequency correlations (r^2) between markers were used to estimate pairwise LD values. GWAS analysis between phenotypic data (Average of IT scores in evaluated replications) and SNP marker data was done based on a mixed linear model (MLM) incorporating genotypes, phenotypes and Q and K matrices [MLM (Q + K)]. A LOD value > 3 was used as a threshold *p*-value for SNP-marker-trait associations [31].

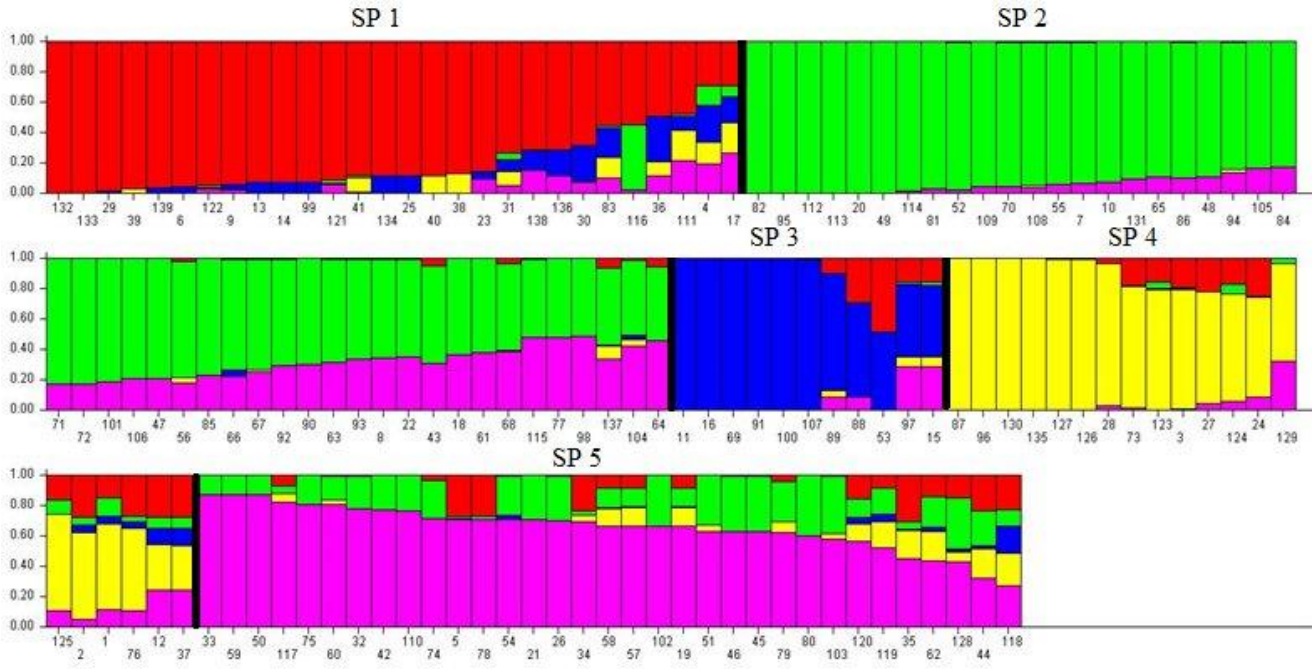
3. Results

3.1. Response of Durum Wheat Genotypes to Stripe Rust (SR)

The number and percent of accessions giving R, MR, MS, and S reactions to the two *Pst* races are given in Figure 1. Of the 139 accessions investigated, 33 (23.7%) and 7 (5.0%) were resistant; 57 (41.0%) and 54 (38.8%) were moderately resistant; 23 (16.5%) and 39 (28.0%) were moderately susceptible; and 28 (20.1%) and 39 (28.0%) were susceptible to races *Pstv40 and Pstv-37*, respectively. Figure 1 shows that 18 (12.9%), 10 (7.2%), 30 (21.6%), and 21 (15.1%) accessions were resistant, moderately resistant, moderately susceptible, and susceptible to both races, respectively. The responses of each accession against the two *Pst* races is presented in Table 1.

3.2. Population Structure

Population structure analysis showed that the durum wheat panel was comprised of five subpopulations (SPs) with 22, 44, 8, 18, and 30 accessions (Figure 2). Seventeen accessions did not belong to any subpopulation. Accessions resistant to one or both races were found in each subpopulation. The genetic divergence among the identified SPs was estimated through pairwise F_{ST} and varied between 0.30 (SP 3) and 0.78 (SP 1). With respect to genetic distance between SPs, SP 1 and SP 5 had the largest distance (0.29), whereas SP 2 with both SP 3 and SP 5 has the smallest distance (0.16).



Subpopulation	Number of accessions	F_{ST}	Genetic distance between subpopulations				
			SP 1	SP 2	SP 3	SP 4	SP 5
SP 1	22	0.57	-	0.25	0.18	0.31	0.29
SP 2	88	0.67		-	0.16	0.19	0.16
SP 3	8	0.30			-	0.21	0.17
SP 4	18	0.96				-	0.22
SP 5	30	0.78					-

Figure 2. Population structure analysis based on 139 durum wheat accessions and 6280 SNP markers.

3.3. LD Decay Analysis and Markers Significantly Associated with Stripe Rust Resistance

All chromosomes fell into five groups based on the genetic distance between them (Table 2). The markers with distance <5 cM showed the highest values for LD. Within this group, the highest LD was observed for chromosome 6A, while the lowest LD was found for chromosomes 1B and 6B. Chromosomes 3A, 5B, and 5A had the highest LD values when inter-marker distances were >50 cm. To identify genomic regions associated with SR resistance, we conducted a GWAS using 6280 SNP markers with a mixed linear model (MLM) on the 139 accessions. As shown in the Manhattan plots and quantile-quantile (Q-Q) plots, the MLM model was well fitted to the data and showed less deviation from the expected *p*-values (plots not shown).

A total of 6280 markers were utilized for GWAS of stripe rust responses, and 230 markers were found significant. One hundred and fifty-three SNPs were significantly associated with resistance to *Pstv-37*, among which 100 were distributed across the B sub-genome and 53 across the A sub-genome. Chromosomes 5B, 6B, and 7B contained the greatest number of significant markers within the B sub-genome with 16, 21, and 29, respectively. With respect to sub-genome A, chromosomes 1A and 4A had the greatest number of significant SNPs with 13 and 10, respectively. Seventy-seven significant associated markers were found in response to race for *Pstv-40*. Chromosomes 4A, 1A, and 3B had the greatest number of significant associated SNP markers with both races, respectively. A summary of the marker associations for stripe rust resistance to the two races of *Pst* is presented in Table S1. The percentage of phenotypic variation (R^2) accounted for by significant SNPs ranged from 4 to 18%. Only three marker-trait

associations showed an R^2 higher than 10% (Table S1). Of the 230 marker-trait associations identified in this study, only 30 were found in response to both races of *Pst* (Table 3).

Table 2. Average linkage disequilibrium (r^2) at different marker distances for 139 durum wheat accessions.

Chromosome	Numbers of SNPs	Length (cM)	* Average Linkage Disequilibrium (r^2) between Pair of Markers				
			D < 5 cM	D = 5–10 cM	D = 10–20 cM	D = 20–50 cM	D > 50 cM
1A	181	106	0.214	0.094	0.069	0.048	0.035
1B	249	101.5	0.127	0.044	0.043	0.039	0.034
2A	206	119.7	0.147	0.041	0.092	0.037	0.032
2B	391	144.2	0.149	0.083	0.065	0.043	0.041
3A	184	163.4	0.264	0.099	0.051	0.052	0.047
3B	340	137	0.139	0.052	0.055	0.038	0.035
4A	168	161.8	0.238	0.029	0.33	0.023	0.039
4B	150	107	0.147	0.044	0.036	0.044	0.048
5A	193	106.7	0.217	0.077	0.049	0.037	0.061
5B	270	179.6	0.25	0.062	0.038	0.033	0.031
6A	240	120.2	0.317	0.047	0.153	0.04	0.034
6B	231	110.4	0.127	0.046	0.041	0.038	0.039
7A	251	165.9	0.149	0.043	0.043	0.031	0.04
7B	267	142	0.145	0.049	0.042	0.041	0.032

* The data presented in this table are based on LD analysis for a subset of genotyping data (3232 SNPs) with a known position on chromosomes.

Table 3. List of SNP markers significantly associated with stripe rust resistance to both races *Pstv-37* and *Pstv-40* of *Puccinia striiformis* f. sp. *tritici*.

Trait	Marker	Position	Chr.	Marker	Position	Chr.	Marker	Position	Chr.
Pstv-37	BobWhite_c53978_99	115	6A	IACX8074	97	1B	wsnp_Ex_c2617_4864441	61	4A
Pstv-40		115	6A		97	1B		61	4A
Pstv-37	BobWhite_c7786_376	64	2B	IACX8294	116	7B	wsnp_Ex_c2617_4864955	61	4A
Pstv-40		64	2B		116	7B		61	4A
Pstv-37	BobWhite_c8428_346	63	1A	IACX9290	97	1B	wsnp_Ex_c54395_57291841	61	4A
Pstv-40		63	1A		97	1B		61	4A
Pstv-37	CAP7_c4064_162	108	5A	Kukri_c93635_290	61	4A	wsnp_Ex_c6044_10590220	61	4A
Pstv-40		108	5A		61	4A		61	4A
Pstv-37	Ex_c5759_628	63	1A	RAC875_c5556_328	45	1B	wsnp_Ex_c7002_12063325	115	6A
Pstv-40		63	1A		45	1B		115	6A
Pstv-37	Excalibur_c24041_794	63	1A	Tdurum_contig12525_769	116	7B	wsnp_Ex_c7002_12063380	115	6A
Pstv-40		63	1A		116	7B		115	6A
Pstv-37	Excalibur_c32735_603	64	4A	Tdurum_contig44173_572	55	6B	wsnp_Ex_c7550_12907422	61	4A
Pstv-40		64	4A		55	6B		61	4A
Pstv-37	Excalibur_c7002_314	115	6A	Tdurum_contig47269_904	55	6B	wsnp_Ex_c831_1625061	9	5B
Pstv-40		115	6A		55	6B		9	5B
Pstv-37	Excalibur_rep_c110429_536	140	7B	Tdurum_contig7981_70	55	6B	wsnp_Ku_c16522_25425455	55	6B
Pstv-40		140	7B		55	6B		55	6B
Pstv-37	GENE-0416_480	97	1B	wsnp_Ex_c12818_20334501	61	4A	wsnp_Ku_c30381_40208899	61	4A
Pstv-40		97	1B		61	4A		61	4A

4. Discussion

The development of new genomic tools can facilitate advances in breeding technology, leading to greatly improved crop varieties that can, in turn, enhance global food security under the challenges of climate change [32]. Biotic stresses are one of the many consequences arising from changes in climate [33]. Stripe rust is considered one of the most important biotic stresses that can negatively affect wheat production worldwide [34]. Hence, it is important to screen wheat germplasm in response to the different virulence types of *Pst* present in different regions of the world [35].

In this study, we evaluated a panel of unique durum wheat landraces, predominating from Iran, to two races of *Pst* at the seedling stage in the glasshouse. We observed a high level of phenotypic variation among the tested accessions (Table 1), a result consistent with previous reports on the response of durum wheat germplasm to stripe rust [12,23,36–38].

Landraces and crop wild relatives are considered rich sources of new genes and alleles for traits in breeding programs given their high level of genetic diversity [39]. In this investigation, about one half of the evaluated landraces showed a range of responses from resistant to moderately resistant to both *Pst* races (Figure 1). Although most of the durum landraces included in this work were sampled from Iran, several others collected from Italy (5 samples), Portugal (1 sample), Argentina (1 sample), and Iraq (1 sample) also were resistant or moderately resistant to races *Pstv-37* and *Pstv-40* (Table 1).

Genetic diversity is a key requirement in any breeding program. Thus, estimation of the extent of genetic diversity and evaluation of natural population structure are important parameters for initiating genetic studies and utilizing plant genetic resources in breeding programs [40]. Population structure is an important factor for association analysis [41]. The results from STRUCTURE analysis using SNP data indicated that the durum panel was comprised of five sub-groups (Figure 2) and that 17 accessions (12%) showed a level of admixture. The grouping of the durum landraces into subpopulations was not in accordance with their geographic origins. Previously, Mehrabi et al. [22] reported a high level of molecular and morphological diversity in durum germplasm from Iran. In a study conducted by Lin et al. [42], a high level of genetic diversity was found using SNP data in a durum wheat population. In an investigation of genetic diversity within a global set of durum landraces, Kabbaj et al. [43] reported a high level of genetic variability using SNP markers.

After revealing a high level of genetic variability in the durum panel, we used a MLM model to incorporate population structure results with phenotypic data in the GWAS analysis to reduce false positive errors [44]. Based on the Q-Q plot, false positive associated markers could be successfully minimized in the association analysis of stripe rust reactions. Linkage disequilibrium (LD) is an important factor in the determination of the power of GWAS. In the present study, LD values were estimated for all chromosomes of the two sub-genomes A and B (Table 2). Like other studies, we found more rapid decay of LD in the A sub-genome compared to the B and D sub-genomes [23,45–47].

Based on a total of 6642 SNP markers, 230 significant associations were found for reaction to *Pst* races *Pstv-37* and *Pstv-40*. Most of the associated markers for reaction to race *Pstv-37* were located on the B genome, while most for reaction to race *Pstv-40* were positioned on the A genome (Table S1). A recent meta-QTL analysis revealed that most stripe rust resistance loci are located on the B genome [48], which agrees with the results from the current study. Additionally, Pradhan et al. [23] identified more QTLs/defense genes against stripe rust on the B genome than on the other sub-genomes of bread wheat. In another study, Kumar et al. [49] also reported a high number of significant marker-trait associations on different chromosomes of the B sub-genome.

The R^2 values for the detected associations were low to moderate (range of 3.7% and 17.8%). The three significant associations found with markers *IAAV5873*, *tplb0033f11_1381*, and *Tdurum_contig10100_523* explained a high level of variation (R^2 values 18%, 14%, and 11%, respectively) for reaction to race *Pstv-37* (Table S1). This may be attributed to markers capturing complex allelic interactions and/or specific alleles [50]. Among the 230 significantly associated SNP markers identified in this study, 30 conferred resistances to both *Pst* races (Table 3). Loci conferring resistance to multiple *Pst* races are more desirable in breeding programs. Moreover, the multiple significant associations were mainly located on chromosomes 1A, 4A, 5A, 6A, 1B, 2B, 6B, and 7B. Many other studies have also reported these chromosomes to harbor a large number of significant MTAs and QTLs for stripe rust resistance. For instance, Zegeye et al. [51] identified several stripe rust resistance QTLs on 2B and 5A. Muleta et al. [52] identified stripe rust resistance QTLs on 1B and 2B. Furthermore, Ye et al. [53] identified several important QTLs on the long arms of 1B, 5A, 1A, 5A, 6A, and 6B. Li et al. [15] had also reported one QTL on 1B in durum wheat which confers adult plant resistance. Recently, Pradhan et al. [20] mapped several QTLs at different genomic locations, i.e., 1B, 1A, and 6B.

5. Conclusions

Durum wheat is considered a major crop in the Mediterranean region. Identification of novel alleles for stripe rust resistance is a key requirement for enhancing the resistance of new cultivars. In this study, we found a high level of variation for stripe rust resistance in a durum wheat panel originating mostly from Iran. Population stratification refers to differences in allele frequencies among extracted sub-populations due to systematic differences in ancestry rather than the association of markers with traits. Consideration of genetic structure coefficients along with a kinship matrix of genotypes is an important technical procedure that was carried out in this work to significantly reduce the rate of false positives. Within the investigated germplasm, 40 accessions (23 Iranian and 5 foreign) were immune against race *Pstv-40*, while 37 samples (16 Iranian and 5 foreign) were immune against race *Pstv-37*. Hence, this germplasm may be useful in programs aimed at pyramiding genes to achieve more durable stripe rust resistance. Our data also revealed 30 significant marker-trait associations for both races of *Pst* used in this study. These markers may be useful for breeders employing marker-assisted selection for stripe rust resistance. Ongoing research in this area will facilitate further advances in genomic selection using informative markers for stripe rust resistance and their application in the rapid screening of resilient genotypes for breeding programs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app12104963/s1. Table S1: List of significant SNP markers associated with two isolates of stripe rust.

Author Contributions: Conceptualization, A.A.M., B.J.S.; methodology, A.A.M.; investigation, A.A.M., O.M. and M.R.; writing—original draft preparation, A.P.-A. and A.A.M.; writing—review and editing, A.P.-A., A.A.M. and B.J.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported in part by the Minnesota Agricultural Experiment Station Project No. MIN-22-085. Open access funding is provided by the University of Minnesota.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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