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Identification of Candidate Genes and Genomic Regions Associated with Adult Plant Resistance to Stripe Rust in Spring Wheat

Amira M. I. Mourad [1,2,*], Mohamed A. Abou-Zeid [3], Shamseldeen Eltaher [4], P. Stephen Baenziger [5] and Andreas Börner [2,*]

1 Department of Agronomy, Faculty of Agriculture, Assiut University, Assiut 71526, Egypt
2 Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstrasse 3, D-06466 Seeland, Germany
3 Wheat Disease Research Department, Plant Pathology Research Institute, ARC, Giza 12619, Egypt; m.abouzeid@arc.sci.eg
4 Department of Plant Biotechnology, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City, Sadat 79, Egypt; shams.eltaher@gebri.usc.edu.eg
5 Department of Agronomy and Horticulture, University of Nebraska–Lincoln, Lincoln, NE 68583, USA; pstephen.baenziger@gmail.com
* Correspondence: amira_mourad@aun.edu.eg or mourad@ipk-gatersleben.de (A.M.I.M.); boerner@ipk-gatersleben.de (A.B.)

Abstract: Wheat stripe rust (caused by *Puccinia striiformis* f. sp. *tritici*) is a major disease that damages wheat plants and affects wheat yield all over the world. In recent years, stripe rust became a major problem that affects wheat yield in Egypt. New races appeared and caused breakdowns in the resistant genotypes. To improve resistance in the Egyptian genotypes, new sources of resistance are urgently needed. In the recent research, a set of 95 wheat genotypes collected from 19 countries, including Egypt, were evaluated for their resistance against the Egyptian race(s) of stripe rust under field conditions in the two growing seasons 2018/2019 and 2019/2020. A high genetic variation was found among the tested genotypes. Single marker analysis was conducted using a subset of 71 genotypes and 424 diversity array technology (DArT) markers, well distributed across the genome. Out of the tested markers, 13 stable markers were identified that were significantly associated with resistance in both years (p-value ≤ 0.05). By using the sequence of the DArT markers, the chromosomal position of the significant DArT markers was detected, and nearby gene models were identified. Two markers on chromosomes 5A and 5B were found to be located within gene models functionally annotated with disease resistance in plants. These two markers could be used in marker-assisted selection for stripe rust resistance under Egyptian conditions. Two German genotypes were carrying the targeted allele of all the significant DArT markers associated with stripe rust resistance and could be used to improve resistance under Egyptian conditions.

Keywords: marker-assisted selection; single marker analysis; stripe rust; coefficient of infection; DArT markers

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1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important crops all over the world [1,2]. During the life cycle of wheat, plants are attacked by different pathogens, including stripe rust (caused by *Puccinia striiformis* f. sp. *tritici*), which is considered a major global threat to wheat production [3–5]. Infection by stripe rust pathogens can occur at any time during the wheat growing season, from the one-leaf stage to maturity, resulting in a decreasing number of kernels/head and kernel mass/plant [6]. Applying fungicides can control stripe rust. However, fungicides are expensive, could be ineffective if not applied on time and harm the environment. On the other hand, planting resistant genotypes is a more effective, economical and easy method in controlling stripe rust disease [7,8].

In recent years, new stripe rust races appeared and spread rapidly worldwide; these races are highly aggressive and can survive under high-temperature conditions [9]. These new races broke the resistance in some Egyptian important genotypes such as Misr_2, Giza_168 and Sakha_61 [10]. Due to this situation, a search for new sources of resistance against the Egyptian rust race(s) is urgently required. Due to the low diversity of the genetic background in Egyptian wheat genotypes, the identification of new sources of resistance in worldwide spring wheat genotypes is urgently needed. Identified new sources of resistance could be used in future breeding programs to accelerate stripe rust resistance in Egyptian wheat.

The first step in breeding resistant genotypes is understanding the genetic control of the resistance [11,12]. At present, there are 80 genes identified for resistance to stripe rust in wheat, and more than 300 temporarily designated genes and QTL have been reported [13–15]. Furthermore, more than 160 quantitative trait loci (QTLs) have been designated in 49 regions on the 21 wheat chromosomes controlling stripe rust resistance [16,17]. All these resistance genes/QTLs could be classified into two different types: seedling resistance genes/QTLs (also known as all-stage resistance (ASR)) and adult plant resistance genes/QTLs (APR). An ASR gene is effective against a specific race or a small number of races and provides resistance beginning from the seedling growth stage through to the entire growth cycle. On the other hand, APR genes are race non-specific and are expressed only at the adult growth stage [6,7,18]. Identification and mapping of both ASR and APR resistance genes help wheat breeders to combine both resistance types into a single genetic background and hence achieve durable and long-lasting stripe rust protection in cultivars [6–8].

Many genetic approaches could be used in mapping resistance genes in plants, such as QTL mapping and genome-wide association study (GWAS). Both methods identify marker alleles associated with the targeted trait. Different types of molecular markers could be used to conduct these analyses, such as simple sequence repeat (SSR), diversity arrays technology (DArT), sequence-tagged sites (STS) or single nucleotide polymorphisms (SNPs). DArT markers were reported as a marker technology that provides high genome coverage, reduces the complexity of the genome and is cost-competitive [19]. This technology has recently gained increasing attention, although it was developed many years ago [20–23]. It has been used widely in mapping QTLs associated with different traits in plants, including stripe rust resistance in wheat [16,24–27]. The objectives of this study were to (1) identify the genetic diversity of the Egyptian and other spring wheat genotypes in their resistance to the Egyptian race(s) of stripe rust, (2) understand the genetic control of the resistance against the Egyptian race(s) of stripe rust, (3) identify some DArT markers and genomic regions associated with stripe rust resistance to be used in marker-assisted selection for stripe rust resistance in wheat and (4) select the best resistant genotypes to be used in improving stripe rust resistance under Egyptian conditions in future breeding programs.

2. Materials and Methods

2.1. Plant Materials

In this study, a set of 95 spring bread wheat genotypes were used to understand the genetic control of wheat resistance to the Egyptian race(s) of stripe rust. These genotypes represented new and old cultivars from 19 different countries collected by the USDA-ARS, Aberdeen, ID, USA (Figure S1 and Table S1). Seeds of all the tested genotypes were obtained from the USDA-ARS, Aberdeen, ID, USA, except seeds of the Egyptian genotypes that were obtained from the Egyptian Governorate.

2.2. Evaluation of Stripe Rust Resistance

The tested genotypes were evaluated for their resistance to the Egyptian race(s) of *P. striiformis* f. sp. *tritici* at the adult growth stage. The evaluation was conducted under field conditions using natural infection. The experiments were conducted for two years (2018/2019 and 2019/2020) at Sids Agricultural Research Station, Egypt. Both experiments

were planted using a randomized complete block design. In each growing season, three replications were planted. Each tested genotype was represented in a single one-meter row with 30 cm row spacing in each replication. Furthermore, the experiment was surrounded by a spreader area planted with a mixture of Morocco and Thatcher, two highly susceptible varieties, to make sure that infection would occur. The spreader area was inoculated artificially with a mixture of urediniospores collected from the Egyptian fields in the previous growing seasons. The infection of the tested genotypes occurred naturally from the surrounding spreaders.

Adult plant resistance was evaluated using two different parameters, disease severity (DS) and infection type (IT) [28]. IT was recorded according to the Stakman et al., (1962) [29] scale (I, R, MR, MS and S), as described by Roelfs et al., (1992) [30]. It was then converted to numbers as described by Abou-Zaid and Mourad 2021 [31]. To calculate the coefficient of infection (CI), DS was multiplied by the converted IT as described by Shewaye, et. al. (2018) and Abouzeid and Mourad (2021) [31,32]. Based on the original IT, the genotype was considered resistant if it had MR, R or immune ITs (0–0.4). However, based on the DS, resistant genotypes should have 10% or less of leaf coverage. Therefore, genotypes that had CI% $\leq$ 4% were considered as resistant genotypes.

2.3. Statistical Analysis of Stripe Rust Resistance

The analysis of variance (ANOVA) was calculated for the CI values using R software [33], using the following model:

$$Y_{ijk} = \mu + g_i + r_j + y_k + gy_{ik} + e_{ijk}$$

where Y_{ijk} is an observation of genotype i in replication j which was planted in year k, μ is the general mean; g_i, r_j, and y_k are the main effects of genotypes (fixed effects), replications (random effects) and years (random effects), respectively; gy_{ik} is the interaction of the genotypes in the two years; e_{ijk} is the error. The broad-sense heritability (H^2) was calculated as described in Abou-Zeid and Mourad 2021 [31]

2.4. Genotyping of the Tested Genotypes Using DArT Markers

In the recent study, 71 genotypes of the tested genotypes were genotyped using 424 DArT markers. The DArT markers' information was a part of a stripe rust resistance study carried out by Maccaferri et. al. (2015) [34] in fields in the U.S.A.. Marker information is available on the website of the U.S. National Plant Genome system (https://npgsweb.ars-grin.gov/gringlobal/search.aspx), (accessed on 9 December 2021). Available DArT markers were used to calculate polymorphic information content (PIC) using PowerMarker software [35], using the following formula:

$$PIC = 1 - \sum_{j=1}^{n} P_{ij}^2 - \sum_{j=1}^{n=1} \sum_{k=j+1}^{n} 2P_{ij}^2 P_{ik}^2$$

where P_{ij} and P_{ik} are the frequencies of j_{th} and k_{th} alleles for marker i, respectively.

Besides this, principal component analysis (PCA) was used for the tested genotypes based on the available DArT markers using TASSEL software [36].

2.5. Single Marker Analysis (SMA) of Stripe Rust Adult Plant Resistance

CI data, as well as the available data of the 424 DArT markers of the tested 71 genotypes, were used to perform SMA to identify DArT markers significantly associated with adult resistance in the tested genotypes. Single marker analysis was performed using PowerMarker software V 3.25 [35]. For each significant marker, the phenotypic variation explained by the marker, as well as the allele effect of each marker, was estimated using TASSEL 5.0 software [36].

2.6. Gene Models Underlying Each Significant DArT Marker and Their Annotations

The sequence of the significant DArT markers was obtained from Diversity Array Technology, available at https://www.diversityarrays.com/technology-and-resources/sequences. The sequences of these markers were blasted against the wheat genome using the EnsemblPlants database available at this link: https://plants.ensembl.org/Triticum_aestivum/Tools/Blast. The best blasts were detected based on the chromosomal location of the DArT marker, the highest length of the blast, the highest percentage of identity (ID%) and the lowest *p*-value. In order to compare the chromosomal position of the best blast and the different available gene models with the highest confidence, possible gene models controlling the resistance were identified. The distance between the significant markers and high-confidence gene models was visualized using MapChart software [37]. Using the international wheat genome sequencing consortium (IWGSC), the functional annotation of these gene models was investigated and their association with stripe rust resistance was examined. Furthermore, the expression of the identified gene models was compared under controlled conditions with disease conditions at adult plant stages using the Wheat Expression Browser "http://www.wheat-expression.com/", (accessed on 9 December 2021).

3. Results

3.1. Phenotypic Variation for Stripe Rust Resistance in the Tested Genotypes

Highly significant differences were identified among the tested genotypes for their resistance against the Egyptian race(s) of stripe rust pathogen, based on the analysis of variance (ANOVA). Highly significant differences were found between the years. The genotype x years interaction was highly significant, while no significant differences were found among the replications. Broad-sense heritability was 0.63 across the two years (Table 1). A highly significant correlation was found between the two years (r = 0.61, p-value > 0.01 (Figure S2).

Table 1. Analysis of variance for stripe rust resistance in the 95-spring wheat genotypes.

Source	d.f	M.S.
Years (Y)	1	27122.56 **
Replications (R)	2	1839.41
Genotypes (G)	86	3799.96 **
GY	85	1402.18 **
GYR	289	941.92
Heritability	63.10	

** $p < 0.01$.

The percentage of the coefficient of infection (CI) ranged from 0% to 100% in both years (2018/2019 and 2019/2020) (Figure 1). The susceptible check "Morocco" showed a very susceptible response to stripe rust, with a CI% of 100% and 93.33% in 2018/2019 and 2019/2020, respectively. Also, the susceptible check "Thatcher" had a CI% of 100% in 2018/2019 but was absent in 2019/2020. The number of susceptible genotypes (with a percentage of CI 50% or more) was higher in 2018/2019 than in 2019/2020, with a total number of 43 and 19 in 2018/2019 and 2019/2020, respectively. Out of the 19 genotypes that were susceptible in 2019/2020, 18 were susceptible in both 2018/2019 and 2019/2020 (Figure 2a and Table S2). On the other hand, 22 and 20 genotypes were resistant to stripe rust (CI $\leq$ 4%) in 2018/2019 and 2019/2020, respectively. Only ten genotypes had the same resistant reaction to stripe rust in both years (Figure 2b). These resistant genotypes are from five different countries: Egypt (three genotypes), Germany (two genotypes), Saudi Arabia (one genotype), the United Kingdom (one genotype) and the U.S.A (three genotypes). (Table S3).

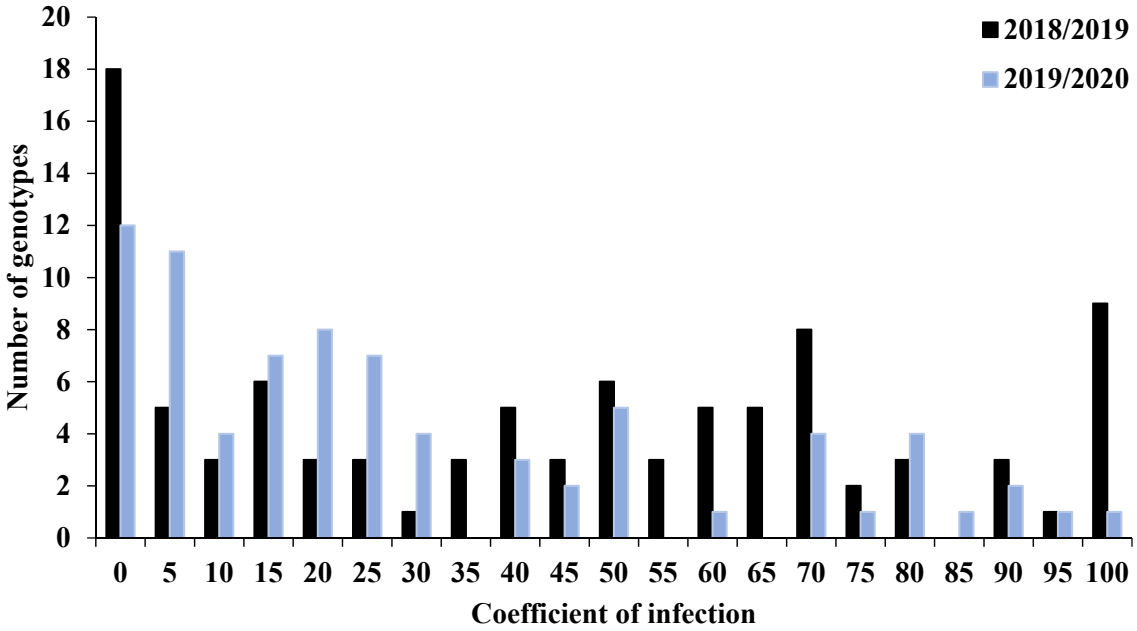

Figure 1. The response of the 95-tested genotypes to stripe rust at the adult plant stage under the Egyptian conditions at 2018/2019 and 2019/2020.

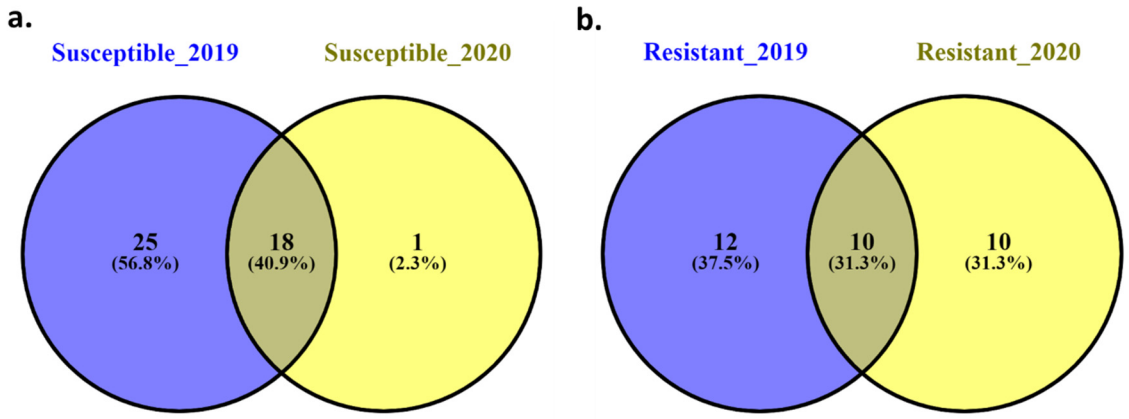

Figure 2. The Venn diagram represents the number of genotypes with the same response in the two evaluation years (2018/2019 and 2019/2020). (**a**) number of susceptible genotypes with a percentage of coefficient of infection (CI%) of 50% or more, (**b**) number of resistant genotypes with a CI% of 4% or less.

3.2. Marker Distribution and Principal Coordinate Analysis

A total number of 424 polymorphic DArT markers were available for 71 genotypes. Out of these markers, 382 (90.09%) had a known chromosomal position, and they covered the whole wheat genome. The B genome was found to carry the highest number of markers, with a percentage of 45% (171 markers). The lowest number, on the other hand, was located on genome D, with a percentage of 17% (66 markers). There were 42 markers located on unknown chromosomal positions with a percentage of 10%. The total number of markers/chromosomes ranged from one marker on chromosome 4D to 38 makers on chromosome 3B (Figure S3a). The PIC value ranged from 0.1 (four markers) to 0.5 (30 markers) across the chromosomes. The majority of markers showed 0.4 of PIC value with a total number of 142 markers (Figure S3b).

The result of principle-component analysis (PCA) classified the tested genotypes into two groups, with a size of nine and 86 genotypes for groups 1 and 2, respectively. The common ten resistant genotypes are located in the second group, while common

susceptible genotypes were distributed between both groups with the number of four and 14 genotypes in group 1 and group 2, respectively (Figure 3).

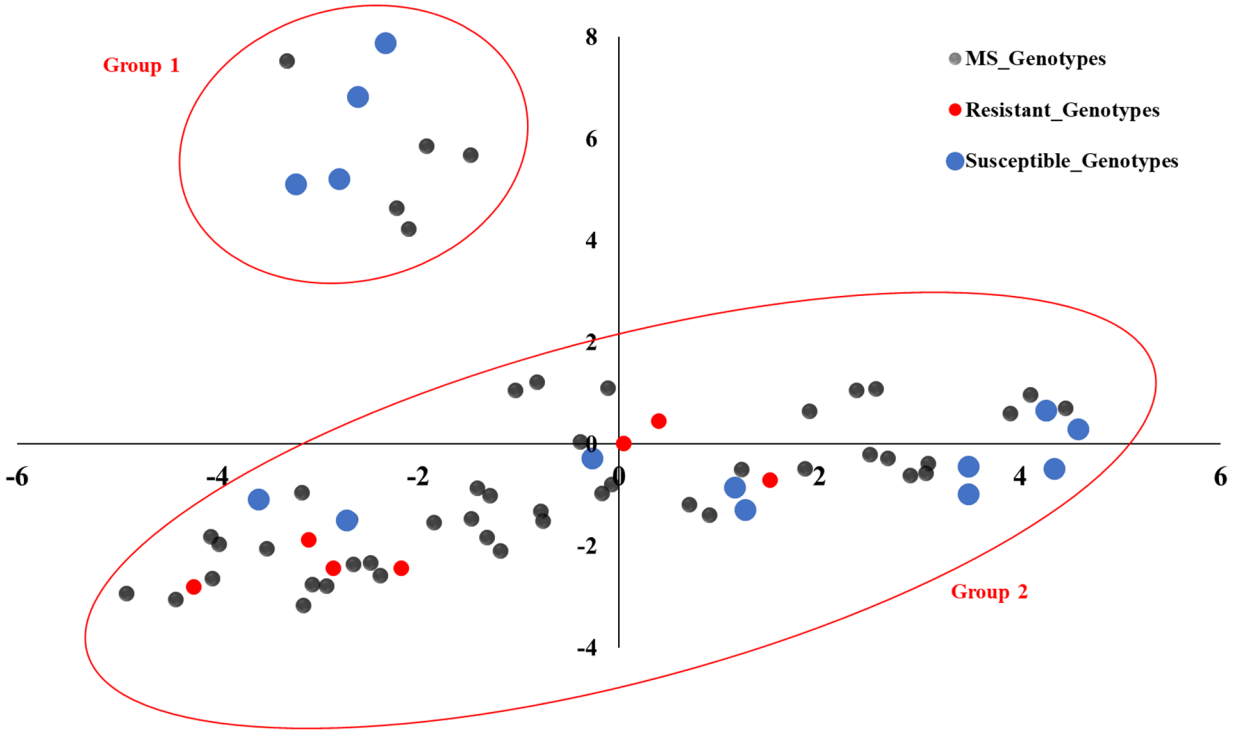

Figure 3. Principle coordinate analysis of the studied spring wheat genotypes based on the 424 DArT markers.

3.3. Single Marker Analysis (SMA) of Adult Plant Resistance to Stripe Rust

Single marker analysis identified 39 and 48 DArT markers significantly associated with stripe rust resistance (p-value $\leq$ 0.05) in 2018/2019 and 2019/2020, respectively (Figure 4, Tables S4 and S5). Out of these identified markers, 13 markers were stable and significantly associated with the resistance in both years. These stable markers were each located on chromosomes 2A, 2B, 3B, 3D, 5A, 5B and 6D, and two markers were located on chromosomes 6B, 7A and 7D, with the p-value ranging from 0.000 to 0.037 in 2018/2019 and from 0.000 to 0.048 in 2019/2020 (Table 2 and Figure 4b). The phenotypic variation explained by these markers (R^2) ranged from 4.38% (WPT-743380) to 17.21% (WPT-9088) in 2018/2019 and from 0.09% (WPT-5736) to 20.30% (WPT-9589) in 2019/2020. Marker "WPT-9088" had the highest allelic effect, increasing stripe rust resistance by a percentage of 36.09% and 27.63% in 2018/2019 and 2019/2020, respectively. Meanwhile, marker "WPT-9589" had the highest allelic effect associated with decreasing resistance, with a value of 30.85% and 32.14% in 2018/2019 and 2019/2020, respectively.

Table 2. Significant stable DArT markers for stripe rust resistance across the tested years (2018/2019 and 2019/2020) based on single marker analysis (p-value 0.05).

DArT Marker	Chromosome	*p*-Value		Allele Effect (%)		R^2 (%)	
		2019	2020	2019	2020	2019	2020
WPT-3976	2A	0.015	0.044	−24.36	−19.21	11.55	9.80
WPT-5736	2B	0.037	0.048	1.66	13.89	4.55	0.09
WPT-9088	3B	0.000	0.002	−36.09	−27.63	17.21	15.00
WPT-0485	3D	0.005	0.027	−23.89	−20.14	10.50	10.24
WPT-9094	5A	0.010	0.004	19.02	24.98	7.58	16.81

Table 2. *Cont.*

DArT Marker	Chromosome	*p*-Value		Allele Effect (%)		R^2 (%)	
		2019	2020	2019	2020	2019	2020
WPT-9724	5B	0.030	0.046	−16.66	−13.81	6.38	5.90
WPT-9256	6B	0.033	0.001	−17.84	−26.54	6.95	19.42
WPT-9589		0.001	0.000	30.85	32.14	13.38	20.30
RPT-7068	6D	0.016	0.013	20.38	22.04	8.37	13.42
WPT-4835	7A	0.009	0.023	−24.21	−16.99	13.96	9.79
WPT-6447		0.002	0.002	−25.38	−24.05	14.96	17.55
WPT-743380	7D	0.009	0.015	19.10	13.37	4.38	10.98
WPT-744976		0.008	0.018	15.26	19.11	5.59	10.71

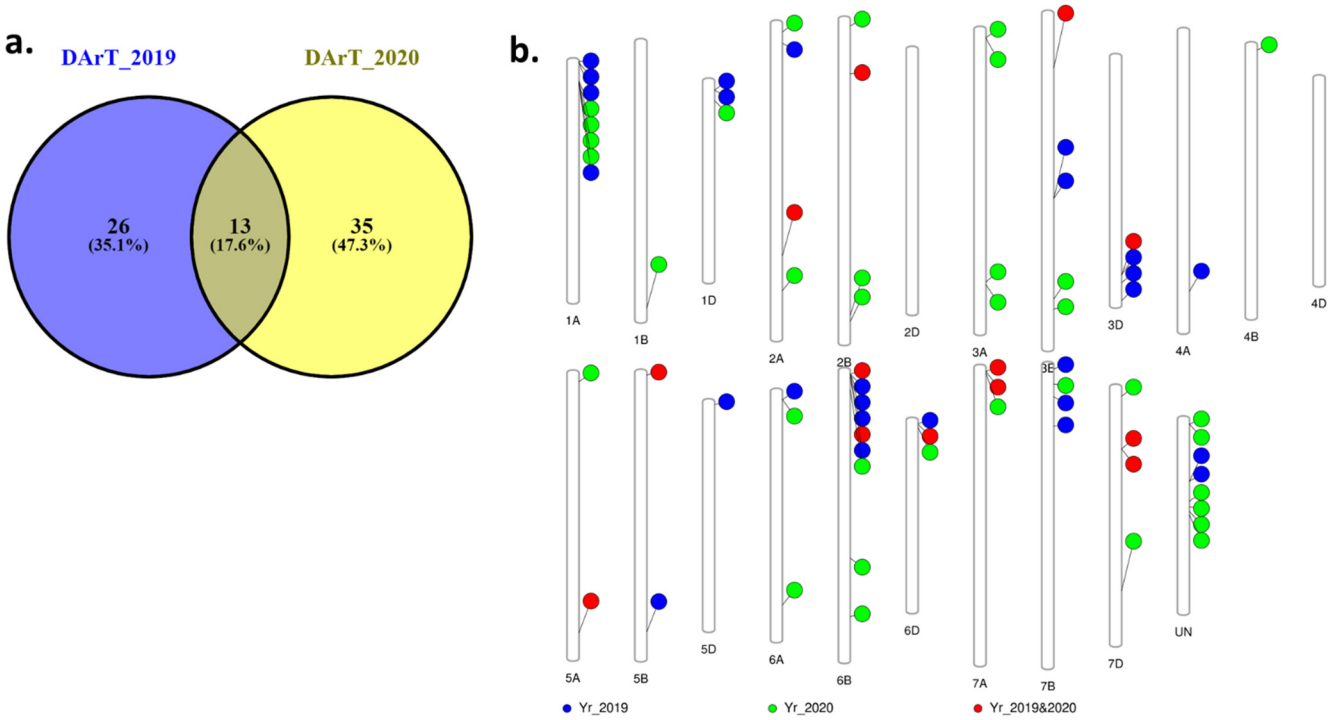

Figure 4. Single marker analysis for stripe rust resistance in the tested genotypes: (**a**) the number of significant markers in each year, (**b**) Distribution of the chromosomal locations of the significant markers across the 21 wheat chromosomes.

3.4. Validation of the Identified Genomic Regions Associated with Stripe Rust Resistance

To validate the association between the significant markers and stripe rust resistance, the sequence of the significant DArT markers was obtained from the Diversity Array Technology website for all the 13 significant stable markers expect WPT-3976 (2A) and RPT-7068 (6D), which did not have available sequence information. These sequences were blasted against the wheat genome using the EnsemblPlants database. Based on the chromosomal location of the DArT markers, the length of the marker and the identified sequence, the highest percentage of identity (ID%) and the lowest *p*-value, the possible positions of the eleven significant DArT markers were detected (Table 3). Furthermore, gene models with high confidence located near or within the detected position were investigated. Out of the eleven markers, four markers (WPT-9088 (3B), WPT-0485 (3D), WPT-9589 (6B), and WPT-4835 (7A)) were located neither near nor within high-confidence gene models (Table 3). Five markers were located very near to high-confidence gene models (WPT-5736 (2B), WPT-9256 (6B), WPT-6447 (7A), WPT-743380 (7D) and WPT-744976 (7D)). Two markers were located within high-confidence gene models WPT-9094 on chromosome 5A

and WPT-9724 on chromosome 5B (Figure 5). The functional annotation of all identified gene models has a strong relation to disease resistance in plants (Table 3).

Table 3. Possible chromosomal location of the 11 significant DArT markers, gene models that are located within or near the DArT markers and their functional annotation based on the PlantEnsemble database.

Marker	Chro.	Marker Length (bp)	Blast Length (bp)	Blast Position	ID%	*p*-Value	Gene Model	Gene Position (bp)	Gene Annotation
WPT-5736	2B	494	34	798127191-798127269	97.10%	6.1×10^{-6}	TraesCS2B02G625200 TraesCS2B02G625300	798128178-798132084 798134799-798139436	UniProtKB-W5B9H0 E3 ubiquitin-protein ligase
WPT-9088	3B	518	510	47651816-47652325	100%	0	NA	NA	NA
WPT-0485	3D	376	376	605639199-605639574	98.9	0	NA	NA	NA
WPT-9094	5A	518	516	536984272-536984787	98.40%	0	TraesCS5A02G327200	536984557-536989401	5′-3′ exoribonuclease 3
WPT-9724	5B	573	573	20040488-20041060	99.8%	0	TraesCS5B02G020900	20040488-20041060	Glutathione S-transferase
WPT-9256	6B	748	143	711392100-711392242	97.2	1.20×10^{-63}	TraesCS6B02G452700	711372963-711375917	Hepatocellular carcinoma-associated antigen 59 family protein, expressed
WPT-9589		572	101	715201767-715201867	96	1.1×10^{-38}	NA	NA	NA
WPT-6447	7A	568	120	691517984-691518114	85.5	5.30×10^{-19}	TraesCS7A02G502500	691521202-691522644	Eukaryotic aspartyl protease family protein
WPT-4835		574	120	691517990-691518109	86.7	2.2×10^{-21}	NA	NA	NA
WPT-743380	7D	869	791	8220754-8221544	99.9	0	TraesCS7D02G018400	8223605-8224570	NBS-LRR disease resistance protein-like protein

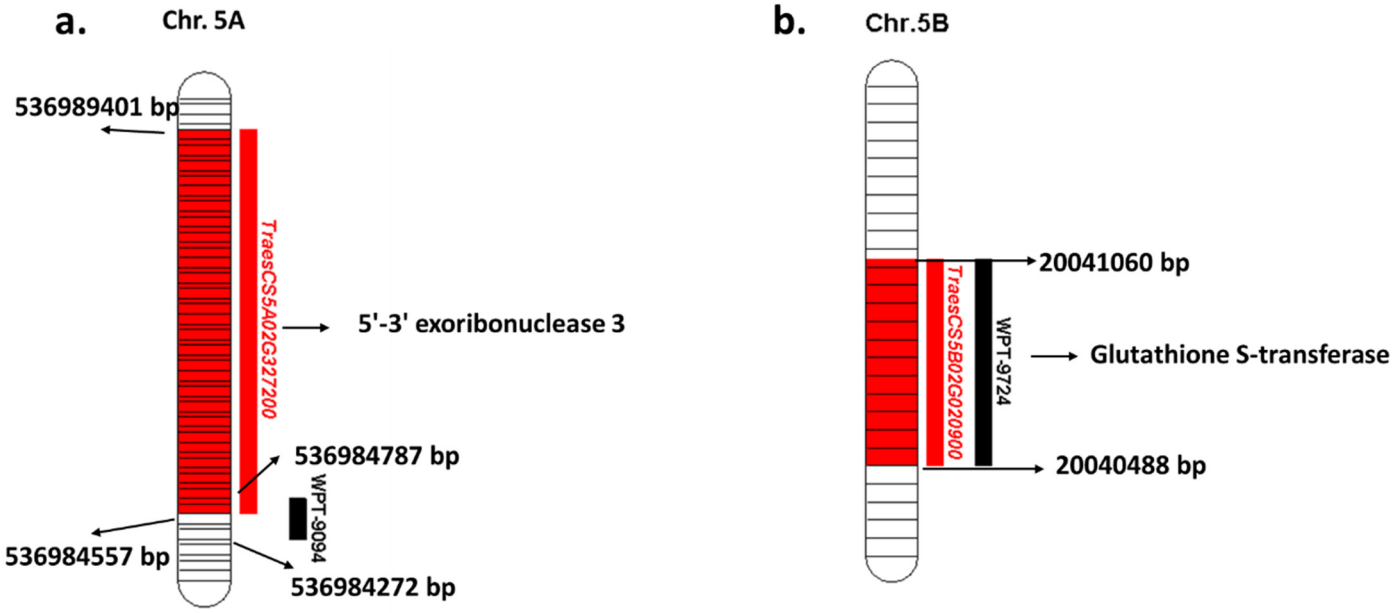

Figure 5. The chromosomal position of the significant DArT markers identified on chromosome 5A (**a**) and chromosome 5B (**b**), the identified gene models that harboring these markers in bp and their functional annotation based on the international wheat genome sequencing consortium (IWGSC V.2).

In the remaining part of our manuscript, we focused only on the significant markers harboring high-confidence gene models. As a result, two markers–WPT-9094 (susceptible effect, 5A), and WPT-9724 (resistant effect, 5B)–were taken into account. The presence of these two markers and their coefficient of infection were investigated in the resistant and susceptible genotypes. The percentage of agreement between the presence of each marker and the expected reaction based on the marker effect was calculated in each group of genotypes (Figure 6). A high percentage of agreement was found for both markers in the resistant genotypes, with a percentage of 85.7%. Meanwhile, a lower percentage of agreement for both markers was found in the susceptible genotypes, with a percentage of 53.33% and 60.00% for WPT-9094 and WPT-9724, respectively.

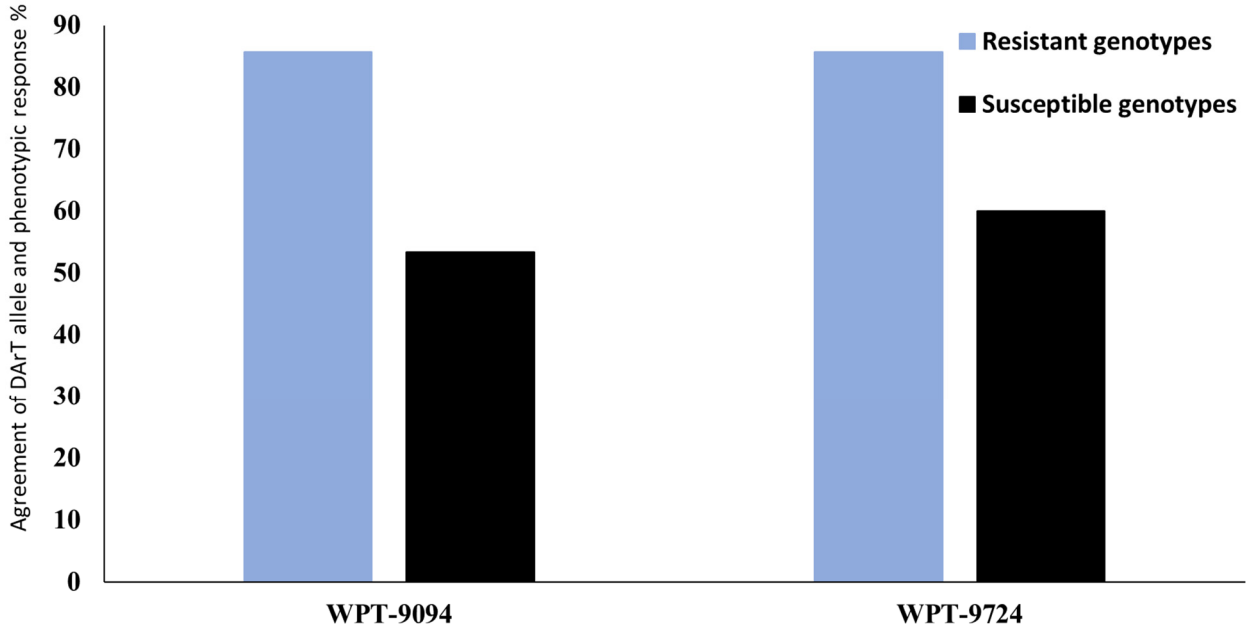

Figure 6. Percentage of agreement between the phenotypic reaction (resistant and susceptible) of the tested genotypes and the presence of the significant DArT markers in the genome of the tested genotypes.

Furthermore, the expression of the two identified gene models harboring significant markers was investigated using the Wheat-Expression database. Higher expression of both genes was found at the reproductive growth stage for both genes under disease conditions compared with the controls. However, the same expression of the TraesCS5A02G327200 gene model was found under both controlled and disease conditions at the vegetative growth stage. Besides this, very little expression was found for the TraesC25B02G020900 gene model under disease conditions with a value of 0.011 tpm compared with 0.00 tpm under controlled conditions (Figure 7).

3.5. Selection of Superior Genotypes for Stripe Rust Resistance in the Studied Materials

To genetically emphasize resistance in the common resistant genotypes listed in Table S3, the number of targeted alleles of the 13 DArT markers associated with the resistance was investigated in each genotype (Figure 8a). Unfortunately, no available DArT marker data were available for the three highly resistant Egyptian genotypes. The two German resistant genotypes (PI_313101 and PI_343730) contained all the targeted alleles for the 13-significant DArT markers. The Saudi Arabian genotype PI_574346 had the lowest number of targeted alleles (seven alleles). The number of targeted alleles in the genotypes from the U.K. and the U.S. ranged from eight to 12 alleles.

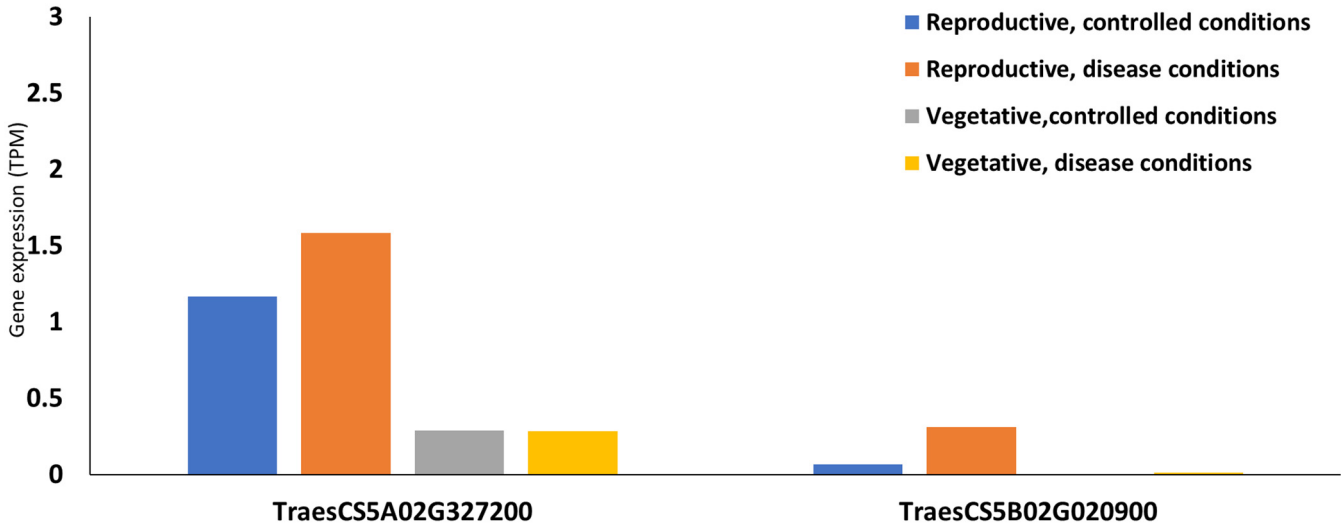

Figure 7. The expression of the two gene models harboring significant DArT markers in transcripts per million (TPM). Blue and orange columns represent the expression of the gene models at the reproductive growth stage under controlled and disease conditions, respectively, while grey and yellow columns represent the expression of the two models at the vegetative growth stage under the same conditions.

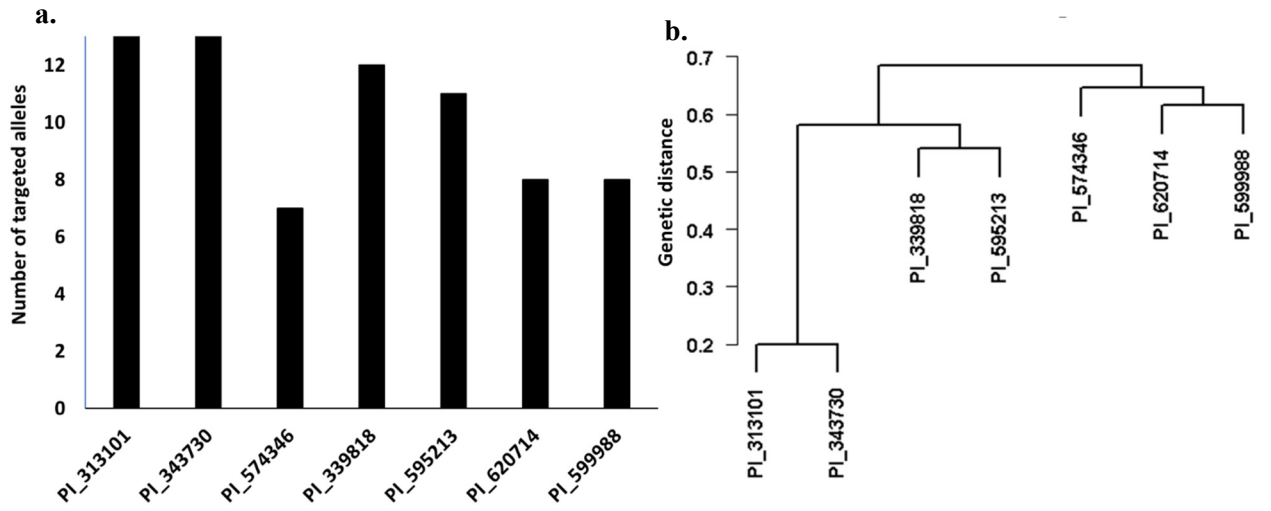

Figure 8. (**a**) Number of targeted alleles of DArT markers significantly associated with stripe rust resistance in the highly resistant genotypes (with a coefficient of infection of 4% or less), (**b**) The genetic distance between each pair of the high resistance genotypes with a coefficient of infection of 4% or less.

The genetic distance between each pair of the resistant genotypes was calculated to confirm their suitability to be crossed for improving stripe rust resistance (Figure 8b and Table S6). The genetic distance ranged from 0.20 between the two German genotypes (PI_313101 and PI_343730) to 0.74 between the German genotype PI_313101 and the American genotype PI_599988. The Saudi Arabian genotype PI_574346 was genetically near to the two American genotypes PI_620714 and PI_599988, while the third American genotype PI_595213 was found to have a small genetic distance from the English genotype PI_339818.

4. Discussion

The appearance of new stripe rust races has led to a lack of resistance in many Egyptian wheat genotypes. This situation threatens wheat production in Egypt, North Africa and the Middle East. As a result, new sources are urgently needed to improve resistance in the Egyptian wheat germplasm. The evaluation of the recent panel that represents new and old cultivars of spring wheat from different 19 countries will enable the identification of

the possible source of resistance genes and enhance the future of wheat breeding in the Middle East.

4.1. Genetic Variation of Stripe Rust Resistance in the Tested Genotypes

The high percentage of CI% (more than 93.3%) in both susceptible checks–Morocco and Theater–indicated that the natural infection occurred sufficiently in both years and that our evaluation is accurate. Highly significant differences were found amo ng the tested genotypes in their adult plant resistance to the Egyptian races of stripe rust which indicated the success of our evaluation and the high genetic variation that existed in our tested materials (Table 1). The percentage of CI ranged from 0–100% in both years (2018/2019–2019/2020), confirming the high genetic variation. The presence of high genetic variation among the tested genotypes is very useful in selecting the resistant genotypes against the Egyptian stripe rust race(s). Highly significant differences were found between the years, and the genotypes x years interaction indicated the different responses of the genotypes in 2018/2019 and 2019/2020. The high degree of broad-sense heritability (H^2_B = 0.63) indicated that most of the phenotypic variation in stripe rust resistance is due to genotypic variation. This concluded that the phenotypic variation is stable, and that the selection of highly resistant genotypes will be successful in future breeding programs. The presence of a highly significant correlation between the two years (r = 0.61, p-value <0.01) could serve to increase the resistance in the different years under the Egyptian environmental conditions, as well as suggesting that selection in one year could be a good predictor of the next year.

Out of the tested genotypes, ten genotypes were resistant to stripe rust in both years, with a percentage of CI of 4% or less. These genotypes are cultivars from five different countries: Saudi Arabia (one genotype), the United Kingdom (one genotype), Germany (two genotypes), Egypt (three genotypes) and the United States (three genotypes) (Table S2). The three Egyptian genotypes (Misr-2, Sids-14 and Shandweel-1) were previously reported to be resistant to the Egyptian race(s) of stripe rust, indicating the reliability of our dataset [38–40]. However, a CI% of 4% was found in Sids-14 in the 2019/2020 growing season, indicating the beginning of the overcoming of resistance in these genotypes. As a result, there is an urgent need to improve resistance in the Egyptian genotypes. Understanding the genetic control of resistance in these three genotypes will enable wheat breeders to improve resistance by increasing genes, controlling resistance against the Egyptian races. Genotypes from the remaining four countries (Germany, Saudi Arabia, the United Kingdom and the U.S.A) could be used to improve resistance in the Egyptian germplasm if they contain different genetic systems that control the resistance. Moreover, integrating exotic resistant genotypes will help to increase genetic diversity, which could be highly useful not only for stripe rust resistance but also for other agronomic features.

4.2. Genetic Analysis of Adult Plant Resistance to Stripe Rust in the Tested Genotypes

To effectively utilize the resistance presented in the recently tested genotypes, it is important to understand the genetic characterization of stripe rust resistance. In the recent study, 424-DArT markers of 71 genotypes were used in the genetic analysis to identify the genetic control of the stripe rust adult plant resistance. DArT markers have been reported as a cost-effective and high-throughput marker system that has been used to characterize different agronomic traits, as well as biotic and abiotic stress resistance in wheat using association mapping (AM) [24,41–46]. The AM relies mainly on testing the association between a single marker and a phenotypic trait. This could be done using different kinds of analysis, such as genome-wide association study (GWAS), which requires more than 100 genotypes to be efficient [47], and SMA, which could be done using any number of genotypes. In the recent study, the number of genotypes (71) is appropriate for SMA analysis [48]. Furthermore, studied DArT markers are well distributed across the three hexaploid wheat genomes (A, B, and D), with the highest number of markers on genome B and the least number on genome D (Figure S3a). Previous studies concluded

that genome B is the most diverse wheat genome, while genome D is the least diverse genome [49–56]. The average PIC value of the studied marker is 0.35, which is quite similar to previous studies [50,56,57]. This PIC value was reported as a good indicator of informative markers [58]. Based on the good distribution of the DArT markers and the appropriate PIC value, we can conclude that this set of DArT markers is highly appropriate for AM studies.

The PCA classified the tested genotypes into two groups. The resistant genotypes were located in the same group, which concluded the genetic similarity of the ten resistance genotypes despite their different origin. This could be illustrated by the gene flow of the different genotypes among the different regions. The high genetic similarity of the ten resistant genotypes concluded that improving stripe rust resistance in the Egyptian wheat background could be done using this set of genotypes. As a result, we can conclude that the studied panel is a highly diverse one, with a minimum effect of population structure on it, which makes this panel highly appropriate for AM [59].

4.3. Identification of Genomic Regions Associated with Stripe Rust Adult Plant Resistance

SMA identified a set of 13 stable DArT markers associated with stripe rust resistance in both years (Figure 4 and Table 2). Based on the chromosomal location of the identified DArT markers, there are several genomic regions distributed on ten different chromosomes controlling the resistance. Previous studies concluded that QTLs that explain less than 10% of the phenotypic variation are minor QTLs, while major QTLs explain more than 10% of the phenotypic variation [60–62]. In our study, the identified QTLs/genomic regions could be classified into minor QTLs (seven in 2018/2019 and four in 2019/2020) and major QTLs (six in 2018/2019 and nine in 2019/2020). The seven genomic regions which differ from minor to major effects in both years confirm the significant interaction between the genotypes and the years (GxY), as the response of the same genotype differed from year to year. Based on the allelic effect of the identified markers, six markers were found to be significantly associated with increasing the susceptibility of the genotypes to stripe rust, while the remaining seven markers were associated with increasing the resistance (Table 2). The identified significant markers were distributed across the wheat genome, which indicates the presence of many QTLs controlling resistance across the wheat genome. Previous studies concluded that there are many QTLs controlling stripe rust resistance in wheat, and that these QTLs are distributed across the wheat genome, which confirmed our results [31,63–69].

To further investigate the genetic association between the significant markers and stripe rust resistance, genomic regions carrying the sequence of the significant DArT markers were investigated. The available sequences were blasted against the wheat genome using the EnsemblPlants database to determine the exact position of these significant markers. EnsemblPlants was recorded as an integrative approach that provides the useful genome-sequence information of several plant species, including wheat [70]. The result of the sequence blasts in the EnsemblPlants database should be taken carefully, and filtration should be done based on the percentage of identity, *p*-value and blast length [70]. In our recent study, the high percentage of identity (ID%) that ranged from 85.5 to 100%, the highest blast length and low *p*-values indicated that the results obtained in this study could be trusted. Out of the 13 significant DArT markers, five were located near high-confidence gene models annotated to be related with disease resistance in plants, and two markers (WPT-9094 (5A), and WPT-9724 (5B)) were located within high-confidence gene models based on the IWGSC v2.0 database. The ID% of these two markers were very high, with a percentage of 98.4 and 99.8% for each marker, respectively. In addition, the blast lengths of both markers were very high, indicating that the identified position is the exact position of the targeted markers.

The DArT marker "WPT-9094" on chromosome 5A was found to be overlapping TraesCS5A02G327200 gene model with a size of 230bp, indicating that this marker is a part of the identified gene model. The functional annotation of this gene model was

associated with 5′-3′ exoribonuclease 3. The putative function of this protein is the exosome complex exonuclease RRP46 (Ribosomal RNA-processing protein 46), which is a protein involved in rRNA processing and a component of the exosome 3′–>5′ exonuclease complex. Recent studies concluded that some ribosomal genes are involved in plant defense and cell death [71–73]. Therefore, the abundance of ribosomal RNA and protein modifies the plant's metabolism, destroys the plant's defense system and facilitates cell-death development upon the pathogen attack [74]. The functional annotation of this gene model is in agreement with the allele effect of the associated DArT marker, which confirms our results. Furthermore, the WPT-9094 DArT marker was mapped on the position 58.3 cM on the long arm of chromosome 5A, and was surrounding two QTLs controlling stripe rust adult plant resistance [66]. Two stripe rust resistance genes were previously mapped on 5AL, *Yr48* and *Yr34* [69]. However, comparing the position of their linked markers (WPT-7061 and KASP markers) and the position of WPT-9094, they were found to be far from each other and harboring different gene models (TraesCS5A02G558500, for WPT-7061 marker). In addition, previous studies identified major stable QTLs on the long arm of chromosome 5A (220–226 cm), controlling stripe rust adult plant resistance [75]. Based on this study, the identified gene model is far away from previously reported gene models associated with stripe rust resistance. Comparing our results with those of previous studies, we can conclude that the gene model identified by the WPT-9094 marker could be a novel gene associated with stripe rust adult plant resistance. The higher percentage of agreement between the absence of the significant DArT marker in the resistance genotypes (85.7%) and lower agreement with the presence of this marker in the susceptible genotypes concluded that the WPT-9094 marker could be used for MAS for stipe rust resistance under Egyptian conditions. More studies should be conducted to provide more information about the gene model and the identified DArT marker.

The sequence of the DArT marker "WPT-9724" on chromosome 5B was found to be the same sequence as that of the gene model TraesCS5B02G020900. This gene model was found to be functionally annotated as Glutathione S-transferase. This enzyme plays an important role in plant-pathogen interaction, which increases plant resistance against pathogens by the detoxification of toxic lipid hydroperoxides that accumulate during infections and induce a systemic resistance response (ISR) to subsequent pathogen infections [76]. The allele effect of the DArT marker was found to increase resistance and reduce stripe rust symptoms, which confirms the function annotation of the linked gene model. The DArT marker "WPT-9724" has been mapped on the long arm of the 5B chromosome with the position of 199.7 cM [77]. It was previously reported to be associated with stem rust resistance in wheat [78]. This DArT marker was not associated with stripe rust resistance under U.S. conditions based on Maccaferri et al.'s (2015a) QTL map, which concluded that stripe rust races are different in the U.S.A. from those that exist in Egypt, and both have two different genetic systems controlling their resistance in wheat. Some stripe rust resistance genes such as *Yr3*, in addition to many QTLs controlling stripe rust resistance, were mapped on chromosome 5B, which supported our results. However, no information is available about the effectiveness of the *Yr3* resistance gene against the Egyptian race(s). Based on our result, the gene model TraesCS5B02G020900 seems to be a novel stripe rust resistance gene that could be markedly selected using the DArT marker WPT-9724. The high agreement between the presence of this DArT marker in the resistant genotypes (85.7%) and the absence of the marker in the susceptible genotypes (60%) could be explained as the resistant alleles masking other resistance alleles, although when they are present, other resistant alleles could be involved. However, these results concluded that more caution should be taken when using this DArT marker in MAS for stripe rust resistance in wheat. More studies are needed to provide more information about the association between the DArT marker and the identified gene model.

4.4. Selection of Superior Genotypes to Stripe Rust Resistance in the Currently Studied Genotypes

To confirm the possibility of improving stripe rust resistance against the Egyptian race(s) using the currently studied materials, the number of the targeted alleles of the common significant DArT markers was investigated in each one of the high resistance genotypes. Unfortunately, no DArT markers were available for the three Egyptian resistant genotypes. As a result, we could not confirm the possibility of improving resistance in the current Egyptian genotypes. However, we would be able to select the best genotypes to be crossed together in order to pyramid many resistance genes against the Egyptian race(s). Genotypes with as many as pyramid resistance genes could be used directly as an Egyptian cultivar if they are highly adapted to the Egyptian environment, or could be crossed with the Egyptian genotypes after confirming that they carry different resistance alleles. The presence of different targeted alleles/genotypes confirms that resistance against the Egyptian race(s) of stripe rust could be improved using the current materials. The two German genotypes seem to be the best genotypes to improve resistance against the Egyptian race, as they carry all the targeted alleles of the thirteen DArT markers.

The genetic distance between the two German genotypes and the five remaining genotypes was high and ranged from 0.56 to 0.74. Previous studies concluded that crossing between genotypes with a high genetic distance produces lines with a high combining ability and distinct alleles controlling the trait [79]. Therefore, crossing between any of these two German genotypes and the remaining five genotypes will be useful. In addition, the genetic distance between each pair of the remaining five genotypes was high, confirming the successful crossing to pyramid many resistant genes in one genotype.

5. Conclusions

In conclusion, the high genetic variation in the 95 genotypes tested for their resistance against the Egyptian race(s) of stripe rust indicates that stripe rust-resistant genotypes could be selected from the studied materials. A set of thirteen DArT markers significantly associated with the resistance were identified. Out of these significant markers, two markers, WPT-9094 on chromosome 5A and WPT-9724 on chromosome 5B, were located within gene models functionally annotated to be associated with disease resistance in the plant. These two markers could be used in MAS for stripe rust resistance in wheat. The two gene models seem to be novel genes controlling resistance, because to our knowledge their locations do not match the location of any genes previously associated with stripe rust resistance. The two highly resistant German genotypes could be used to improve resistance against the Egyptian race(s) of stripe rust, as they contain all the targeted alleles of the significant DArT markers and are genetically highly distant from the remaining resistant genotypes; thus crossing will be successful.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11122585/s1, Figure S1: Histogram represents the number of genotypes/country of the 95-spring wheat genotypes used in the current study, Figure S2: Correlation between the coefficient of infection (CI%) in 2018/2019 and 2019/2020, Figure S3: Histogram represents the distribution of the 424-DArT markers in the spring wheat tested genotypes (a) and the distribution of polymorphic information content (PIC) values of the tested markers, Histogram represents the distribution of polymorphic information content (PIC) values of the tested markers (b). Table S1: List of the 95-spring wheat genotypes used in the current study, their country of origin, and their response to the stripe rust infection (disease severity and infection type) in the two growing seasons. Table S2: Common susceptible genotypes (50% or more) in both years. Table S3: Common resistant genotypes (4% or less) in both years. Table S4: Significant DArT markers for stripe rust resistance in the season of 2018/2019, Table S5: Significant DArT markers for stripe rust resistance in the season of 2019/2020, Table S6: The genetic distance between each pair of the high resistance genotypes base on the 424 DArT markers.

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the manuscript. The authors agreed to be accountable for the content of the work. All authors have read and agreed to the published version of the manuscript.

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References

1. Juliana, P.; Singh, R.P.; Singh, P.K.; Poland, J.A.; Bergstrom, G.C.; Huerta, J.; Bhavani, S.; Crossa, J.; Sorrells, M.E. Genome-wide association mapping for resistance to leaf rust, stripe rust and tan spot in wheat reveals potential candidate genes. *Theor. Appl. Genet.* **2018**, *131*, 1405–1422. [CrossRef] [PubMed]
2. Food and Agriculture Organization of the United Nations. FAO FAOSTAT Database. 2017. Available online: https://www.fao.org/home/en (accessed on 9 December 2021).
3. Riaz, A.; Athiyannan, N.; Periyannan, S.K.; Afanasenko, O.; Mitrofanova, O.P.; Platz, G.J.; Aitken, E.A.B.; Snowdon, R.J.; Lagudah, E.S.; Hickey, L.T.; et al. Unlocking new alleles for leaf rust resistance in the Vavilov wheat collection. *Theor. Appl. Genet.* **2018**, *131*, 127–144. [CrossRef]
4. Wellings, C.R. Global status of stripe rust: A review of historical and current threats. *Euphytica* **2011**, *179*, 129–141. [CrossRef]
5. Kumar, S.; Archak, S.; Tyagi, R.K.; Kumar, J.; Vk, V.; Bhagat, I.; Meeta, M.; Bains, N.S.; Chowdhury, A.K.; Saha, B.C.; et al. Evaluation of 19,460 Wheat Accessions Conserved in the Indian National Genebank to Identify New Sources of Resistance to Rust and Spot Blotch Diseases. *PLoS ONE* **2016**, *11*, e0167702. [CrossRef]
6. Chen, X.M. Epidemiology and control of stripe rust [Puccinia striiformis f. sp. tritici] on wheat. *Can. J. Plant Pathol.* **2005**, *27*, 314–337. [CrossRef]
7. Chen, X. High-temperature adult- plant resistance, key for sustainable control of stripe rust. *Am. J. Plant Sci.* **2013**, *2013*, 608–627. [CrossRef]
8. Chen, X.M. Integration of cultivar resistance and fungicide application for control of wheat stripe rust Symposium contribution/Contribution à un symposium Integration of cultivar resistance and fungicide application for control of wheat stripe rust. *Can. J. Plant Pathol.* **2014**, *36*, 311–326. [CrossRef]
9. Markell, S.G.; Milus, E.A. Emergence of a novel population of Puccinia striiformis f. sp. tritici in eastern United States. *Phytopathology* **2008**, *98*, 632–639. [CrossRef]
10. Elbasyoni, I.S.; El-Orabey, W.M.; Morsy, S.; Baenziger, P.S.; Al Ajlouni, Z.; Dowikat, I. Evaluation of a global spring wheat panel for stripe rust: Resistance loci validation and novel resources identification. *PLoS ONE* **2019**, *14*, e0222755. [CrossRef]
11. Eltaher, S.; Baenziger, P.S.; Belamkar, V.; Emara, H.A.; Nower, A.A.; Salem, K.F.M.; Alqudah, A.M.; Sallam, A. GWAS revealed effect of genotype$\times$ environment interactions for grain yield of Nebraska winter wheat. *BMC Genomics* **2021**, *22*, 2. [CrossRef] [PubMed]
12. Bhavani, S.; Singh, P.K.; Qureshi, N.; He, X.; Biswal, A.K.; Juliana, P.; Dababat, A.; Mourad, A.M.I. Globally Important Wheat Diseases: Status, Challenges, Breeding and Genomic Tools to Enhance Resistance Durability. In *Genomic Designing for Biotic Stress Resistant Cereal Crops*; Kole, C., Ed.; Springer: New Delhi, India, 2021; pp. 59–128. ISBN 9783030758790.
13. Feng, J.; Wang, M.; See, D.R.; Chao, S.; Zheng, Y.; Chen, X. Characterization of novel gene Yr79 and four additional quantitative trait loci for all-stage and high-temperature adult-plant resistance to stripe rust in spring wheat PI 182103. *Phytopathology* **2018**, *108*, 737–747. [CrossRef]
14. Nsabiyera, V.; Bariana, H.S.; Qureshi, N.; Wong, D.; Hayden, M.J.; Bansa, U.K. Characterisation and mapping of adult plant stripe rust resistance in wheat accession Aus27284. *Theor. Appl. Genet.* **2018**, *131*, 1459–1467. [CrossRef] [PubMed]
15. Huang, S.; Liu, S.; Zhang, Y.; Xie, Y.; Wang, X.; Jiao, H.; Wu, S.; Zeng, Q.; Wang, Q.; Singh, R.P.; et al. Genome-wide wheat 55K SNP-based mapping of stripe rust resistance loci in wheat cultivar shaannong 33 and their alleles frequencies in current chinese wheat cultivars and breeding lines. *Plant Dis.* **2021**, *105*, 1048–1056. [CrossRef]
16. Maccaferri, M.; Zhang, J.; Bulli, P.; Abate, Z.; Chao, S.; Cantu, D.; Bossolini, E.; Chen, X.; Pumphrey, M.; Dubcovsky, J. A Genome-wide association study of resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*) in a worldwide collection of hexaploid spring wheat (*Triticum aestivum* L.). *G3 Genes | Genomes | Genet.* **2015**, *5*, 449–465. [CrossRef]
17. Li, J.; Dundas, I.; Dong, C.; Li, G.; Trethowan, R.; Yang, Z.; Hoxha, S.; Zhang, P. Identification and characterization of a new stripe rust resistance gene Yr83 on rye chromosome 6R in wheat. *Theor. Appl. Genet.* **2020**, *133*, 1095–1107. [CrossRef] [PubMed]
18. Roland, F.L. Stripe rust of wheat and barley in North America: A retrospective historical review. *Annu. Rev. Phytopathol.* **2002**, *40*, 75–118.

19. Myburg, A.A.; Griffein, A.R.; Sederoff, R.R.; Whetten, R.W. Comparative genetic linkage maps of Eucalyptus grandis, Eucalyptus globulus and their F1 hybrid based on a double pseudo-backcross mapping approach. *Theor. Appl. Genet.* **2003**, *103*, 1028–1042. [CrossRef]
20. Wenzl, P.; Carling, J.; Kudrna, D.; Jaccoud, D.; Huttner, E.; Kleinhofs, A.; Kilian, A. Diversity Arrays Technology (DArT) for whole-genome profiling of barley. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 9915–9920. [CrossRef]
21. Wittenberg, A.H.J.; Van Der Lee, T.; Cayla, C.; Kilian, A.; Visser, R.G.F.; Schouten, H.J. Validation of the high-throughput marker technology DArT using the model plant Arabidopsis thaliana. *Mol. Gen. Genom.* **2005**, *274*, 30–39. [CrossRef] [PubMed]
22. Akbari, M.; Wenzl, P.; Caig, V.; Carling, J.; Xia, L.; Yang, S.; Uszynski, G.; Mohler, V.; Lehmensiek, A.; Kuchel, H.; et al. Diversity arrays technology (DArT) for high-throughput profiling of the hexaploid wheat genome. *Theor. Appl. Genet.* **2006**, *113*, 1409–1420. [CrossRef]
23. Tinker, N.A.; Kilian, A.; Wight, C.P.; Heller-, K.; Wenzl, P.; Rines, H.W.; Bjørnstad, Å.; Howarth, C.J.; Jannink, J.; Anderson, J.M.; et al. New DArT markers for oat provide enhanced map coverage and global germplasm characterization. *BMC Genomics* **2009**, *22*, 39. [CrossRef]
24. Jighly, A.; Oyiga, B.C.; Makdis, F.; Nazari, K. Genome-wide DArT and SNP scan for QTL associated with resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*) in elite ICARDA wheat (*Triticum aestivum* L.) germplasm. *Theor. Appl. Genet.* **2015**, *128*, 1277–1295. [CrossRef]
25. Bemister, D.H.; Semagn, K.; Iqbal, M.; Randhawa, H.; Strelkov, S.E.; Spaner, D.M.; Bemister, D.H.; Semagn, K.; Iqbal, M.; Strelkov, S.E.; et al. Mapping QTL Associated with Stripe Rust, Leaf Rust, and Leaf Spotting in a Canadian Spring Wheat Population. *Crop. Sci.* **2019**, *59*, 650–658. [CrossRef]
26. Chen, T.; Tantasawat, P.A.; Wang, W.; Gao, X.; Zhang, L. Population structure of Chinese southwest wheat germplasms resistant to stripe rust and powdery mildew using the DArT-seq technique. *Ciência Rural. St. Maria* **2018**, *48*, 1–9. [CrossRef]
27. Ahmed, A.A.M.; Mohamed, E.A.; Hussein, M.Y.; Sallam, A. Genomic regions associated with leaf wilting traits under drought stress in spring wheat at the seedling stage revealed by GWAS. *Environ. Exp. Bot.* **2021**, *184*, 104393. [CrossRef]
28. Peterson, R.; Campbell, A.; Hannah, A. A Diagrammitic scale for estimating rust intensity on leaves and stems of cereals. *Can. J. Res.* **1948**, *26*, 496–500. [CrossRef]
29. Stakman, E.C.; Stewart, D.M.; Loegering, W.Q. *Identification of Physiologic Races of Puccinia Graminis Var. Tritici*; USDA_ARS: Washington, DC, USA, 1962.
30. Roelfs, A.P.; Singh, R.P.; Saari, E.E. *Rust Diseases of Wheat: Concepts and Methods of Disease Management*; CYMMIT: Texcoco, Mexico, 1992; ISBN 968612747X.
31. Abou-Zeid, M.A.; Mourad, A.M.I. Genomic regions associated with stripe rust resistance against the Egyptian race revealed by genome-wide association study. *BMC Plant Biol.* **2021**, *21*, 42. [CrossRef] [PubMed]
32. Shewaye, Y.; Taddesse, W. Association mapping of seedling and adult plant resistance for stripe rust resistance in spring bread wheat (*Triticum aestivum* L.). *Adv. Crop. Sci. Technol.* **2018**, *6*, 2–11. [CrossRef]
33. R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. 2020. Available online: https://www.R-project (accessed on 9 December 2021).
34. Maccaferri, M.; Ricci, A.; Salvi, S.; Milner, S.G.; Noli, E.; Martelli, P.L.; Casadio, R.; Akhunov, E.; Scalabrin, S.; Vendramin, V.; et al. A high-density, SNP-based consensus map of tetraploid wheat as a bridge to integrate durum and bread wheat genomics and breeding. *Plant Biotechnol. J.* **2015**, *13*, 648–663. [CrossRef] [PubMed]
35. Liu, K.; Muse, S.V. PowerMarker: An integrated analysis environment for genetic marker analysis. *Bioinformatics* **2005**, *21*, 2128–2129. [CrossRef] [PubMed]
36. Bradbury, P.J.; Zhang, Z.; Kroon, D.E.; Casstevens, T.M.; Ramdoss, Y.; Buckler, E.S. TASSEL: Software for association mapping of complex traits in diverse samples. *Bioinformatics* **2007**, *23*, 2633–2635. [CrossRef] [PubMed]
37. Voorrips, R. MapChart: Software for the graphical presentation of linkage maps and QTLs. *J. Hered.* **2002**, *93*, 77–78. [CrossRef] [PubMed]
38. Omara, R.; Abu Aly, A.A.M.; Abou-zeid, M. Characterization of partial resistance to stripe rust (Puccinia striiformis f. sp. tritici) in some Egyptian wheat cultivars. *J. Plant Prot. Path. Mansoura Univ.* **2018**, *9*, 111–119. [CrossRef]
39. Minaas, E.S.; Walid, M.E.O.; Reda, I.O. Seedling and adult plant resistance to leaf rust in some Egyptian wheat genotypes. *Afr. J. Agric. Res.* **2016**, *11*, 247–258. [CrossRef]
40. Shahin, A.A.; Omar, H.A.; El-Sayed, A.B.B. Characterization of Yr18/Lr34 partial resistance gene to yellow rust in some Egyptian wheat cultivars. *Egy. J. Plant Prot. Res.* **2018**, *6*, 1–9.
41. Mourad, A.M.I.; Sallam, A.; Belamkar, V.; Wegulo, S.; Bai, G.; Mahdy, E.; Bakheit, B.; El-wafa, A.A.; Jin, Y.; Baenziger, P.S. Molecular marker dissection of stem rust resistance in Nebraska bread wheat germplasm. *Sci. Rep.* **2019**, *9*, 11694. [CrossRef]
42. Mourad, A.M.I.; Sallam, A.; Belamkar, V.; Wegulo, S.; Bowden, R.; Jin, Y.; Mahdy, E.; Bakheit, B.; El-Wafaa, A.A.; Poland, J.; et al. Genome-wide association study for identification and validation of novel SNP markers for Sr6 stem rust resistance gene in bread wheat. *Front. Plant Sci.* **2018**, *9*, 380. [CrossRef]
43. Letta, T.; Olivera, P.; Maccaferri, M.; Jin, Y.; Ammar, K.; Badebo, A.; Salvi, S.; Noli, E.; Crossa, J.; Tuberosa, R. Association mapping reveals novel stem rust resistance loci in durum wheat at the seedling stage. *Plant Genome* **2014**, *7*, 1–13. [CrossRef]
44. Cane, M.A.; Maccaferri, M.; Nazemi, G.; Salvi, S.; Francia, R.; Colalongo, C.; Tuberosa, R. Association mapping for root architectural traits in durum wheat seedlings as related to agronomic performance. *Mol. Breed.* **2014**, *34*, 1629–1645. [CrossRef]

45. Marone, D.; Panio, G.; Ficco, D.B.M.; Russo, M.A.; De Vita, P.; Papa, R.; Rubiales, D.; Cattivelli, L.; Mastrangelo, A.M. Characterization of wheat DArT markers: Genetic and functional features. *Mol. Gen. Genom.* **2012**, *287*, 741–753. [CrossRef] [PubMed]
46. Ollier, M.; Talle, V.; Laure, A.; Zoé, B.; Bihan, L.; Duerr, S.; Lemmens, M.; Goudemand, E.; Robert, O.; Louis, J.; et al. QTL mapping and successful introgression of the spring wheat-derived QTL Fhb1 for Fusarium head blight resistance in three European triticale populations. *Theor. Appl. Genet.* **2020**, *133*, 457–477. [CrossRef]
47. Kumar, J.; Pratap, A.; Solanki, R.K.; Gupta, D.S.; Goyal, A.; Chaturvedi, S.K.; Nadarajan, N.; Kumar, S. Genomic resources for improving food legume crops. *J. Agric. Sci.* **2012**, *150*, 289–318. [CrossRef]
48. Alqudah, A.M.; Sallam, A.; Baenziger, P.S.; Börner, A. GWAS: Fast-forwarding gene identification and characterization in temperate Cereals: Lessons from Barley–A review. *J. Adv. Res.* **2020**, *22*, 119–135. [CrossRef]
49. Dubcovsky, J.; Dvorak, J. Genome plasticicty a key factor in the success of polyploid wheat under domestication. *Science* **2007**, *316*, 1862–1866. [CrossRef]
50. Eltaher, S.; Sallam, A.; Belamkar, V.; Emara, H.A.; Nower, A.A.; Salem, K.F.M.; Poland, J.; Baenziger, P.S. Genetic diversity and population structure of F3:6 Nebraska Winter wheat genotypes using genotyping-by-sequencing. *Front. Genet.* **2018**, *9*, 76. [CrossRef]
51. Nielsen, N.H.; Backes, G.; Stougaard, J.; Andersen, S.U.; Jahoor, A. Genetic diversity and population structure analysis of European hexaploid bread wheat (*Triticum aestivum* L.) varieties. *PLoS ONE* **2014**, *9*, e94000. [CrossRef]
52. Röder, M.S.; Korzun, V.; Wendehake, K.; Plaschke, J.; Tixier, M.H.; Leroy, P.; Ganal, M.W. A microsatellite map of wheat. *Genetics* **1998**, *149*, 2007–2023. [CrossRef] [PubMed]
53. Liu, Y.-G.; Tsunewaki, K. Restriction Fragment Length Polymorphism(RFLP) analysis in wheat. II. Linkage maps of the RFLP sites in common wheat. *Japanese J. Genet.* **1991**, *66*, 617–633. [CrossRef] [PubMed]
54. Peng, J.; Korol, A.B.; Fahima, T.; Röder, M.S.; Ronin, Y.I.; Li, Y.C.; Nevo, E. Molecular genetic maps in wild emmer wheat, Triticum dicoccoides: Genome-wide coverage, massive negative interference, and putative quasi-linkage. *Genome Res.* **2000**, *10*, 1509–1531. [CrossRef] [PubMed]
55. Alipour, H.; Bihamta, M.R.; Mohammadi, V.; Peyghambari, S.A.; Bai, G.; Zhang, G. Genotyping-by-sequencing (GBS) revealed molecular genetic diversity of Iranian wheat landraces and cultivars. *Front. Plant Sci.* **2017**, *8*, 1293. [CrossRef]
56. Mourad, A.; Belamkar, V.; Baenziger, P.S. Molecular genetic analysis of spring wheat core collection using genetic diversity, population structure, and linkage disequilibrium. *BMC Genom.* **2020**, *21*, 434. [CrossRef]
57. Allen, A.M.; Barker, G.L.A.; Berry, S.T.; Coghill, J.A.; Gwilliam, R.; Kirby, S.; Robinson, P.; Brenchley, R.C.; D'Amore, R.; Mckenzie, N.; et al. Transcript-specific, single-nucleotide polymorphism discovery and linkage analysis in hexaploid bread wheat (*Triticum aestivum* L.). *Plant Biotechnol. J.* **2011**, *9*, 1086–1099. [CrossRef] [PubMed]
58. Salem, K.F.M.; Sallam, A. Analysis of population structure and genetic diversity of Egyptian and exotic rice (*Oryza sativa* L.) genotypes. *C. R. Biol.* **2015**, *339*, 1–9. [CrossRef]
59. Rostoks, N.; Ramsay, L.; Mackenzie, K.; Cardle, L.; Bhat, P.R.; Roose, M.L.; Svensson, J.T.; Stein, N.; Varshney, R.K.; Marshall, D.F.; et al. Recent history of artificial outcrossing facilitates whole-genome association mapping in elite inbred crop varieties. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 18656–18661. [CrossRef]
60. Mourad, A.M.I.; Sallam, A.; Belamkar, V.; Mahdy, E.; Bakheit, B.; El-wafaa, A.A.; Baenziger, P.S. Genetic architecture of common bunt resistance in winter wheat using genome- wide association study. *BMC Plant Biol.* **2018**, *18*, 280. [CrossRef] [PubMed]
61. Niu, X.; Zhu, Y.; Sun, Z.; Yu, S.; Zhuang, J.; Fan, Y. Identification and validation of quantitative trait loci for grain number in rice (*Oryza sativa* L.). *Agronomy* **2020**, *10*, 180. [CrossRef]
62. Hussain, W.; Baenziger, P.S.; Belamkar, V.; Guttieri, M.J.; Jorge, P.; Easterly, A.; Sallam, A.; Poland, J. Genotyping-by-Sequencing derived high-density linkage map and its application to QTL mapping of flag leaf traits in bread wheat. *Sci. Rep.* **2017**, *7*, 16394. [CrossRef] [PubMed]
63. Klarquist, E.F.; Chen, X.; Carter, A. Novel QTL for stripe rust resistance on chromosomes 4A and 6B in soft white winter wheat cultivars. *Agronomy* **2016**, *6*, 4. [CrossRef]
64. Lara, E.P. Mapping of Genomic Regions Associated with Agronomic Traits and Resistance to Diseases in Canadian Spring Wheat. Ph.D. Thesis, University of Alberta, Edmonton, AB, Canada, 2017.
65. Randhawa, M.; Bansal, U.; Valárik, M.; Klocova, B.; Dolezel, J.; Bariana, H. Molecular mapping of stripe rust resistance gene Yr51 in chromosome 4AL of wheat. *Theor. Appl. Genet.* **2014**, *127*, 317–324. [CrossRef] [PubMed]
66. Lan, C.; Rosewarne, G.M.; Singh, R.P.; Sybil, A.; Huerta-Espino, J.; Basent, B.R.; Zhang, Y.; Yang, E. QTL characterization of resistance to leaf rust and stripe rust in the spring wheat line Francolin # 1. *Mol. Breed.* **2014**, *34*, 789–803. [CrossRef]
67. Rosearne, G.M.; Herrera-Foessel, S.A.; Singh, R.; Huerta-Espine, J.; Lan, C.; He, Z. Quantitative trait loci of stripe rust resistance in wheat. *Theor. Appl. Genet.* **2013**, *126*, 2427–2449. [CrossRef] [PubMed]
68. Cheng, B.; Gao, X.; Cao, N.; Ding, Y.; Gao, Y.; Chen, T.; Xin, Z.; Zhang, L. Genome-wide association analysis of stripe rust resistance loci in wheat accessions from southwestern China. *J. Appl. Genet.* **2020**, *61*, 37–50. [CrossRef] [PubMed]
69. Qureshi, N.; Bariana, H.S.; Zhang, P.; McIntosh, R.; Bansal, U.K.; Wong, D.; Hayden, M.J.; Dubcovsky, J.; Shankar, M. Genetic relationship of stripe rust resistance genes Yr34 and Yr48 in wheat and identification of linked KASP markers. *Plant Dis.* **2018**, *102*, 413–420. [CrossRef] [PubMed]

70. Bolser, D.; Staines, D.M.; Kersey, E.; Kersey, P. Ensembl Plants: Integrating tools for visualizing, mining, and analyzing plant genomics data. In *Plant Bioinformatics: Mthods and Protocols*; Edwards, D., Ed.; Humana Press: New York, NY, USA, 2007; Volume 406, pp. 115–140, ISBN 9781597455350.
71. Quirino, B.F.; Normanly, J.; Amasino, R.M. Diverse range of gene activity during Arabidopsis thaliana leaf senescence includes pathogen-independent induction of defense-related genes. *Plant Mol. Biol.* **1999**, *40*, 267–278. [CrossRef] [PubMed]
72. Chekanova, J.A.; Shaw, R.J.; Wills, M.A.; Belostotsky, D.A. Poly(A) tail-dependent exonuclease AtRrp41p from Arabidopsis thaliana rescues 5.8 S rRNA processing and mRNA decay defects of the yeast ski6 mutant and is found in an exosome-sized complex in plant and yeast cells. *J. Biol. Chem.* **2000**, *275*, 33158–33166. [CrossRef]
73. Stirpe, F.; Battelli, M.G. Ribosome-inactivating proteins: Progress and problems. *Cell. Mol. life Sci.* **2006**, *63*, 1850–1866. [CrossRef]
74. Xi, L. A Negative Regulator of Ribosomal RNA Processing Specifies R-Gene-Independent Cell Death in Barley-Powdery Mildew Interactions. Diploma Thesis, Iowa State University, Ames, IA, USA, 2008.
75. Rosa, S.B.; Zanella, C.M.; Hiebert, C.W.; Brûlé-Babel, A.L.; Randhawa, H.S.; Shorter, S.; Boyd, L.A.; McCallum, B.D. Genetic characterization of leaf and stripe rust resistance in the Brazilian wheat cultivar toropi. *Phytopathology* **2019**, *109*, 1760–1768. [CrossRef]
76. Gullner, G.; Komives, T.; Király, L.; Schröder, P. Glutathione S-Transferase enzymes in plant-pathogen interactions. *Font. Plant Sci.* **2018**, *9*, 1836. [CrossRef]
77. Singh, A.; Knox, R.E.; DePauw, R.M.; Singh, A.K.; Cuthbert, R.D.; Campbell, H.L.; Shorter, S.; Bhavani, S. Stripe rust and leaf rust resistance QTL mapping, epistatic interactions, and co-localization with stem rust resistance loci in spring wheat evaluated over three continents. *Theor. Appl. Genet.* **2014**, *127*, 2465–2477. [CrossRef]
78. Singh, A.; Knox, R.E.; DePauw, R.M.; Singh, A.K.; Cuthbert, R.D.; Campbell, H.L.; Singh, D.; Bhavani, S.; Fetch, T.; Clarke, F. Identification and mapping in spring wheat of genetic factors controlling stem rust resistance and the study of their epistatic interactions across multiple environments. *Theor. Appl. Genet.* **2013**, *126*, 1951–1964. [CrossRef] [PubMed]
79. Bertan, I.; De Carvalho, F.I.F.; Oliveira, A.C. De Parental selection strategies in plant breeding programs. *J. Crop Sci. Biotechnol.* **2007**, *10*, 211–222.