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Discovering stable genomic regions linked to powdery mildew adult-plant resistance in wheat

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Wheat powdery mildew (WPM) is a devastating disease that causes significant yield losses worldwide. In Egypt, a lake in immune cultivars was reported and little is known about its genetic control. In this study, two different plant sets (the isoline set and the test set) were evaluated for WPM adult plant resistance (APR-WPM) over three cropping seasons (2021-2022, 2022-2023, and 2023-2024). The results of the isoline set revealed that the *Pm1b*, *Pm34*, *Pm36*, *MIAG12*, and *NACG13* resistance genes were stable among the three cropping seasons. The results of the evaluated set revealed highly significant differences among the cropping seasons as well as among the tested genotypes, suggesting the presence of different *Blumeria graminis* f. sp. *tritici* (*Bgt*) races and the potential to select highly resistant genotypes. Eight resistant genotypes belonging to five different countries, excluding Egypt, were identified. A genome-wide association study (GWAS) was performed using each cropping season separately as well as the average of the three seasons. GWAS was done using two different SNP data sets, GBS and 25K-SNP array. A total of 86 significant markers associated with resistance were identified. Among these markers, only four markers were stable across at least two seasons. Three gene models harboring these markers were detected and functionally annotated to be linked with disease resistance. Gene enrichment analysis revealed that each gene model regulated a single network of different biological processes and molecular function pathways. Among the resistant genotypes, one Sudanese genotype was selected for its strong WPM resistance and high yield potential. This genotype was also found to be highly adapted to the Egyptian environment and a high-yielding genotype. The incorporation of this genotype into future breeding programs could increase the resilience of Egyptian wheat germplasm.

1 Introduction

Among cereal crops, wheat (*Triticum aestivum*) is considered the most important crop that provides food for 35% of the world's population (Paux et al., 2008; Mourad et al., 2019; Mondal et al., 2021). Wheat powdery mildew (WPM, caused by *Blumeria graminis* f. sp *tritici* (*Bgt*)) is one of the most devasting foliar diseases affecting wheat worldwide and causes significant yield losses of 62% (Costamilan, 2005; Maxwell et al., 2009; El-Shamy et al., 2012; Huang et al., 2013; Singh et al., 2016; Jabran et al., 2023, 2024). In Egyptian fields, WPM occurs annually and has become more threatening than rust diseases (El-Shamy and Mohamed, 2022). The severity of fungal diseases, such as WPM, may increase the vulnerability of wheat to other biotic and abiotic stresses (Figueroa et al., 2018). Moreover, climate change is expected to exacerbate fungal disease outbreaks in the near future (Kissoudis et al., 2014). Therefore, greater efforts are needed to manage this disease effectively. Similarly, few studies have focused on WPM management under Egyptian conditions. These studies revealed the presence of *Pm* genes in Egyptian wheat germplasms and characterized different *Bgt* races in Egyptian fields (El-Shamy et al., 2012, 2016; Emara et al., 2016; Elsayed and Elkot, 2020; Draz et al., 2022; Mourad et al., 2024a).

As a fungal disease, WPM resistance can be classified into two main types: race-specific resistance (also known as seedling resistance) and race-non-specific resistance (also known as adult plant resistance) (Roelfs et al., 1992; Bhavani et al., 2021; Cheng et al., 2022). Pyramiding multiple seedling and adult plant resistance genes in the same genotype can provide a high level of broad-spectrum resistance to combat this serious disease (Bajgain et al., 2015; Mourad et al., 2018). Fungicide application is a potential method for WPM control. However, because of its harmful environmental effects, especially its long-term use, alternative control methods are needed (Goswami et al., 2018). One environmentally friendly approach is biological control, which involves the use of nonpathogenic organisms such as *Trichoderma asperellum* (T34) (Mourad et al., 2024c), *Aspergillus chevalieri* (BYST01) (Zhang et al., 2024), and *Bacillus* spp (Matzen et al., 2019; Shahin et al., 2019). However, further studies are needed to identify effective biological control agents for widespread use. Plant breeding has long been an effective method for identifying and pyramiding WPM resistant genes in wheat germplasm (Liang et al., 2019; Andersen et al., 2020; Bhavani et al., 2021). To date, 68 WPM resistant genes have been detected and mapped onto wheat chromosomes (He et al., 2021). However, some of these genes are linked to undesirable traits (Hurni et al., 2014). Therefore, identifying additional effective resistance genes or genomic regions is essential for improving WPM resistance.

Association mapping (AM) is an effective approach for detecting novel genomic regions controlling specific traits. Owing to advancements in sequencing methods in recent years, genome-wide association studies (GWASs) have been widely used to improve various traits in wheat (Alqudah et al., 2020). However, the number of GWASs conducted in the last five years to identify genomic regions controlling WPM resistance is very low (Kang et al., 2019; Mohler and Stadlmeier, 2019; Simeone et al., 2020; Miedaner et al., 2020; Alemu et al., 2021; Du et al., 2021; Hinterberger et al., 2022; Jin et al., 2022; Kaur et al., 2023; Mourad et al., 2024a; Bhadana et al., 2025). Interestingly, only one GWAS has been conducted to comprehensively understand the genetic control of WPM at the seedling stage under Egyptian conditions (Mourad et al., 2023). Additionally, numerous databases for functional annotations, gene expression, and gene enrichment have recently emerged, providing valuable insights into the functions of different wheat genomic regions (Bolser et al., 2007; Pearce et al., 2015; Ge et al., 2022). One of the valuable databases for enhancing the GWAS results is *EnsemblePlants* which provides detailed information on the gene models present in the same position as the significant SNP markers (Bolser et al., 2007). These gene models could be submitted to *Knetminer* (Hassani-Pak et al., 2021) and *ShinyGo* (Ge et al., 2022) databases to understand more about their role in controlling the studied trait based on their functional annotation. Therefore, integrating GWAS results with data from these databases will enhance our understanding of the genetic control of this trait. However, when GWAS and database findings are utilized to study disease resistance, it is crucial to consider the yield performance of the tested genotypes to avoid the development of highly resistant genotypes with low yield potential.

The objectives of this study are to: (1) investigate the genetic control of adult plant resistance to WPM (APR-WPM) under Egyptian field conditions; (2) identify marker-trait associations for WPM resistance across multiple cropping seasons; (3) detect stable genomic regions associated with the resistance; and (4) Select WPM-resistant genotypes with high yield potential for future breeding programmes.

2 Materials and methods

2.1 Plant materials

In this study, two distinct sets of plant materials were evaluated for WPM-APR: the "tested set" and the "isoline set". The tested set consisted of 196 spring wheat genotypes. A detailed description of this set has been provided previously (Esmail et al., 2023b; Mourad et al., 2024c). Briefly, it represents a highly diverse collection from 22 countries worldwide (Supplementary Table S1). Among these genotypes, 35 were Egyptian, including breeding lines, old cultivars, and new cultivars. Seeds of the Egyptian genotypes were sourced from Egyptian governorate, whereas seeds of the non-Egyptian genotypes were obtained from the USDA-ARS, Aberdeen, ID, the U.S.A.

The isoline set included 20 distinct lines, each carrying a different powdery mildew resistance gene (Supplementary Table S2). This set was included to identify the possible *Pm* resistant genes against the Egyptian *Bgt* races. Seeds for this set were obtained from Dr. Abdelrazek Abdelrhim at Minia University, Minia, Egypt, and had been previously evaluated for powdery mildew resistance under Egyptian field conditions (Abdelrhim et al., 2018).

2.2 Field evaluation of powdery mildew adult plant resistance

The powdery mildew resistance of the plants in the tested set and the isoline set was evaluated across three cropping seasons: 2021-2022, 2022-2023, and 2023-2024. The evaluations were conducted at the Experimental Farm of Sakha Agricultural Research Station, Agricultural Research Center, Egypt (31°5′12″N, 30°56′49″E). The experimental layout was carried out using a randomized complete block design (RCBD) with three replications per cropping season. The genotypes were sown at the end of November (optimum sowing date) each year.

Each tested genotype was planted in a 3-meter-long row spaced 30 cm apart, with 5 g of seeds per row. During each cropping season, the experiment area was surrounded by a 1.5 m² belt of the highly powdery mildew-susceptible variety "Jagalene". Artificial inoculation was conducted on this surrounding belt, which served as an infection spreader to ensure a uniform distribution of Bgt spores. Inoculation involved the use of Bgt conidia collected from naturally infected fields in the surrounding area. This was done by removing the propagated conidiospores from the infected plants and dispreading them over the spreader belt (Draz et al., 2023). Standard agronomic practices were applied according to recommendations for wheat cultivation in Egyptian fields.

The disease severity (DS) of powdery mildew was recorded once the susceptible check was fully covered with Bgt spores via a modified Cobb scale (0–9) as described previously in Peterson et al. (1948). The percentage of the leaf surface covered by mycelia was determined via Cobb's scale as follows: 0% = immune (no visible sign of infection); 1 = 10% of the leaf area covered by mycelia; 2 = 20%; 3 = 30%; 4 = 40%; 5 = 50%; 6 = 60%; 7 = 70%; 8 = 80%; and 9 = 90%. Genotypes with a percentage < 20% were considered resistant.

2.3 Statistical analysis of powdery mildew adult plant resistance

Analysis of variance (ANOVA) was conducted by combining DSs from the three cropping seasons, and PLABSTAT software (Utz, 1997) was used via the following method:

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

where Y_{ijk} is an observation of genotype i in replication j, which was planted in experiment k; μ is the general mean; g_i, r_i, and E_k are the main effects of genotypes (random effects), replications, and experiments (fixed effects), respectively; and e_{ijk} is the error.

Furthermore, for each experiment, the following model was used for ANOVA:

$$Y_{ij} = \mu + r_j + g_i + gr_{ij} + e_{ij}$$

where Y_{ij} is an observation of genotype i in replication j; μ is the general mean; g_i and r_j are the main effects of genotypes and replications, respectively; and e_{ij} is the error. The BLUP values

were calculated following the same model using the lme4 R package (Bates et al., 2015). BLUPs were used to adjust the phenotypic data scored across different seasons as well as in each season. Broad-sense heritability was calculated via the following formula:

$$H^2 = \sigma_G^2 / (\sigma_G^2 + \frac{\sigma_{GR}^2}{r})$$

where σ_G^2 and σ_R^2 are the variances of the lines and the residuals, respectively. r is the number of replicates.

2.4 Genome-wide association study for resistance, gene models controlling WPM-APR resistance, functional annotation, and gene enrichment

To determine the genetic control of powdery mildew resistance, GWAS was conducted using DS-BLUP values of each cropping season separately as well as the average of the three cropping seasons together. The rMVP package of R software (Yin et al., 2021) was used to run the GWAS. This package allows three different models of GWAS as follows: the generalized linear model (GLM), the mixed linear model (MLM), and the fixed and random model Circulating Probability Unification (FarmCPU), which includes principle component analysis (PCA) and/or kinship as a covariate. The best model for each experiment was determined on the basis of the Quantil-Quantil-Plot (QQ-plot), which represents the deviation of the distribution of the observed -log$_{10}$ (P-value) from the expected value. The significant SNP markers were identified as those whose P-value was ≤0.001 (-log$_{10}$≥3.00) (Ahmed et al., 2022; Mourad et al., 2024b).

Furthermore, gene models harboring significant markers were detected on the basis of the base-pair position of the markers and the presence of gene models in the same position via the $EnsemblePlants$ database (Bolser et al., 2007). Despite the $EnsemblePlants$ database utilizing the International Wheat Genome Sequencing Consortium (IWGSC) RefSeq v2.0 assembly for SNP positioning, gene annotation was based on IWGSC RefSeq v1.0, which currently offers more comprehensive and functionally curated gene models. While the coordinate systems between v1.0 and v2.0 are broadly compatible in well-anchored regions, minor differences in gene structure or gene IDs may occur. Furthermore, gene enrichment of the common gene models in both evaluations was detected based on the biological process, molecular function, and cellular component pathways via the $ShinyGo$ 0.76 database (Ge et al., 2022). To identify the most important pathways, a cutoff false discovery rate (FDR) p-value < 0.01 was applied.

2.5 Selection of superior genotypes to improve WPM-APR in Egyptian wheat germplasm

The most resistant genotypes were those with DS<20% in all three cropping seasons. Moreover, the genetic distance between

each pair of selected superior genotypes was calculated via neighbor-joining via TASSEL V5.2.19 software (Bradbury et al., 2007) and visualized as a phylogenetic tree via the iTol database (Letunic and Bork, 2021). For each evaluated genotype, the thousand kernel weight (TKW, g) was scored by measuring the weight of 100 kernels from each genotype in two cropping seasons, 2022-2023 and 2023-2024.

2.6 Yield and yield components of the selected genotypes under non-infected field conditions

The yield and yield components of the same tested plant materials (196 spring wheat genotypes) were evaluated in noninfected fields at the field of the Faculty of Agriculture, Assuit University, Asyut, Egypt (27°11′20.36″N, 31°10′06.45″E), for three cropping seasons: 2021–2022, 2022–2023, and 2023–2024. For each cropping season, the seeds of each tested genotype were sown in rows with a length of 1.5 m. The distance between the seeds in each row was 10 cm. The experimental design was a randomized complete block design (RCBD) with two replications in each cropping season. The irrigation and agricultural practices were performed as recommended for the wheat plants. To ensure the absence of infection, fungicides were applied as recommended.

The following traits were recorded for each genotype in each replication/cropping season: heading date (HD), plant height (PH, cm), spike length (SPL, cm), grain number of spikes (GN/SP), number of spikelets per spike (NS/SP), grain yield per spike (GY/SP, g), and thousand kernel weight (TKW, g).

3 Results

3.1 Evaluation of powdery mildew isoline set

Among the 20 evaluated isolines, none were immune in the three cropping seasons (Figure 1). However, NC06BGTAG13 line (carrying *NCAG13* gene) was immune in two cropping seasons (2022-2023 and 2023-2024) and resistant (DS = 5%) in the third cropping season. Moreover, 5-BIL29, NC06BGTAG12, NC96BGTD7, NC96BGTD3, and NC99BGTAG11 isolines (carrying *Pm36*, *MIAG12*, *Pm34*, *Pm35*, and *Pm37* gene, respectively) were resistant in at least two cropping seasons (DS<20%). MocZlatka (carrying *Pm1b* gene) was immune in only two cropping seasons (2021-2022 and 2023-2024), whereas it is not resistant in 2022-2023 (DS<20%). The remaining 13 isolines were not resistant in any cropping season.

3.2 Variation in WPM-APR in the tested plant set

The ANOVA revealed highly significant differences in WPM-APR among the three tested cropping seasons (Supplementary

FIGURE 1
The response of the tested powdery mildew isolines in each cropping season under Egyptian field conditions.
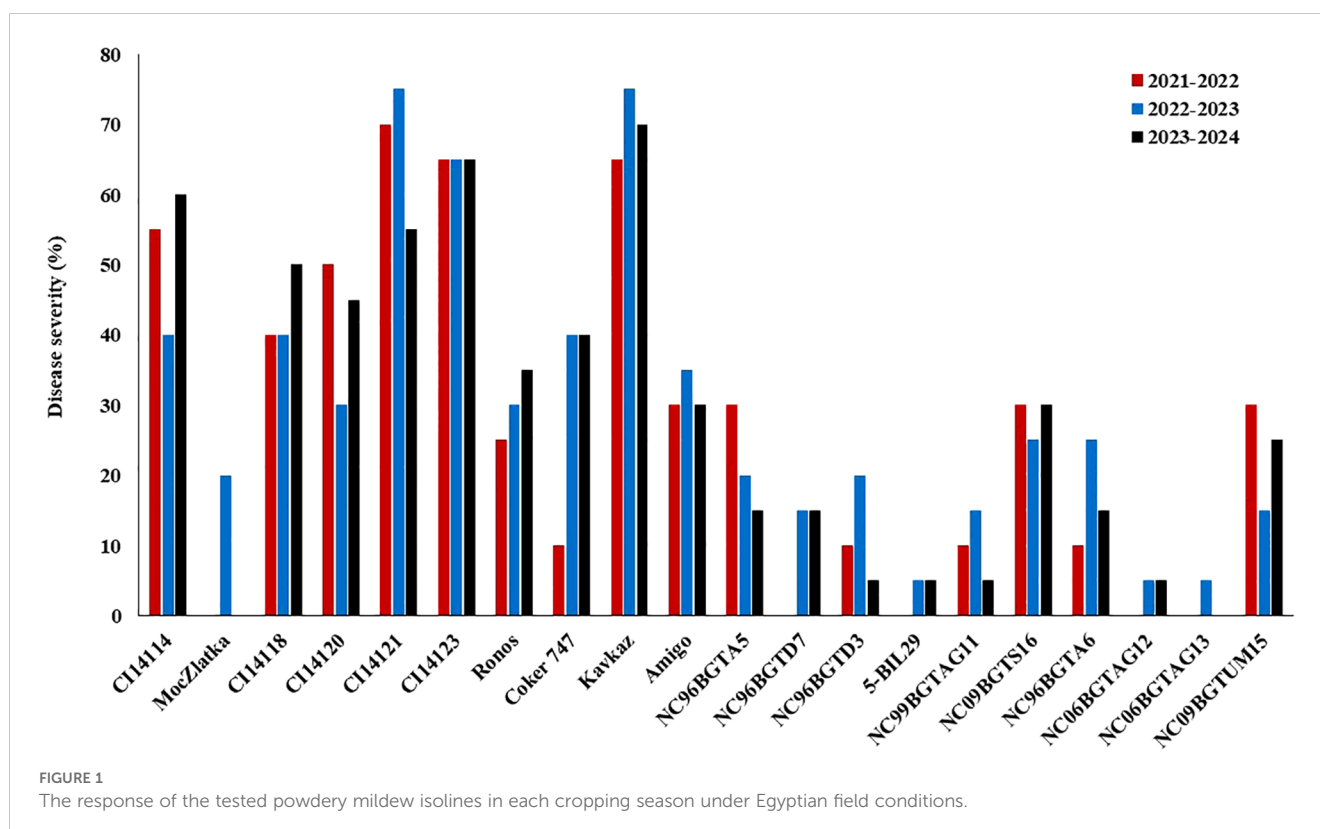
TABLE 1 Phenotypes of powdery mildew adult plant resistance in the three years of evaluation (2022, 2023, and 2024) representing the minimum, maximum, and mean of the resistance in each cropping season as well as standard deviation (SD), coefficient of variance (CV), and broad-sense heritability (H^2).

Cropping season	Min	Max	Mean	SD	C.V	H^2
2021/2022	13.33%	80.00%	48.02%	19.37	40.33%	0.89
2022/2023	13.33%	86.67%	44.84%	19.77	44.09%	0.92
2023/2024	3.33%	83.33%	37.55%	19.04	50.70%	0.94

Table S3). Furthermore, a highly significant G×Y interaction was detected. The resistance of the tested genotypes significantly differed during each cropping season (Supplementary Tables S4-S6). The disease severity ranged from 13.33% to 80%, with an average of 48.02% in the first cropping season (Table 1). However, it ranged from 13.33% to 86.67%, with an average of 44.84%, and from 3.33% to 83.33%, with an average of 37.55%, in 2022-2023 and 2023-2024, respectively. High degrees of broad-sense heritability (H^2) were detected in the three cropping seasons, with values of 0.89, 0.92, and 0.94 in the 2021-2022, 2022-2023, and 2023-2024 cropping seasons, respectively (Table 1). It was clearly noticed that the disease severity of WPM infection was almost the same in 2021-2022 and 2022-2023 cropping seasons. While the DS in 2023-2024 cropping season was completely different from 2021-2022 and 2022-2023 (Figure 2).

None of the tested genotypes were immune in any cropping season (Figure 3a). However, eight, 11, and 37 genotypes were found to be resistant to WPM (DS<20%) in 2021-2022, 2022-2023,

and 2023-2024, respectively. Notably, the majority of the tested genotypes were susceptible to WPM, with 188, 185, and 159 genotypes in 2021-2022, 2022-2023, and 2023-2024, respectively. Among the resistant genotypes in each cropping season, eight genotypes were resistant in all three seasons (Figure 3b). These genotypes were from Germany (three genotypes), U.S.A. (two genotypes), Sudan (one genotype), Kenya (one genotypes), and the U.K (one gneotype) (Table 2). The DS ranged from 6.67% for the German genotype "Carstens Sommerweizen" in 2023-2024 to 16.67% for the eight genotypes in both 2021-2022 and 2022-2023. Interestingly, none of the tested 35 Egyptian genotypes were immune or resistant to WPM during the three cropping seasons (Supplementary Figure S1). However, Giza-171 and Sohag 5 were resistant in two cropping seasons (DS<20%) and nonresistant in the third season. The remaining 33 Egyptian genotypes were found to be resistant in only one cropping season or susceptible in the three cropping seasons.
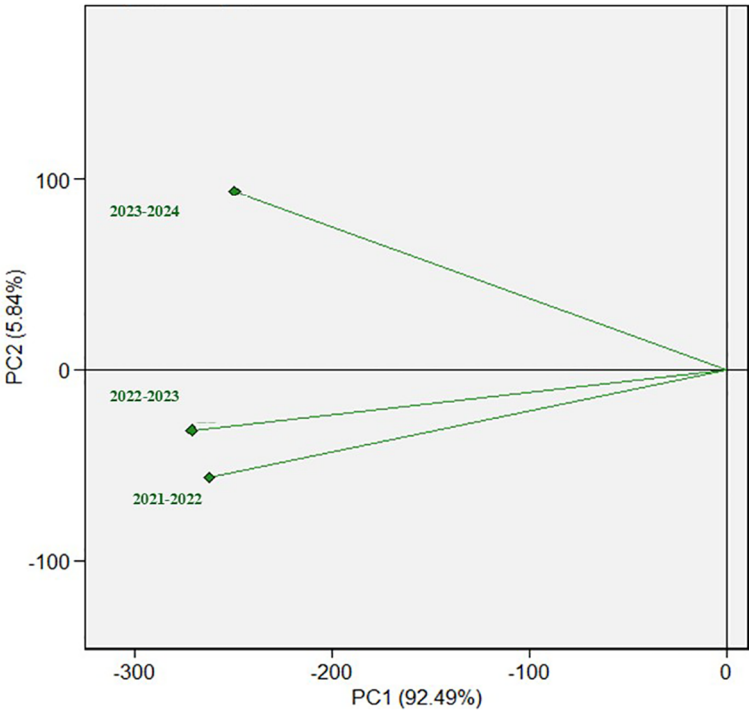

FIGURE 2
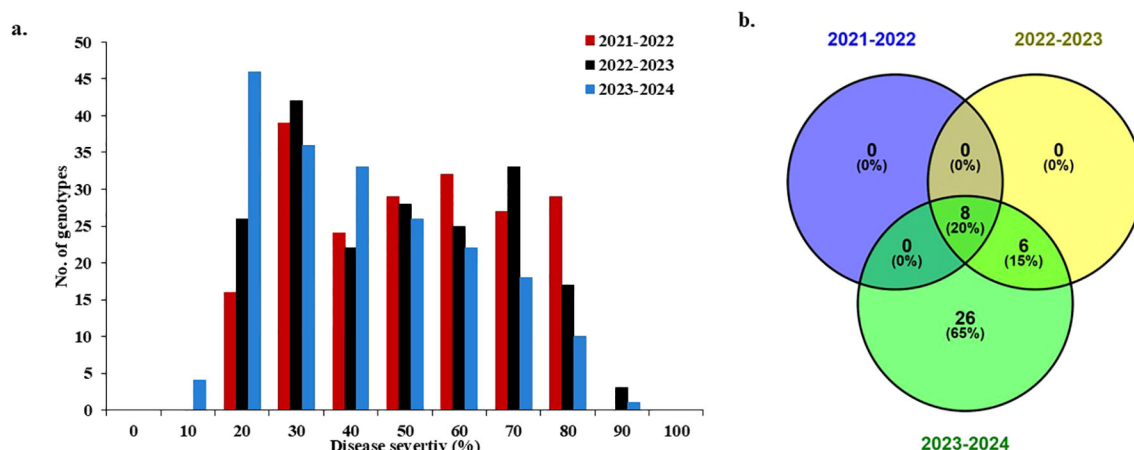The relationship among disease severity in the three cropping seasons.

FIGURE 3
The response of the 196-spring wheat genotypes to powdery mildew infection at the adult growth stage in the three evaluation growing seasons:
(a) histogram represents the distribution of disease severity (%) in each growing season. **(b)** number of resistant genotypes (with a value of disease
severity<20%) in each growing season and the number of commonly resistant genotypes in the three growing seasons.

3.3 Association mapping for WPM-APR

3.3.1 GWAS for WPM-APR in each cropping season

Owing to the highly significant differences in WPM-APR among the three cropping seasons, GWAS was carried out for each cropping season separately. Furthermore, GWAS was run on the basis of the average of the three cropping seasons to identify genomic regions commonly controlling the resistance of WPM-APR. Owing to the presence of three different subpopulations in the tested set (Mourad et al., 2020), PCA and/or kinship were included in each GWAS model. For each season, three different GWAS models were used for each SNP marker dataset. The Q-Q and Manhattan plots of the best model for each marker set in each cropping season are presented in Supplementary Figures S2, S3. The summarized results of the GWAS for each cropping season are presented in Table 3. The detailed results are presented in Supplementary Table S7.

A total of 26 markers were significantly associated with resistance in the 2021-2022 cropping season on the basis of both

25K-SNPs and GBS-SNPs (Table 3). These markers were distributed among 13 different chromosomes. The percentage of phenotypic variation explained by these markers ranged from 0.1% to 10.66%. The resistant alleles of these markers were found to reduce disease severity, ranging from -5.92 to -2.34, and were located within 11 different gene models. Among these gene models, only three were major genes, as they explained more than 10% of the phenotypic variation.

Furthermore, 37 significant markers that were distributed among 14 chromosomes were found to be significantly associated with resistance in the 2022-2023 cropping season. These markers explained 0.03–16.38% of the phenotypic variation. The allele effects of these markers ranged from -7.80 to -2.63. A total of 17 genes harbored these significant markers, with only four major genes.

The lowest number of significant markers associated with resistance was identified in the 2023-2024 cropping season, with 19 markers. These markers were distributed among eight chromosomes and explained 0.92–13.61% of the phenotypic variation. The target alleles of these markers reduced disease

TABLE 2   List of common resistant genotypes (DS<20%) in the three cropping seasons 2022, 2023, and 2024, their country of origin, and plant ID.

Genotype name	Genotype ID	Country	DS% in 2022	DS% in 2023	DS% in 2024
Nelin	–	Sudani	16.67	13.33	13.33
Koga II	PI243191	Germany	16.67	13.33	10.00
Kolben II	PI321700	Germany	13.33	16.67	13.33
Carstens Sommerweizen	PI180617	Germany	13.33	16.67	6.67
Equator KTI	PI191638	Kenya	16.67	13.33	13.33
Solitaire	PI520532	United Kingdom	13.33	16.67	10.00
OR9630064	PI620714	United States	16.67	16.67	13.33
Spinkcota	PI583670	United States	16.67	13.33	13.33

TABLE 3 Summary of significant markers associated with powdery mildew adult plant resistance in each cropping season, as well as across three seasons, in the evaluated 196 spring wheat genotypes using the iSelect 25K SNP array (25K SNPs) and genotyping-by-sequencing (GBS) marker datasets.

Experiment	Marker set	No. of sig. markers	No of chr	R² (%)	P-value	Allele effects	No. of gene models	No. of major genes
2022	25K-SNPs	15	9	0.95 – 5.58	2.73E-08 – 0.001	(-4.64) – (-2.54)	9	0
	GBS-SNPs	11	7	0.1 – 10.66	1.61E-09 – 0.001	(-5.92) – (-2.34)	2	3
	Total	26	13	0.1 – 10.66	1.61E-09 – 0.001	(-5.92) – (-2.34)	11	3
2023	25K-SNPs	17	8	0.03 – 11.02	1.79E-06 – 0.001	(-7.80) – (-2.63)	8	1
	GBS-SNPs	20	13	0.84 – 16.38	9.90E-10 – 0.001	(-6.92) – (-3.29)	9	3
	Total	37	14	0.03 – 16.38	9.90E-10 – 0.001	(-7.80) – (-2.63)	17	4
2024	25K-SNPs	11	6	0.92 – 7.56	9.50E-08 – 0.001	(-7.15) – (-3.04)	10	0
	GBS-SNPs	8	5	6.33 – 13.61	3.48E-05 – 0.001	(-9.49) – (-6.28)	4	2
	Total	19	8	0.92 – 13.61	9.50E-08 – 0.001	(-9.49) – (-3.04)	14	2
Across the three cropping seasons	25K-SNPs	14	9	0.50 – 9.49	3.81E-10 – 0.001	(-6.67) – (-2.46)	11	0
	GBS-SNPs	13	8	0.86 – 13.16	2.19E-08 – 0.001	(-7.46) – (-3.21)	6	2
	Total	27	9	0.50 – 13.16	3.81E-10 – 0.001	(-7.46) – (-2.46)	17	2

severity with a range of -9.49 to -3.04. A number of 14 gene models were found to harbor these significant markers with only two major genes.

On the basis of the average of the three cropping seasons, 27 markers were significantly associated with the resistance and were distributed among 9 chromosomes. These markers explained 0.50–13.16% of the phenotypic variation. Furthermore, the range of DS reduction due to the presence of the target alleles of these markers was -7.46 to -2.46. The significant markers were located within 17 gene models. Among these gene models, only two genes were found to harbor markers with a major effect.

Notably, the significant markers associated with WPM-APR in the three cropping seasons as well as the average over the three years were distributed across all 21 wheat chromosomes except 4A, 3D, and 6D (Figure 4a). The largest number of significant markers were found on the chromosomes 2B and 7B, with 11 markers each. The lowest number of markers was found on chromosomes 3A, 4B, 4D, 5D, and 7D, which carry one marker for each chromosome. The chromosomal locations of the five significant markers are not known; thus, they are located on unknown chromosomes.

It was noted that two stable markers were found to be significantly associated with WPM under all conditions (three years and the average over the three years as well). One stable marker was significant in 2021-2022, 2023-2024, and the average, while one was significant in 2021-2022, 2022-2023, and the average (Figure 4b). These four common markers are located on four different chromosomes: 1A, 2B, 3A, and 5A (Table 4). Moreover, four, one, and two markers were common between 2021-2022 and the average, 2022-2023 and the average, and 2023-2024 and the average, respectively.

Chromosomes 1A, 2B, and 5A, which carried the four common markers across the three cropping seasons, also presented many significant markers, with 4, 11, and 9 markers, respectively (Figure 4a). Therefore, linkage disequilibrium (LD) between the significant markers and the common significant marker on each chromosome was investigated. Notably, no significant LD was detected between any of the common significant markers and the other significant markers detected on the same chromosome (Supplementary Figure S4).

3.3.2 Gene models harboring significant markers associated with WPM-APR

A total of 11, 17, 14, and 17 genes were found to harbor significant markers associated with resistance in 2021-2022, 2022-2023, 2023-2024, and the average, respectively (Table 3). The complete list of these genes and their functional annotations are presented in detail in Supplementary Table S7. Notably, the functional annotation of these genes is associated with disease resistance and plant-pathogen interactions, such as glutathione S-transferase produced by *TraesCS3B02G536100*, NBS–LRR–like resistance protein produced by *TraesCS1D02G064200*, glucan endo-1,3-beta-glucosidase produced by *TraesCS7A02G069700*, and serine/threonine–protein kinase produced by the *TraesCS2A02G537600* gene.

The four common significant markers associated with resistance in at least two different cropping seasons were found to be located within three different gene models (Table 4). Two genes,
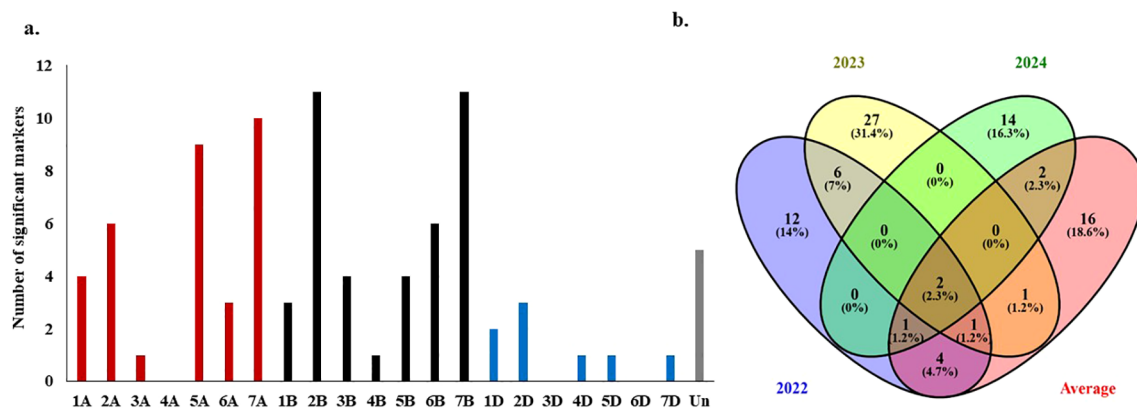
FIGURE 4
Significant markers associated with powdery mildew adult plant resistance in the evaluated 196-spring wheat genotypes: **(a)** number of signifcant markers in each growing season as well as the average of the three growing seasons, **(b)** number of total significant markers associated with the resistance on each one of the 21-wheat chromosomes.

TraesCS2B02G569400 and *TraesCS5A02G314000*, harbored markers associated with resistance in the three cropping seasons. The third gene, *TraesCS3A02G282800*, harbored a marker associated with resistance in the 2021-2022 and 2023-2024 cropping seasons. Furthermore, the *TraesCS2B02G569400* gene was found to have a major effect on resistance in the three cropping seasons. The functional annotation of this gene model revealed that it controls heavy metal transport/detoxification superfamily proteins. On the other hand, the two other genes had minor effects and controlled the production of rhomboid-like proteins and nonspecific serine/threonine protein kinases by the *TraesCS5A02G314000* and *TraesCS3A02G282800* genes, respectively.

Gene enrichment of the three common gene models revealed 12 biological process (BP) and six molecular function (MF) pathways associated with resistance (FDR<0.05) (Figure 5a). The BP pathways were associated with cell death, negative regulation of biological processes, proteolysis, and apoptotic processes. The MF pathways were associated with serine-type peptidase, endopeptidase activity, and serine hydrolase activity. The 12 BP pathways worked in three different networks, each controlled by one gene model (Figure 5b). The six MF pathways worked in two different networks controlled by only two genes from the three identified common genes. Furthermore, *TraesCS2B02G569400* was reported to be directly related to the response of wheat to fungi and bacteria and the downregulation of the defense response (Figure 5c). The *TraesCS3A02G282800* gene (known as *SnRK1*) was reported to be associated with the defense response to bacteria, fungi, and insects in *Arabidopsis thaliana*. No relationship was found between *TraesCS5A02G314000* and disease resistance.

3.4 Selection of superior resistant and high-yield genotypes

The number of targeted alleles and different alleles of the significant markers associated with resistance were investigated in the eight selected genotypes as well as the two most resistant Egyptian genotypes. The number of target alleles ranged from 20 in the American genotype "Spinkcota" to 31 in the Egyptian genotype "Giza 171". The Sudanese genotype "Nelin" carried 24 target alleles, which is quite similar to the other Egyptian genotype "Sohag 5" (Figure 6a). The lowest number of different alleles was 7, which was found between "Carstens Sommerweizen" and both "Koga II" and "Kolben II". The highest number of different marker alleles was 25 between "Spinkcota" and both "Koga II" and "Kolben II" (Figure 6b). For the two Egyptian genotypes, the highest number of different alleles was 22 between "Solitaire" and both genotypes as well as between "Sohag 5" and both "Koga II" and "Kolben II". Nelin had 13 different markers between itself and both "Sohag 5" and "Giza 171".

Moreover, the genetic distance between the eight selected genotypes as well as the Egyptian genotypes was calculated and presented as a matrix (Supplementary Table S8) and phylogenetic tree (Figure 6c). Three clusters were identified to contain all ten genotypes. The two Egyptian genotypes were located in the same cluster as the Sudanese genotype "Nelin" and the two American genotypes. The Kenyan genotypes were located alone in a separate cluster. The German and English genotypes were located in the same cluster together. The greatest distance was "0.451" between the Egyptian genotype "Sohag 5" and the English genotype "Solitaire". The lowest value of genetic distance was found between the two German genotypes "Koga II" and "Kolben II", with a value of 0.014.

To provide more understanding of the possibility of using the eight common resistant genotypes in future breeding programs, the yield of these genotypes in comparison with the best two resistant Egyptian cultivars (Sohag 5 and Giza 171) was investigated in disease-infected and noninfected fields (Figure 7). Obviously, the Sudanese genotype "Nelin" had greater TKW in both cropping seasons in infected fields (Figure 7a) and the three cropping seasons under a controlled field (Figure 7b). Furthermore, the yield of the Kenyan genotype "Equator KTI" was almost the same as that of the two Egyptian genotypes in 2022-2023 under disease conditions and

TABLE 4 List of significant markers commonly associated with powdery mildew adult plant resistance among different cropping seasons and their average (Avr), their chromosomal location, p-value, phenotypic variation explained by marker (R²), target allele, allele effect, and gene models harboring them.

Significant marker	Chr	Position	Cropping season				R² (%)				Target allele	Allele effect				Gene model	Functional annotation
			2022	2023	2024	Avr	2022	2023	2024	Avr		2022	2023	2024	Avr		
S2B_760015865	2B	760015865	✓	✓	✓	✓	10.09	10.54	13.61	13.16	C	-3.24	-4.50	-8.83	-3.79	TraesCS2B02G569400	Heavy metal transport/detoxification superfamily protein
AX-108793797	5A	524672679	✓	✓	✓	✓	5.58	9.53	6.08	7.46	C	-3.21	-4.46	-3.48	-4.10	TraesCS5A02G314000	Rhomboid-like protein
Ra_c88203_468	3A	511075441	✓		✓	✓	0.95	NA	1.43	0.85	T	-2.56	NA	-4.72	-4.65	TraesCS3A02G282800	Non-specific serine/threonine protein kinase
S1A_12130376	1A	12130376	✓	✓	✓	✓	9.9	8.62	6.09	9.75	T	-3.41	-5.16	12.00	-5.63	no gene model	(no gene model)

in 2021-2022 under noninfected conditions. However, it has an extremely low yield in the 2023-2024 cropping season under powdery mildew-infected fields as well as in 2022-2023 and 2023-2024 under noninfected fields.

Furthermore, the yield components of the ten selected genotypes were measured under noninfected field conditions (Supplementary Figure S5). Notably, the Sudanese genotype "Nelin" had a heading date (HD) similar to that of the Egyptian genotypes, with values of 99 and 88 days in the 2022-2023 and 2023-2024 cropping seasons, respectively. The remaining resistant genotypes had higher HD values than the Egyptian genotypes did, except for "Spinkcota" (Supplementary Figure S5a). The PH values of the Egyptian genotypes were 101.5 cm and 88.5 cm for Giza 171 and 98.5 cm and 92 cm for Sohag 5 in 2022-2023 and 2023-2024, respectively. Nelin was slightly taller than the two Egyptian genotypes were, with PH values of 104 cm, 114.75 cm, and 112.67 cm in the 2021-2022, 2022-2023, and 2023-2024 cropping seasons, respectively (Supplementary Figure S5b). The remaining genotypes could be classified into two groups: shorter genotypes (Solitaire, OR9630064, and Spinkcota) and very tall genotypes in at least one cropping season (Koga II, Kolben II, Carstens Sommerweizen, and Equator KTI). All eight selected genotypes presented spike length (SPL) values within the range of the Egyptian genotypes (8.67–14.33 cm in 2021-2022, 8.5–13.5 cm in 2022-2023, and 8.17–14.34 cm in 2023-2024), except for "OR9630064", which had a longer spike in 2022-2023 (16.5 cm) (Supplementary Figure S5c). The number of spikes per plant (NS/SP) was similar for all the selected genotypes and the Egyptian genotypes (Supplementary Figure S5d). The GY/SP ratio was lower in all eight resistant genotypes than in the Egyptian genotypes (Supplementary Figure S5e). However, Nelin had the highest GY/SP among the selected genotypes. Similarly, the grain number per spike (GN/SP) was lower in all eight selected genotypes than in the Egyptian genotypes. However, "Spinkcota" and "Nelin" had the highest GN/SP values among the selected genotypes (Supplementary Figure S5f).

4 Discussion

Wheat powdery mildew is a major disease that affects wheat-cropping areas worldwide. Limited information is available regarding resistance to this disease under Egyptian conditions (Mourad et al., 2024a). This study is the first to investigate WPM-APR under Egyptian field conditions. The evaluated plant materials were collected from 22 different countries and have been reported to be genetically diverse and well-adapted to Egyptian fields (Mourad et al., 2020, 2022c). This broad genetic diversity provides valuable resources for improving powdery mildew resistance in Egyptian wheat. WPM-APR was assessed across three cropping seasons (2021-2022, 2022-2023, and 2023-2024). Previous studies have reported the presence of different Bgt isolates in Egyptian fields across different cropping seasons (El-Shamy and Mohamed, 2022; Mourad et al., 2022). Therefore, different isolates have been identified in recent studies.
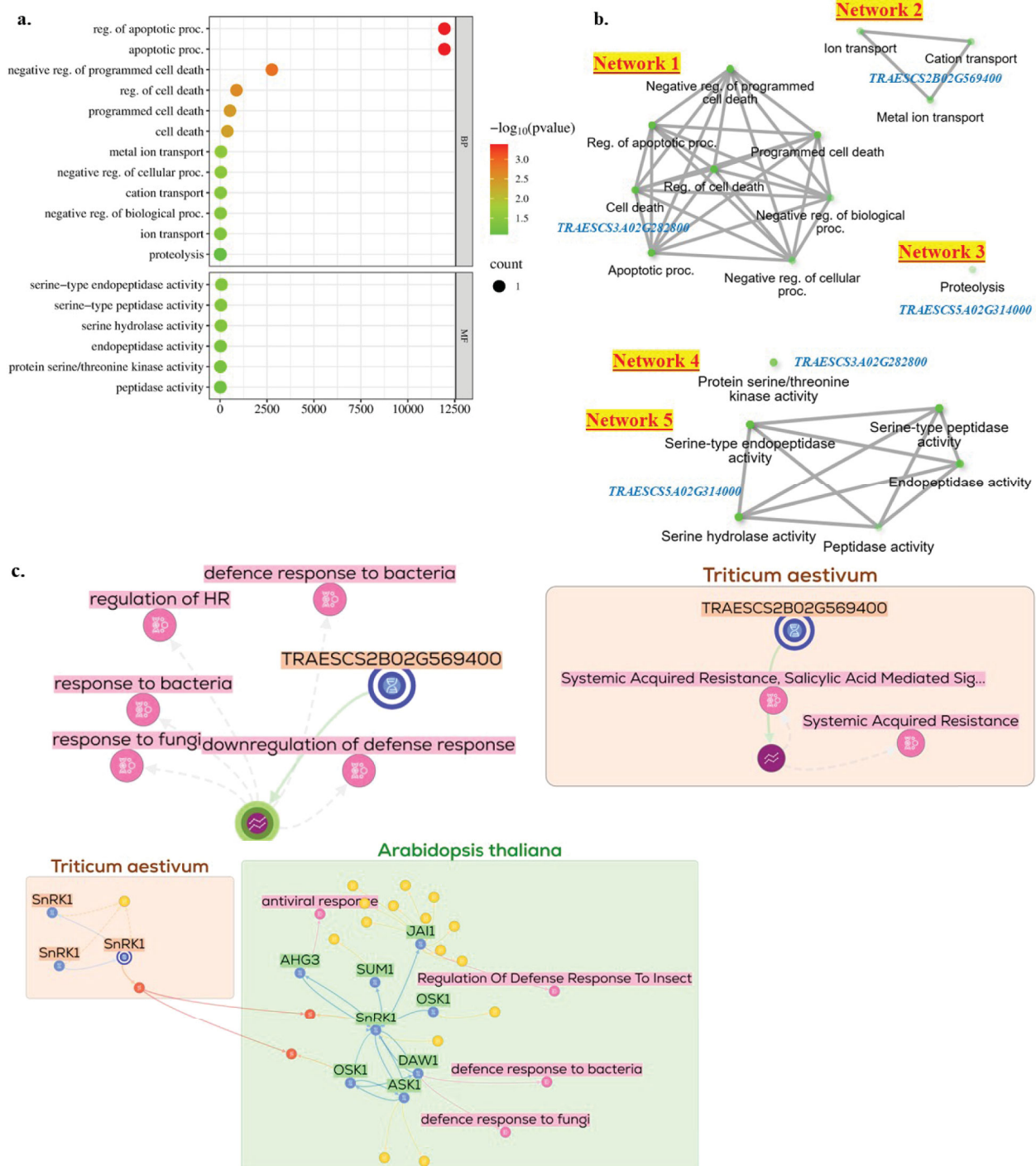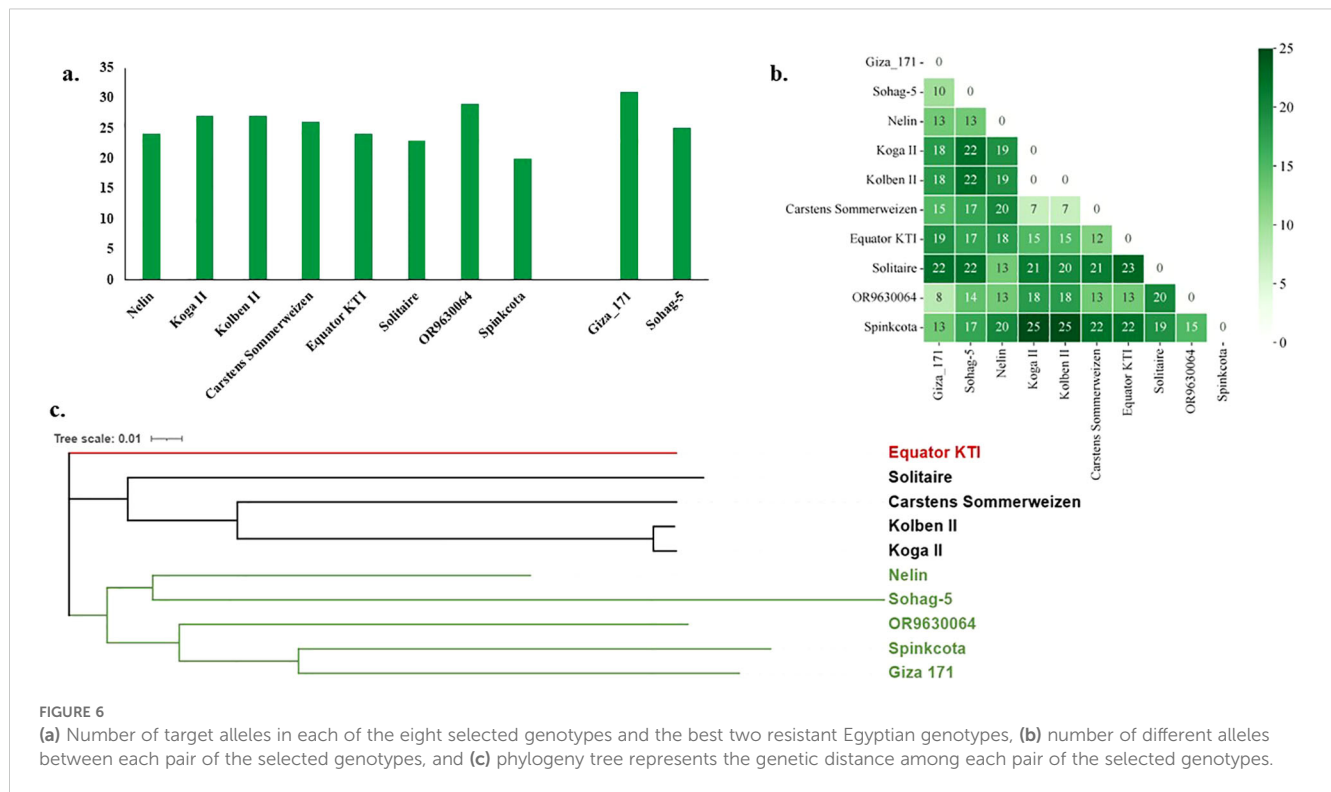
FIGURE 5
Gene enrichment and gene network of the three gene models harbor markers significantly associated with powdery mildew adult plant resistance in the three tested growing seasons (2022, 2023, and 2024): **(a)** enrichment pathways identified based on the biological process pathways (BP) and molecular function (MF), **(b)** gene network of the 12 BP-pathways controlled by the three gene models (Network 1, Network 2, and Network 3) and the six MF pathways (Network 4 and Network 5, **(c)** Network of *TRAESCS2B02G569400* and *TRAESCS3A02G282800* in relation to disease resistance in wheat and Arabidopsis genomes, respectively.

A set of 20 isolines carrying 20 different WPM resistance genes was evaluated during each cropping season. Notably, different responses of most of the evaluated isolines were recorded in each season, confirming the c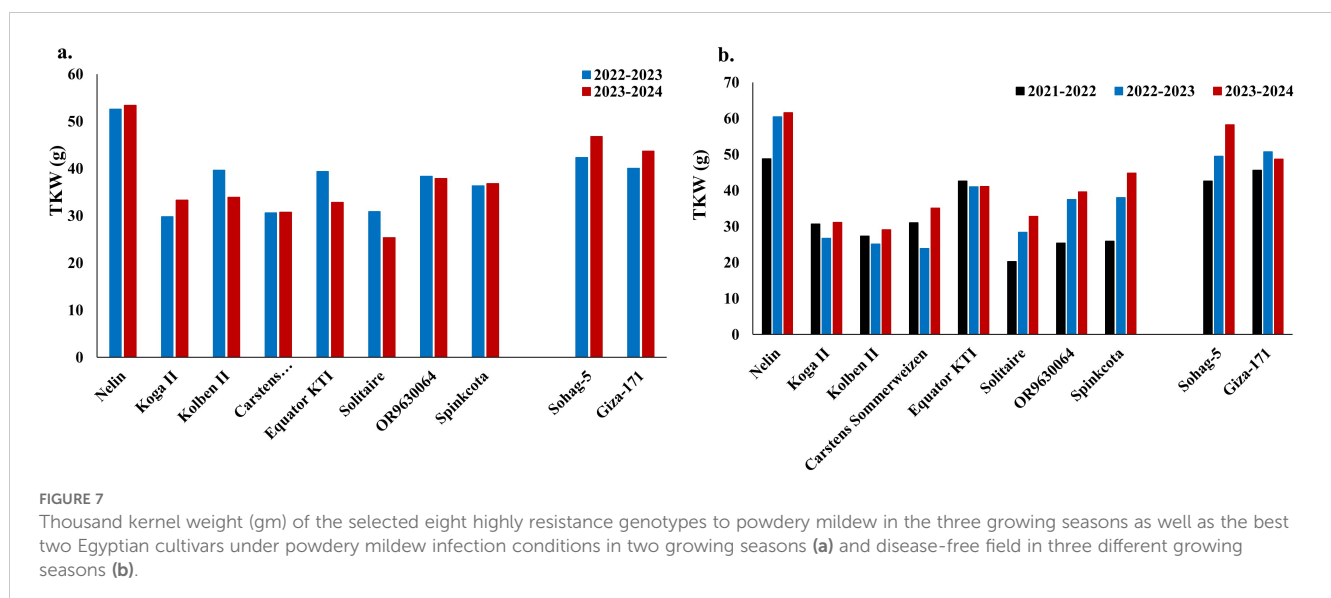hanges occurred in the *Bgt* isolates during each cropping season. Furthermore, Mourad et al. (2022) evaluated the same set of isolates at the seedling growth stage using *Bgt* isolates collected from Egyptian fields in the 2020-2021 and 2021-2022 cropping seasons. A comparison of the results of their study

FIGURE 6
(a) Number of target alleles in each of the eight selected genotypes and the best two resistant Egyptian genotypes, (b) number of different alleles between each pair of the selected genotypes, and (c) phylogeny tree represents the genetic distance among each pair of the selected genotypes.

with the results obtained in the present study revealed that the *NACG13* gene was highly effective against different *Bgt* races in both the seedling and adult growth stages. Furthermore, *Pm1b*, *Pm34*, *Pm36*, and *MIAG12* were resistant to WPM during the two cropping seasons at the seedling and adult growth stages. Previous studies reported the resistance of these WPM resistance genes under Egyptian conditions (El-Shamy et al., 2016; Abdelrhim et al., 2018; El-Shamy and Mohamed, 2022). These five WPM resistance genes could be good sources of WPM resistance among the three cropping seasons.

4.1 Variation in WPM−adult plant resistance in the tested plant material

The highly significant differences in WPM-APR among the three cropping seasons again confirmed the presence of different *Bgt* isolates in each cropping season (Supplementary Table S3). Furthermore, the highly significant differences among the tested genotypes represent the high genetic variation among them and the possibility of selecting highly resistant genotypes using this plant set. The high degrees of broad-sense heritability in each cropping season indicate that the

FIGURE 7
Thousand kernel weight (gm) of the selected eight highly resistance genotypes to powdery mildew in the three growing seasons as well as the best two Egyptian cultivars under powdery mildew infection conditions in two growing seasons (a) and disease-free field in three different growing seasons (b).

phenotypic variation in WPM resistance is mainly due to genotypic variation; thus, the selected resistant genotypes are promising for improving WPM resistance (Table 1). Previous studies reported high degrees of broad-sense heritability for WPM resistance (Hoseinzadeh et al., 2019; Hinterberger et al., 2022; Mourad et al., 2022).

Notably, none of the genotypes in the tested set were immune to WPM during any cropping season, confirming the urgent need to improve WPM in wheat crop under Egyptian field conditions. The lack of immune Egyptian wheat genotypes has been previously reported in the majority of commercial cultivars (El-Shamy et al., 2016; Draz et al., 2019, 2022; Elsayed and Elkot, 2020). Furthermore, most of the genotypes were susceptible to WPM (DS ≥20%). Few genotypes were resistant to WPM, with very few (8 genotypes) common resistant genotypes across the three cropping seasons. These genotypes belong to five different countries, and none of them are Egyptian genotypes, which again confirms the lack of WPM-resistant genotypes in the Egyptian wheat germplasm. As these 8 common genotypes belong to five different countries, crossing these genotypes with Egyptian cultivars could help improve WPM-APR in Egyptian wheat germplasms. The same set was evaluated previously for WPM at the seedling growth stage against Egyptian races, and 19 genotypes were resistant to the different *Bgt* isolates used in this study (Mourad et al., 2023). Among these 19 genotypes, one English genotype, "Solitaire," was resistant at the adult growth stage in our recent study during the three different cropping seasons.

4.2 Association mapping of WPM-APR

Two types of markers, GBS-SNP and 25K-SNP, covering different parts of the wheat genome, were used in the GWAS (Liu et al., 2020; Mourad et al., 2022, 2024b, c; Esmail et al., 2023b). Furthermore, different models have been applied in GWASs, which are effective in identifying significant markers and accounting for the effects of population structure (Turuspekov et al., 2016; Kaler et al., 2020; Muhammad et al., 2020; Kiani et al., 2021). Notably, the best GWAS model varied depending on the phenotypic data and the set of markers used (Supplementary Figures S2, S3). Therefore, testing multiple GWAS models to identify markers significantly associated with the studied trait is highly recommended. The significant markers were identified based on -log10 (*p*-value) = 3.00. It was reported that using more stringent corrections such as Bonferroni can be overly conservative, potentially overlooking biologically meaningful associations, particularly for traits governed by multiple small-effect loci. Our choice was therefore a deliberate compromise to enhance the discovery of candidate loci, with the understanding that the identified associations should be considered suggestive. Therefore, all markers detected in this study at this threshold should be validated in independent populations or through functional characterization to confirm their roles in the traits of interest. There are many earlier studies that validated markers detected at p ≤ 0.001 in different genetic backgrounds such as drought tolerance in wheat, alkaline-saline, and disease resistance (Sallam et al., 2022, 2024; Mourad et al., 2025).

Different significant markers were found to control resistance in each cropping season, with only four common markers, which again

confirmed the presence of different *Bgt* races in each cropping season (Table 3, Figure 3b). Combining the GWAS results from the three cropping seasons with the average, a total of 86 markers across 18 different chromosomes were identified as being significantly associated with resistance. The large number of significant markers across the different chromosomes suggests the complexity of the genetic system controlling the resistance. A similar complex genetic system that controls WPM resistance has been reported previously (Kang et al., 2019; Mohler and Stadlmeier, 2019; Miedaner et al., 2020; Simeone et al., 2020; Alemu et al., 2021; Du et al., 2021; Hinterberger et al., 2022; Jin et al., 2022; Mourad et al., 2023, 2024a). Most of the significant markers presented values of $R^2 < 10\%$, suggesting that WPM-APR is a quantitative trait that is controlled by many genes with minor effects. Furthermore, most of these markers are located within gene models that control the production of proteins and enzymes that increase wheat resistance to diseases, including powdery mildew. Examples include glutathione S-transferase (Gullner et al., 2018), NBS-LRR-like resistance protein (Oladzad et al., 2023), glucan endo-1,3-beta-glucosidase (Perrot et al., 2022), and serine/threonine-protein kinase (Cao et al., 2011). Furthermore, the "S7A_35630468" marker associated with resistance in the 2022-2023 cropping season was previously reported to control WPM seedling resistance via different *Bgt* isolates under Egyptian conditions (Mourad et al., 2023). This marker is located in the *TraesCS7A02G069700* gene, which is functionally annotated to produce glucan endo-1,3-beta-glucosidase, an enzyme that controls disease resistance in wheat, including powdery mildew (Benbow et al., 2020; Li et al., 2020; Yue et al., 2021; Esmail et al., 2023b).

Furthermore, the "wsnp_CAP8_rep_c3844_1896355" marker associated with resistance in the 2023-2024 cropping season was previously reported to control the disease severity and infection type of adult leaf rust plant resistance under Ethiopian conditions (Lhamo et al., 2023). This marker is located within the *TraesCS7A02G529200* gene, which is functionally annotated to control the production of the chaperone protein dnaJ, a protein that has been reported to play an important role in several pathological processes in wheat, such as yellow mosaic virus (Liu et al., 2022).

4.3 Stable genomic regions controlling broad-spectrum resistance to different *Bgt* isolates

Among the many markers significantly associated with resistance, only four were associated with resistance in at least two of the three cropping seasons (Table 4, Supplementary Table S7). This low number of common significant markers represents the complexity of the wheat genome as well as the continuous changes in *Bgt* races. Two markers were associated with WPM resistance in the three cropping seasons, while the other two markers were significantly associated with WPM resistance in only two years, which could be due to the effect of genotype × interaction (Eltaher et al., 2021). These four markers were distributed among four different chromosomes. Interestingly, no linkage disequilibrium (LD) was detected between any of these common markers and other significant markers associated with

resistance in only one cropping season. This further confirms the complexity of the wheat genome and the challenge of developing wheat genotype with broad-spectrum resistance to WPM (Supplementary Figure S4). Therefore, it is highly recommended that more GWASs be conducted on this serious disease to increase our understanding of broad-spectrum resistance to WPM (Mourad et al., 2024a). The four markers can be converted to KASP markers for further validation in different genetic backgrounds. Moreover, this marker can be used for marker-assisted selection under Egyptian conditions. It was reported that genetic validation assesses whether the same QTL or gene is consistently detected across different locations or years and whether its effect remains significant when evaluated in diverse genetic backgrounds (Sallam et al., 2023).

Only three gene models harboring the common significant markers were detected. The functional annotation of these gene models was directly and indirectly associated with disease resistance in wheat. For example, the *TraesCS2B02G569400* gene is functionally annotated to produce heavy metal transport/detoxification superfamily proteins. Previous studies reported that some heavy metal transporter proteins regulate disease resistance (Chu et al., 2022; Liu et al., 2025). Furthermore, the rhomboid-like protein produced by the *TraesCS5A02G314000* gene was previously reported to be an important gene for improving wheat resistance to kernel bunt (Singh et al., 2020) and both stripe and leaf rust diseases (Kumar et al., 2020). The third gene was found to control nonspecific serine/threonine protein kinases, which are key members of many powdery mildew resistance genes, such as *Pm21* (Cao et al., 2011), *Pm13* and *Pm4* (Li et al., 2024), as well as the *Sr34* stem rust resistance gene (Yu et al., 2023).

Through gene enrichment analysis, three biological process (BP) networks and two molecular function (MF) networks were identified. Each network was regulated by a single gene from the identified gene models, suggesting that each gene functions independently in enhancing WPM resistance. *TraesCS3A02G282800* regulates cell death and negatively influences biological processes, playing a crucial role in initiating the plant immune response (Coll et al., 2011). Serine/threonine kinases are among the main kinases that control programmed cell death (apoptosis) (Cross et al., 2000). *TraesCS5A02G314000* controls the activity of serine-type peptidase. This enzyme works in the proteolysis of cell proteins. Proteolysis was found to result in a high level of broad-spectrum resistance without any reduction in plant yield (Liu et al., 2024). *TraesCS2B02G569400* works in one network to control metal ion/cation transport via the BP pathway. However, its role in MF has not been identified.

Furthermore, known *Pm* resistance genes located at the same chromosomal positions as the four stable markers were identified to increase our understanding. On chromosome 1A, the *Pm3*, *Pm17*, and *Pm25* genes have been reported (Chen et al., 1996; Shi et al., 1998; Bougot et al., 2002). However, on the basis of the results of the differential lines, these three genes were not effective against the Egyptian races in the three cropping seasons (Figure 1). Therefore, we can conclude that the stable significant marker on this marker could be associated with a novel resistance gene. The *Pm6*, *Pm26*, *Pm42*, and *M1AB10* genes were mapped to the 2B chromosome. *Pm6* was included in the differential lines tested in this study and was not

effective in resisting Egyptian *Bgt* races. Unfortunately, no seeds for the *Pm26*, *Pm42*, or *M1AB10* genes were available for evaluation in our study. However, a previous study reported that *Pm26* is located on the short arm of the 2B chromosome in the 26573946:27934056 bp region (Hinterberger et al., 2022). Compared with the location of the stable marker on this chromosome (S2B_760015865), *Pm42* is located far from our stable markers (Hua et al., 2009). The *Pm64*, *MIZec1*, and *M1AB10* genes were mapped to the long arm of 2B (Maxwell et al., 2010; Zhang et al., 2019). However, previous studies reported that the "wsnp_Ex_c24135_33382318" and "S2B_754153049" markers overlapped with the chromosomal position of *Pm64* (Lhamo et al., 2025). Hence, our identified marker could not be *Pm64*. Furthermore, the *MIZec1* and *M1AB10* genes were mapped to 797 and 799 Mbp, respectively (Lhamo et al., 2025). Since none of the mentioned genes overlapped with the position of the "S2B_760015865" marker, we can conclude that this marker and its harboring gene are novel or unknown genes. *Pm44* is located on the 3AS chromosome (Jin et al., 2020). Based on our findings, the "Ra_c88203_468" marker was located on the long arm of this chromosome. *Pm2026* and *Pm55* are effective resistance genes that have been mapped to the 5AL chromosome (Xu et al., 2008; Zhang et al., 2016; Liu et al., 2017; Lu et al., 2024). However, no information is available about the base pair location of this gene. Therefore, we could not confirm whether the significant marker on this chromosome was associated with *Pm2026* and *Pm55* due to a lack of information.

4.4 Selection of superior resistant and high-yield genotypes

The best parents for crossing are those with high genetic distance and a high number of different alleles associated with the target allele (Bertan et al., 2007; Mourad et al., 2020; Esmail et al., 2023a; Mourad et al., 2025; Sharshar et al., 2025). Based on this concept, "Solitaire" could be considered the best parent for crossing with the two Egyptian genotypes to improve WPM-APR; however, selecting superior genotypes that are both disease-resistant and high-yielding is challenging. Therefore, it is crucial to consider yield as a selection criterion in addition to a high level of WPM resistance. In this study, eight resistant genotypes were identified as stable across the three cropping seasons. Additionally, two Egyptian genotypes were considered the most resistant Egyptian genotypes, exhibiting resistance in one or two cropping seasons. "Sohag 5" has been reported as a high-yielding durum wheat genotype (Hamada et al., 2016), whereas "Giza 171" is a high-yielding bread wheat genotype resistant to stem and leaf rust (Hamada et al., 2015). Both cultivars displayed an intermediate level of resistance in at least one or two cropping seasons. Therefore, improving their resistance to WPM will result in high-yielding and resistant genotypes. Solitaire had lower yield and yield components than the two other tested genotypes did. Thus, using this genotype as a parent in future breeding programs will increase WPM-APR but may negatively impact the yield of Egyptian wheat germplasm. Among the eight selected stable resistant genotypes, only "Nelin" presented a greater TKW than did the two Egyptian cultivars in both the control and infected fields. Therefore, despite having an intermediate number of different and target alleles and

having a low genetic distance from the two Egyptian genotypes, "Nelin" could be a valuable parent in future breeding programs to improve resistance in Egyptian wheat germplasm.

5 Conclusion

In conclusion, the high genetic variation in WPM-APR found in the tested panel confirms the possibility of selecting highly resistant genotypes. The absence of immune genotypes in the three cropping seasons, especially in the Egyptian wheat genotypes, confirms the urgent need to improve WPM resistance. This study is the first to elucidate the genetic control of WPM-APR under Egyptian conditions. Four stable significant markers across the three cropping seasons were identified. These markers are located on four different chromosomes and work separately in different networks, suggesting that they are four different resistance genes. Eight superior resistant genotypes that belong to five different countries other than Egypt were selected. The Sudanese genotype "Nelin" was found to be the best parent for improving WPM resistance and yield traits in Egyptian wheat germplasm.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

Author contributions

AM: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. AS: Data curation, Writing – review & editing. AB: Writing – review & editing. RH: Writing – review & editing. SE: Writing – review & editing, Data curation.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fagro.2025.1595621/full#supplementary-material

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