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Identification and Genetic Differentiation of Powdery Mildew Resistance of some Egyptian Barley Genotypes

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ABSTRACT

Powdery mildew is recognized as a paramount disease impacting barley crops, resulting in significant yield losses. This research aimed to identify Egyptian barley genotypes that not only possess a high yield potential but also exhibit resistance to powdery mildew. Twelve barley genotypes were evaluated over two consecutive growing seasons, 2021/2022 and 2022/2023, to assess their disease response. Among these, Line 2 showed the highest resistance to powdery mildew, closely followed by Giza 134, Giza 138, and Line 3, with Line 2 emerging as a promising candidate for breeding programs aimed at developing resistant cultivars. On the other hand, Giza 132 and Giza 126 were identified as the most susceptible, whereas Giza 123 and Giza 2000 displayed moderate susceptibility. The study utilized six SSR primers to analyze genetic diversity among the genotypes, with these primers demonstrating varying degrees of resolving power, the average being 2.66. Notably, the primer Bmac0213 was identified as the most effective for genetic diversity analysis, with the highest resolving power value of 4.28. Molecular similarity indices among the barley genotypes varied, ranging from 0.111 to 0.875, as determined by SSR data analysis. These findings contribute valuable insights for breeding strategies aimed at enhancing powdery mildew resistance in barley, highlighting the role of genetic diversity and molecular markers in identifying and developing resistant cultivars

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INTRODUCTION

Barley (*Hordeum vulgare* L.) is globally recognized as the fourth most significant cereal crop, trailing only maize, wheat, and rice in terms of importance. Its utility spans a wide array, serving not just as feed for animals and food for humans, but also in beer brewing and, more recently, as a potential biofuel source. This recognition stems from barley's rich composition of fibers, vitamins, and minerals, underscoring its health benefits (Abdelrady et al., 2024). Furthermore, barley's relatively simple genetic structure, short life cycle, and ability to self-pollinate make it a valuable resource for genetic studies.

Mildews have a biotrophic relationship with their plant hosts; they have a parasitic relationship and only obtain nutrients by leeching them to a living plant, while attempts to culture the fungi in vitro have been unsuccessful (Simon et al., 2015). Blumeria graminis is the powdery mildew responsible for disease in wheat, barley, oats, and rye, and extensive study has been undertaken to elucidate its genomics and host-pathogen interaction (Hacquard et al., 2013; Jankovics et al., 2015). The host specificity can be very high, for example, the grass pathogen B. graminis has a subtype below species level known as 'formae speciales' which infects barley, but not its close relative, wheat, and hence is detonated B.graminis formae. speciales. hordei (or Bgh).

Powdery mildews complete their asexual life cycle within their host. The life cycle of the barley pathogen B.graminis f.sp hordei is initiated by the arrival of the conidia upon the leaf surface, ready for germination. Infection begins with the formation of a primary germ tube followed by a secondary germ tube which innovate from the conidia (Godfrey et al., 2009) in six hours post inoculation (hpi), the secondary germ tube will enlarge into an appressorium, from which a peg forms that will penetrate the cell wall via mechanical force and enzymatic activity (Pryce-Jones et al., 1999). From this breech, a multi-digitated feeding structure, known as the haustoria, grows inside the plant epidermis. If these stages progress successfully than a secondary hypha develops and proliferates on the exterior of the leaf, and conidiophores, carrying spores (conidia) will appear, around 3-7dpi (Kuhn et al., 2016; Noir et al., 2009). From here the conidia are free to spread through the wind to repeat the asexual cycle on new areas of leaf or new plants entirely.

The occurrence of sexual reproduction is relatively rare compared to the asexual cycle, suggested by studies into the PM's genome (Hacquard et al., 2013; Wicker et al., 2013). Sexual reproduction occurs typically at the end of the growth season, allowing the pathogen to survive during inter-crop periods. Ascospores are produced, enclosed in an ascus, that is hosted within a spherical-shaped fruiting body known as the chasmothecium. In the next season, these bodies can be ruptured, to restart the infection cycle (Heffer et al., 2006). It has been suggested that year-round wild grasses, which can even host some f.sp of PM, might also help in the persistence of the pathogen during these periods (Wolfe & Schwarzbach, 1978).

The genetic diversity within barley, highlighted by extensive germplasm collections that include elite varieties, landraces, and wild accessions from diverse geographical origins, positions barley as an exemplary model for plant genetic research. These collections have been well-characterized and are meticulously preserved, presenting a vast reservoir of natural diversity for both breeding and scientific investigation. Historically, the lack of a reference genome sequence impeded the full utilization of these resources in fundamental and applied breeding sciences. This challenge was met by the International Barley Genome Sequencing Consortium, which provided a crucial reference genome, thereby facilitating genetic research and breeding advancements (Mayer et al., 2012). This genomic blueprint has become instrumental in trait isolation, the exploration and exploitation of genetic diversity, and delving into the unique biology and evolutionary history of one of humanity's first domesticated crops. The consortium's efforts have significantly contributed to the development of barley varieties with improved disease resistance and nutritional profiles, aligning with global initiatives to combat climate change and enhance dietary fiber content in grains (Mayer et al., 2012).

Among the challenges barley faces, powdery mildew, caused by Blumeria graminis f. sp. hordei (Bgh), remains a formidable foe. This fungal infection significantly impacts global barley production, affecting both yield and quality. Studies indicate substantial economic losses across different regions, with yield reductions reaching up to 20% in Europe, 30% in North Africa, and 40% in Western Australia, highlighting the critical need for effective disease management and crop improvement strategies (Brown & Hovmøller, 2002; Dean et al., 2012; Piechota et al., 2019). Advances in understanding genetic resistance to powdery mildew have been promising, with recent research identifying genetic markers linked to resistance. This progress emphasizes the potential of breeding resistant barley varieties as a strategic approach to enhancing crop resilience (Czembor et al., 2022; Leisova-Svobodova et al., 2011).

Molecular marker technologies, such as Restriction Fragment Length Polymorphisms (RFLPs) and Microsatellites (SSRs), have been pivotal in these efforts. These technologies facilitate the precise identification of resistance traits, +offering a more accurate estimation of gene locations and reducing the likelihood of phenotypic misidentification (Maroof et al., 1994; Struss & Plieske, 1998; Wright, 1998). SSRs, in particular, are prized for their high polymorphism, enabling the identification of a wide range of genotypes within a population. This capability is invaluable for assessing genetic diversity and selecting breeding parents, providing detailed genetic information crucial for plant breeding efforts to improve disease resistance, genetic diversity, and other desirable traits (Ahmad et al., 2018; Hasan et al., 2021). This research aims to investigate the relationship between natural powdery mildew infections and yield-related traits across twelve barley genotypes. Additionally, it seeks to identify new resistant sources and dependable molecular genetic markers to enhance barley breeding programs. This endeavor contributes to the scientific understanding of barley's genetic resistance to powdery mildew and supports the development of more resilient barley varieties, thereby addressing both economic and environmental sustainability challenges in barley production.

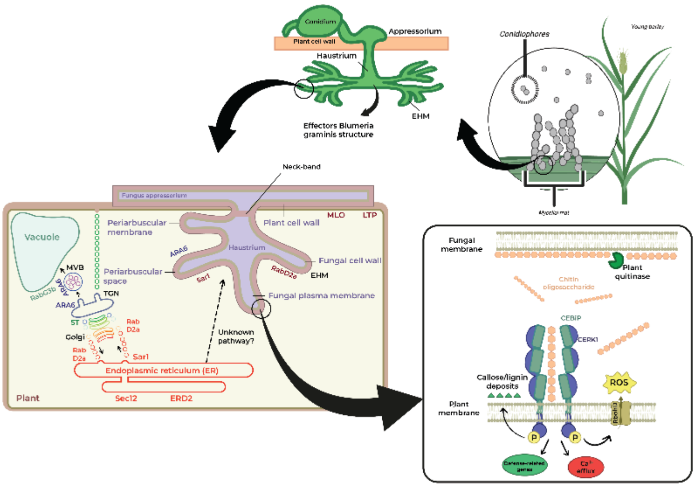


Fig 1. The Internal Structure of the Barley Powdery Mildew Blumeria Graminis F.Sp Hordei and Resistance Signaling

METHODOLOGY

Plant Material

This study evaluated twelve barley genotypes, including three foreign lines from ICARDA and nine local varieties, to show how they responded to powdery mildew. The evaluation took place over two consecutive growing seasons, 2021/2022 and 2022/2023, at the experimental farm of the Faculty of Agriculture, Kafrelsheikh University, Egypt. Name, pedigree, and origins of all barley genotypes used in this study are listed in Table 1.

Table 1. Name and Pedigree of the 12 Studied Barley Genotypes

No	Genotypes	Pedigree
1	Giza 123	Giza 117//FAO86
2	Giza 125	Giza 117/Bahteem 52// Giza 118/FAO 86
3	Giza 126	BaladiBahteem/SD729-por12762-Bc
4	Giza 2000	Cr366-13-1/Giza121
5	Giza 132	Rihane-05//As46/Aths*2" Aths/ Lignee686
6	Giza 133	Carbo/Gustoe
7	Giza 134	Alanda-01/4/WI2291/3/Api/CM67//L2966-69
8	Giza 137	Giza 118 /4/Rhn-03/3/Mr25-//Att//Mari/Aths*3-02
9	Giza 138	Acsad1164/3/Mari/Aths*2//M-Att-73-337-1/5/Aths/ lignee686 /3/Deir Alla 106//Sv.Asa/ Attiki /4/Cen/Bglo."S"
10	Line 1	BLLU/PETUNIA1//CABUA/3/Alanda//Lignee572//Arar
11	Line 2	Lignee527/Chn-01//Gustoe/5/Alanda-01/4/WI2291/3/Api/CM67//L2966-69
12	Line 3	Apm/HC1905//Robur/3/Arar/4/Arar/3/Mari/Aths*2//M-Att-73-337-1/5/Giza2000

Experimental Design

Barley seeds were planted by hand at the rate recommended in Egypt, which is (50 kg/fed.), during the first week of December. Each plot covered an area of (4.2 m2), consisting of six rows that were 3.5 meters long and spaced 20 cm apart. The setup for this experiment followed a randomized complete block design with four repeats. All farming practices were carried out promptly, adhering to the guidelines provided by the Ministry of Agriculture. The natural occurrence of powdery mildew, caused by Blumeria graminis conidia, was assessed under natural infection in the fields surrounded by a highly sensitive variety. The disease's presence was recorded using a 0-9 scale, as established by Leath & Heun,(1990) starting from when the disease first appeared. Observations of disease symptoms were made during the heading stage of the barley. Various data points were collected, including days until maturity, plant height, spike length (cm), number of grains spike-1, number spikes/m2, 1000-grain weight (g), biological yield (kg/fed.), grain yield (kg/fed.), and characteristics of powdery mildew infection.

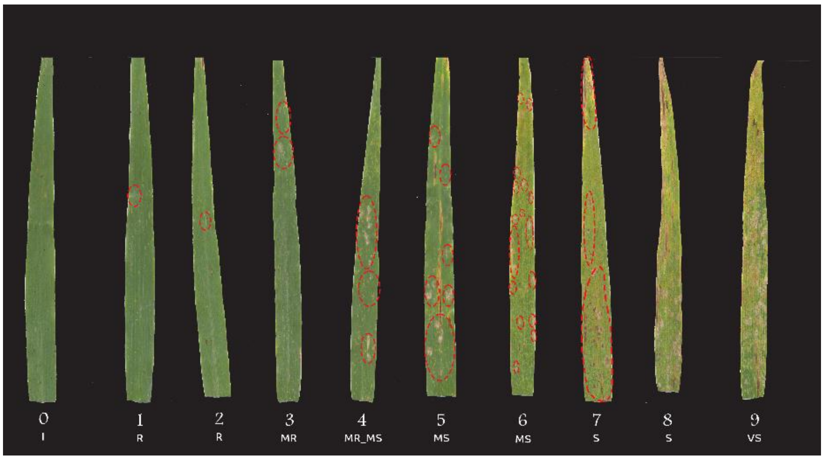


Fig 2. Quantification of Disease Susceptibility on a 0-9 Scale: 0 Indicates Immunity, 1-2 Represent Resistance, 3 Denotes Moderate Resistance, 4 Suggests Transition Between Moderate Resistance and Moderate Sensitivity, 6 Indicates Moderate Sensitivity, 7-8 Reflect Sensitivity, and 9 Designates High Sensitivity

Scanning Electron Microscopy of Fungal Colonization of Buds

Small segments of bract tissue (0.5 × 0.5 cm) surrounding flower buds inoculated with Powdery Mildew (Blumeria graminis f. sp. hordei) and naturally infected bracts were prepared for scanning electron microscopy (SEM). The tissue segments were adhered to a stub using a graphite-water colloidal mixture and Tissue-Tek (O.C.T. Compound). The samples were rapidly frozen in a nitrogen slush for 10–20 seconds and then transferred to the preparation chamber of a cryo system attached to an SEM. The samples were sublimed for 5 minutes at -80°C, sputter-coated with platinum for 30 seconds and moved into the SEM chamber. Images were captured at a working distance of 4 mm, with a scanning resolution of 3072 × 2207 pixels over 128 low-dose scanning passes with drift correction. For SEM sample processing, a double fixation protocol was used. Samples were fixed with 2.5% glutaraldehyde in phosphate buffer (0.1 M, pH 7.0) for over 4 hours, washed, and post-fixed with 1% osmium tetroxide in phosphate buffer for 1-2 hours, followed by washing. Dehydration was done through a graded ethanol series, followed by absolute ethanol. Samples were dehydrated in a critical point dryer. The dehydrated samples were coated with gold-palladium in an ion sputter and observed using SEM. This method ensured high-quality imaging of powdery mildew infection on barley genotypes.

Statistical Analysis

For each experiment, we assessed the elements of variance analysis following the method outlined by Kearsey & Pooni, (1996). We compared the average performance of all traits across genotypes and cultivars in this study, using the (LSD) test at a 5% significance level. All statistical calculations were conducted using the Co-stat Computer Program, adhering to the guidelines set by Snedecor & Cochran, (1967).

Genetic Polymorphism Assessment DNA Isolation and Primer Selection

We extracted DNA from fresh leaves of the twelve barley genotypes using a method based on Cetyl Trimethyl Ammonium Bromide (CTAB), a common procedure for plant samples, according to Murray & Thompson, (1980). To explore genetic differences among the 12 barley genotypes and to identify DNA markers linked to resistance against powdery mildew, six SSR (Simple Sequence Repeat) primers were chosen associated with powdery mildew loci, which are listed in Table 2. These primers were produced by iNtRON Biotechnology, Inc., located in Korea.

Table 2. Barley SSR Primers and Their Sequences

No.	Primer name	Primer sequence	Reference
1	BMAG0872	F: ATGTACCATTACGCATCCA R: GAAATGTAGAGATGGCACTTG	(Ramsay et al., 2000)
2	Bmac0213	F: ATGGATGCAAGACCAAAC R: CTATGAGAGGTAGAGCAGCC	(Ramsay et al., 2000)
3	UMB0503	F: TCCCGGTGCCATATACAAAT R: TTTGATGAAACGAAGGGAAA	(Beaubien & Smith, 2006)
4	GBM1187	F: GCCAAGAGCATCAAGAGGAT R: GTGGGGACTATGTCCGGC	(Hearnden et al., 2007)
5	Bmac0218	F:ATTGCATTGATTAACTCCTACA R: GGGGGAATCTTTGTGTAAG	(Hearnden et al., 2007)
6	Bamac0134	F: CCAACTGAGTCGATCTCG R: CTTCGTTGCTTCTCTACCTT	(Schmalenbach et al., 2008)

F: Forward **R: Reverse**

Amplification Condition

We conducted the amplification reactions with a 20 µl mix that included 1 µl of genomic DNA (at a concentration of about 60 ng/µl), 1 µl of the forward and reverse primer (10 p mole/µl), 10 µl of a 2X PCR Master mix (i-TaqTM from iNtRON Biotechnology), and 7 µl of sterile deionized water (ddH2O). The PCR amplification was performed using a thermal cycler (Perkin Elmer Cetus), programmed as follows: initial denaturation at 94°C for 4 minutes, then 35 cycles consisting of 30 seconds at 94°C, 30 seconds at 55°C for SSR annealing, and 30 seconds at 72°C, with a final extension of 5 minutes at 72°C and 4°C for 99 h (until removing reactions from the thermocycler). then samples were stored at 4°C.

The amplified DNA was analyzed using horizontal gel electrophoresis with 1.5% agarose gel. The electrophoresis ran at 70 volts for 90 minutes. We visualized the bands using a Benchtop UV-transilluminator and captured images with a photo Doc-ItTM imaging system. To determine the molecular size of the amplified products, we compared them with DNA 1 Kb plus ladders (TIANGEN, cat.no. MD113).

Data Analysis

We analyzed the DNA banding patterns produced by SSR (Simple Sequence Repeat) techniques using the GelAnalyzer 3 software. To catalog polymorphic DNA bands, we marked them as present ("1") or absent ("0"), creating a binary data matrix. Utilizing this matrix, we calculated the genetic similarity (GS) among the genotypes with the Nei & Li coefficient (Nei & Li, 1979). Additionally, cluster analysis was conducted using this software, organizing the genotypes based on genetic similarity through the Unweighted Pair Group Method with Arithmetic Mean (UPGMA).

The effectiveness of the primers in differentiating between genotypes was evaluated by calculating their Polymorphic Information Content (PIC) and Resolving Power (RP). The PIC for each locus was determined using the formula $PIC_i = 2f_i(1-f_i)$, where PIC_i is the polymorphic information content at locus i, f_i is the frequency of present bands, and $(1-f_i)$ is the frequency of absent bands, as described by Roldan-Ruiz et al., (2000). The overall PIC for each primer was computed by averaging the PIC values from all loci associated with that primer. The RP was calculated following the method by Prevost & Wilkinson, (1999) using the formula $Rp = \sum Ib$, where Ib signifies informative bands. The informativeness of each band, Ib, was calculated using the formula $Ib = 1 - [2 \times (0.5 - p)]$, with p representing the proportion of genotypes containing the band.

RESULTS

Differential Susceptibility and Resistance to Powdery Mildew in Plant Genotypes

Powdery mildew causes white, powdery growth on the surfaces of leaves, stems, and sometimes fruits. Visual assessments conducted 25 days after artificial inoculation showed in Figure 3 indicate fungal spores which revealed significant variation in powdery mildew infection among different genotypes. This variation indicates differing levels of susceptibility and resistance to the disease. The severity of the disease varies based on the genetic makeup of the host plant and environmental conditions.

Differential susceptibility and resistance among genotypes can be attributed to genetic variation, environmental factors, and plant defense mechanisms. Genetically, some plants possess resistance genes that can recognize and respond to the pathogen, preventing infection or limiting its spread. Environmental conditions such as temperature, humidity, light intensity, and soil composition influence powdery mildew development, with some genotypes showing increased resistance under certain conditions. Additionally, plant defense mechanisms, including physical barriers like leaf wax coatings, chemical defenses such as phytoalexins, and immune responses triggered by pathogen recognition receptors, vary among genotypes and contribute to differences in disease susceptibility.

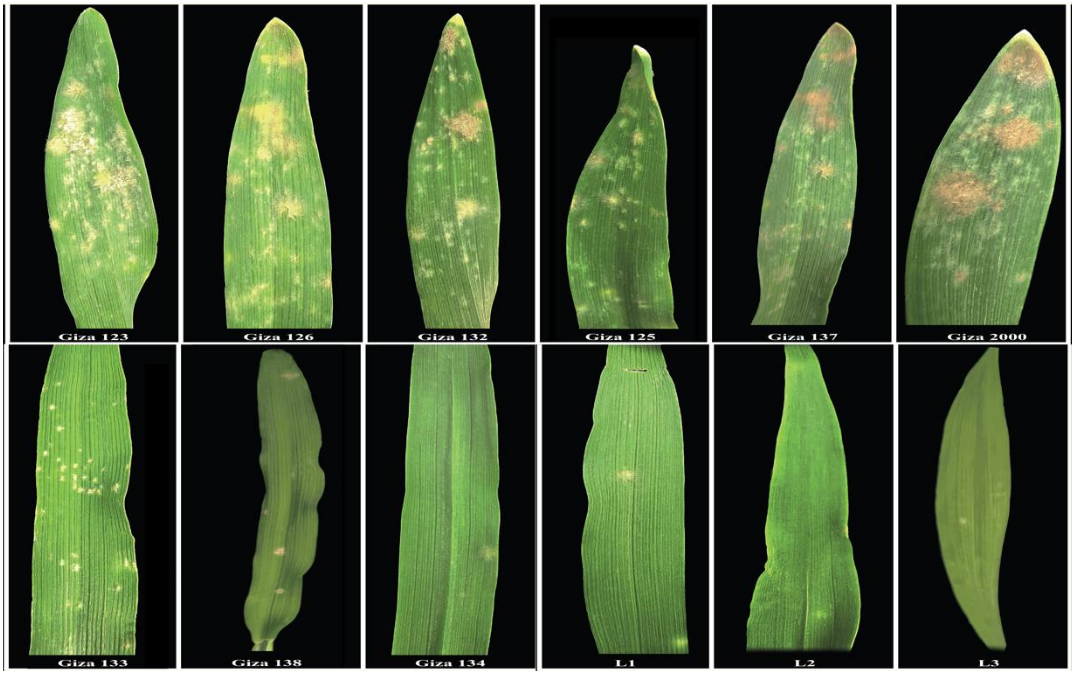


Fig 3. Symptoms of Powdery Mildew Infection in Barley Genotypes

Figure 3 illustrates the symptoms of powdery mildew infection caused by Blumeria graminis f. sp. hordei (Bgh) on various barley genotypes grown in pots. Following artificial inoculation with spores, mold colonies are observed developing on sensitive and moderately sensitive genotypes, with severe infections causing leaf curling in younger plants. Older mold colonies feature a light beige center, as seen in the Giza 2000 genotype. Disease-resistant genotypes exhibit no mold development, maintaining green and robust leaves.

Development and Impact of Powdery Mildew on Plant Tissues

Powdery mildew is a fungal disease affecting various plants, including barley, characterized by white mycelium on plant tissues. It infects young leaflets, older leaves, young shoots, and buds, indicating its ability to spread widely if not managed. Scanning electron microscopy (SEM) studies reveal detailed insights into powdery mildew's development and reproduction. White mycelium appears on both young and older leaves, as well as young shoots (Fig. 4a–d). This mycelium forms conidia, either singly or in chains on conidiophores, which develop directly from the mycelium (Fig.4a,b). The foot cells of conidiophores are distinctly curved (Fig. 4c), aiding adhesion to the plant surface for efficient spore dispersal. On infected flower buds, the mycelium proliferates significantly, leading to prolific spore production (Fig. 4c,d). Spores often adhere to glandular trichomes, specialized hair-like structures on the plant surface that secrete substances like terpenes (Fig. 4e,g), suggesting the fungus may use these secretions for nutrient uptake or other benefits. A critical stage in powdery mildew's lifecycle is spore germination. Under favorable conditions, spores germinate and produce a germ tube that grows towards the plant tissue, facilitating deeper infection (Fig. 4f). This process is essential for spreading the fungus within and between plants.

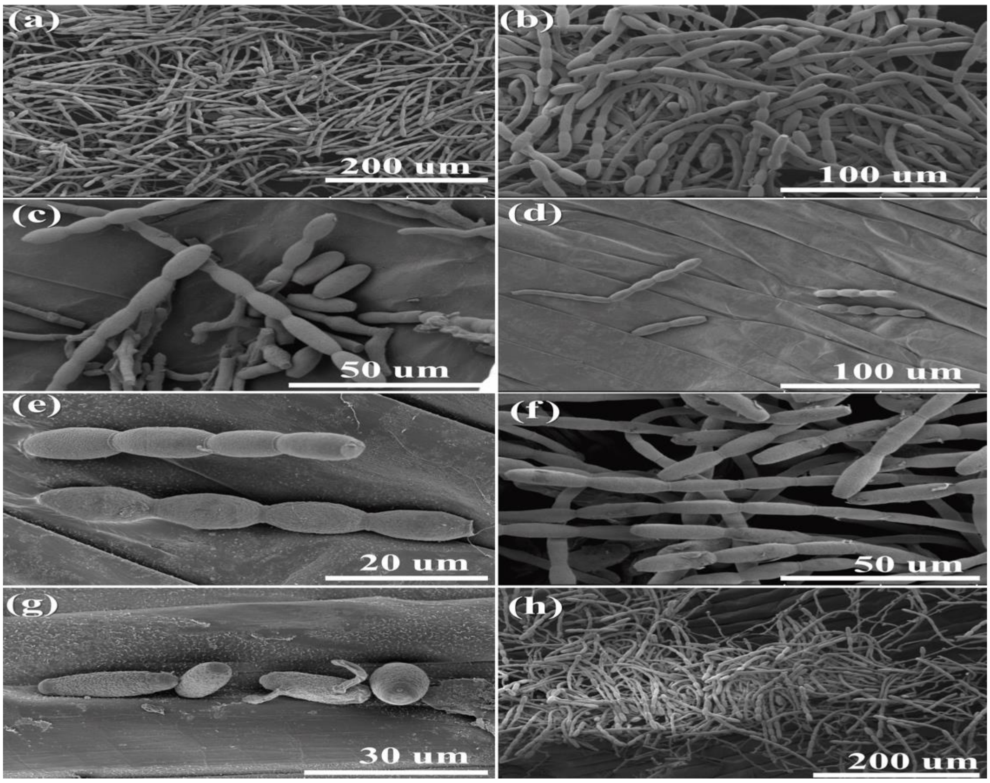


Fig 4. Scanning Electron Microscopy Analysis of Powdery Mildew Infection Development in Studied Genotypes of Barley. (A, B, H) Mycelium and Conidiophore Structures; (C, D, E, F) Conidiophore Production; (G) Conidial Germination

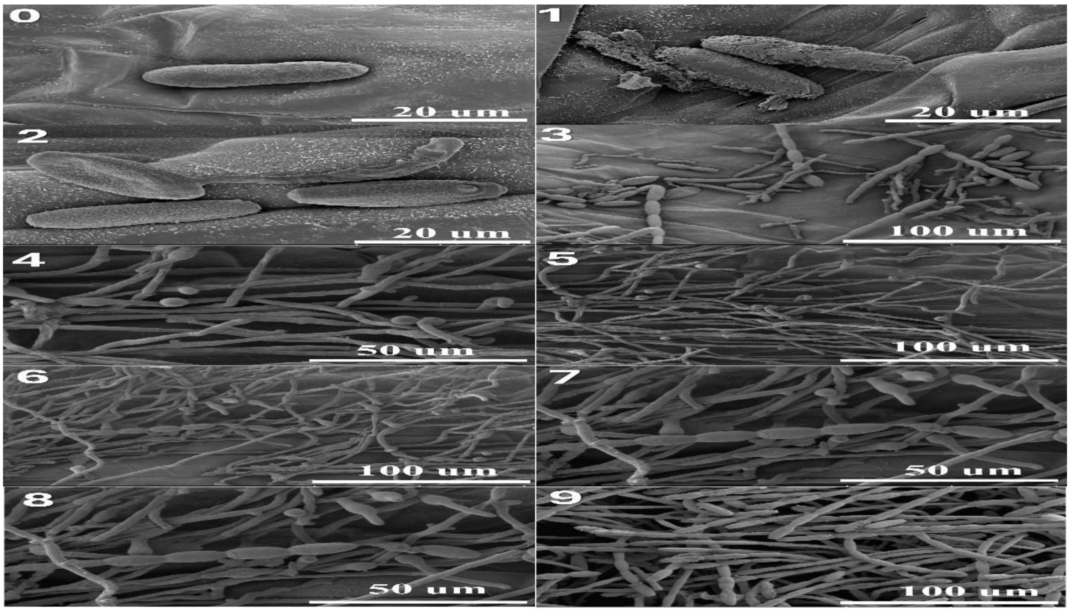


Fig 5. Scanning Electron Microscopy Analysis of Powdery Mildew Infection and Disease Susceptibility Quantification on a 0-9 Scale in Barley Genotypes: 0 Indicates Immunity, 1-2 Represent Resistance, 3 Denotes Moderate Resistance, 4 Suggests Transition Between Moderate Resistance and Sensitivity, 6 Indicates Moderate Sensitivity, 7-8 Reflect Sensitivity, and 9 Designates High Sensitivity

Table 3 shows the mean squares for all the characteristics of the genotypes we studied over two seasons. The results highlighted that the mean squares for the genotypes were significant or highly significant for all traits in both seasons.

Table 3. Estimated Mean Squares of Agronomic Characters for Used Genotypes in the Two Growing Seasons

Source of variation	**d.f**	**Days to maturity**		**Plant height**		**Spike length**	
		2021/22	**2022/23**	**2021/22**	**2022/23**	**2021/22**	**2022/23**
Replications	**3**	3.37ns	0.85 ^{ns}	3.23 ^{ns}	41.20 ^{ns}	0.40 ns	0.36 ns
Genotypes	**11**	19.97**	9.48*	192.94**	154.41**	6.03**	3.92**
Error	**33**	2.60	3.92	6.66	23.49	0.55	0.43
		No. of grains spike^{-1}		**No. of spikes/m^2**		**1000-grain weight**	
		2021/22	**2022/23**	**2021/22**	**2022/23**	**2021/22**	**2022/23**
Replications	**3**	15.36 ns	14.82 ns	84.32 ns	88.68 ns	0.49 ns	1.10 ns
Genotypes	**11**	110.78**	77.67**	892.97**	840.88**	38.59**	41.92**
Error	**33**	7.5	8.41	75.84	118.34	0.54	1.64
		Biological yield		**Grain yield**		**Powdery mildew infection**	
		2021/22	**2022/23**	**2021/22**	**2022/23**	**2021/22**	**2022/23**
Replications	**3**	0.31ns	0.14ns	0.03ns	0.01ns	0.35 ns	5.59*
Genotypes	**11**	1.39**	0.41**	0.27**	0.16**	22.28**	11.71**
Error	**33**	0.37	0.13	0.03	0.02	2.33	1.33

* and ** indicate significant mean squares at 0.05 and 0.01 levels, respectively

Genotype Performance Analysis: Days to Maturity, Plant Height, Spike Length, and Yield Metrics

Figure 6 displays the performances of the 12 genotypes concerning days to maturity, plant height, and spike length. The average values for days to maturity indicated that Line 2 and Giza123 exhibited the most desirable traits for earliness, with Line 2 showing 127 days in the first season and 120.25 days in the second season, while Giza123 showed 127.75 days in the first season and 120.75 days in the second season. Conversely, Giza 132 was the latest maturing genotype, with recorded durations of 135 days in the first season and 126.25 days in the second season.

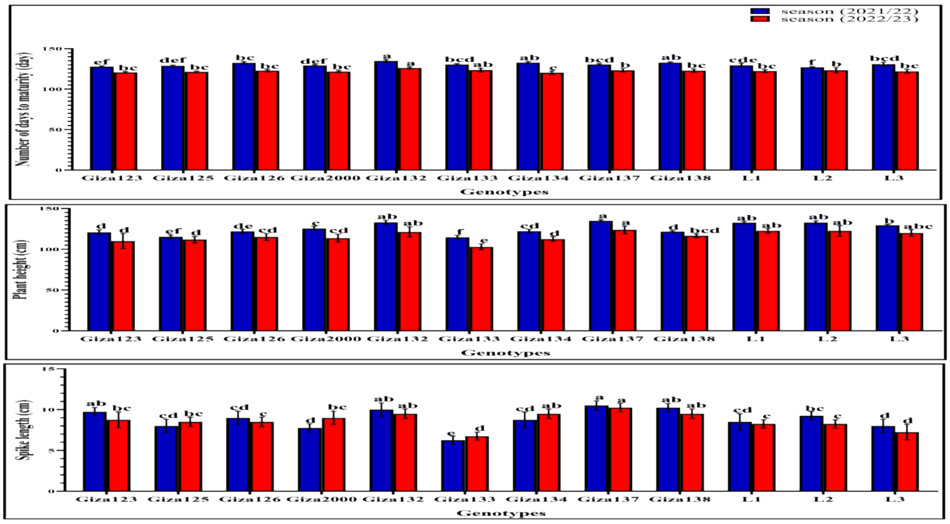


Fig 6. The Days to Maturity (Day), Plant Height (Cm), and Spike Length (Cm) for the Studied Genotypes During the 2021/22 And 2022/23 Seasons

Figure 6 illustrates that there were significant differences in plant height among the 12 barley genotypes during both growing seasons. Specifically, Giza 137, Line 1, and Line 2 were consistently the tallest, with heights of 135.00, 132.50, and 132.75 cm in the first season, and 123.75, 122.50, and 122.50 cm in the second season, respectively. Giza133 was the shortest, with average heights of 114.75 cm in the first season and 103.00 cm in the second season. Regarding spike length, Giza132, Giza 137, and Giza 138 exhibited the longest spikes, with Giza132 having 10.00 and 9.50 cm, Giza 137 having 10.50 and 10.25 cm, and Giza 138 having 10.25 and 9.50 cm in the first and second seasons.

Genotype Performance Analysis: Biological Yield (Kg Plot -1), Number of Grains Spike-1, and Number of Spikes M-2 for the Studied Genotypes

For the number of grains per spike, Figure 7 shows that Giza138, Line 1, and Line 2 produced the highest average numbers in both seasons, with Giza138 having 71.00 and 70.25, Line 1 having 75.00 and 71.25, and Line 2 having 75.50 and 70.50 grains per spike in the first and second seasons, respectively. In terms of the number of spikes per square meter, Line 2 had the highest in the first season with 439.50 spikes, followed by Giza 134 and Giza 133 both with 420.00 spikes, and Giza 138 with 412.50 spikes. In the second season, Giza 2000 led with 409.75

spikes, followed closely by Line 2 with 407.00 spikes. The lowest numbers were recorded by Giza125 and Line 3 in the first season (391.50 spikes) and by Giza123 and Giza 126 in the second season (368.00 and 373.00 spikes, respectively). For the weight of 1000 grains, Giza 138 had the heaviest, averaging 61.20 and 60.95 g in the first and second seasons, respectively. Line 2 followed with weights of 57.29 and 57.58 g in the respective seasons. In terms of biological yield, Giza 138, Giza 134, and Line 2 were consistently among the top three performers across both seasons. Furthermore, Figure 7 reveals significant variations in biological yield (kg plot -1), with Line 2 (7.16 kg) and Giza 138 (7.17 kg) delivering the highest yields in the first season, and Giza 132 (5.69 kg) followed by Giza 134 (5.67 kg) leading in the second season. Giza 123, Giza 125, and Line 1 had the lowest yields in the first season, while Giza 123, Giza 125, and Giza 2000 were the lowest in the second season.

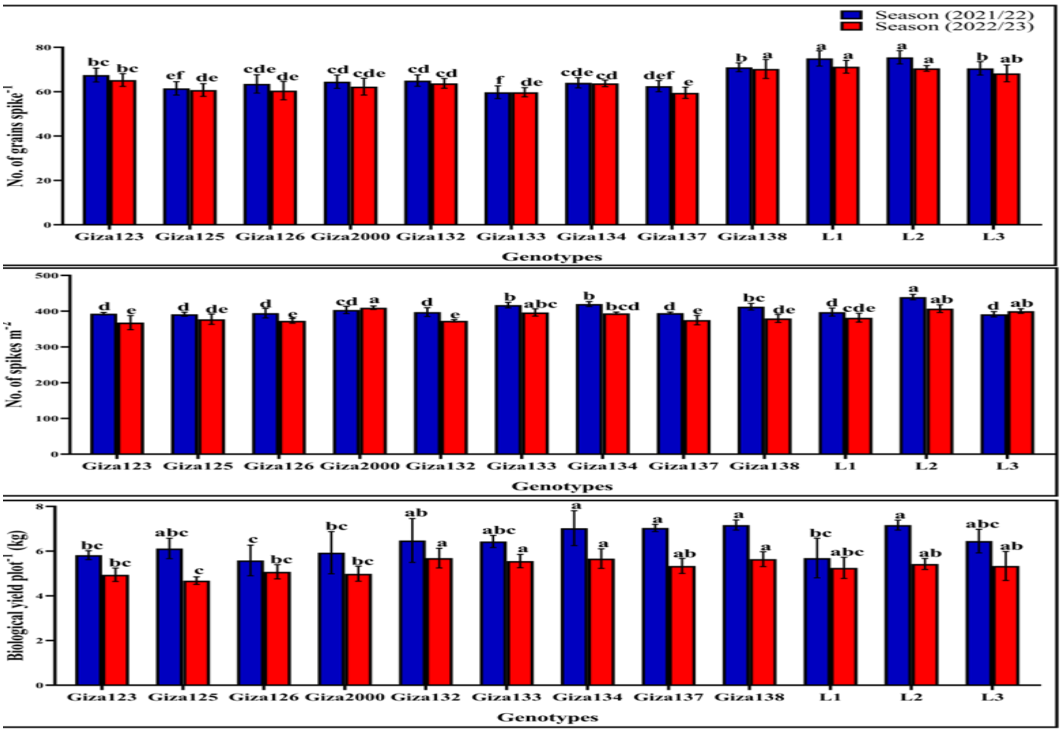
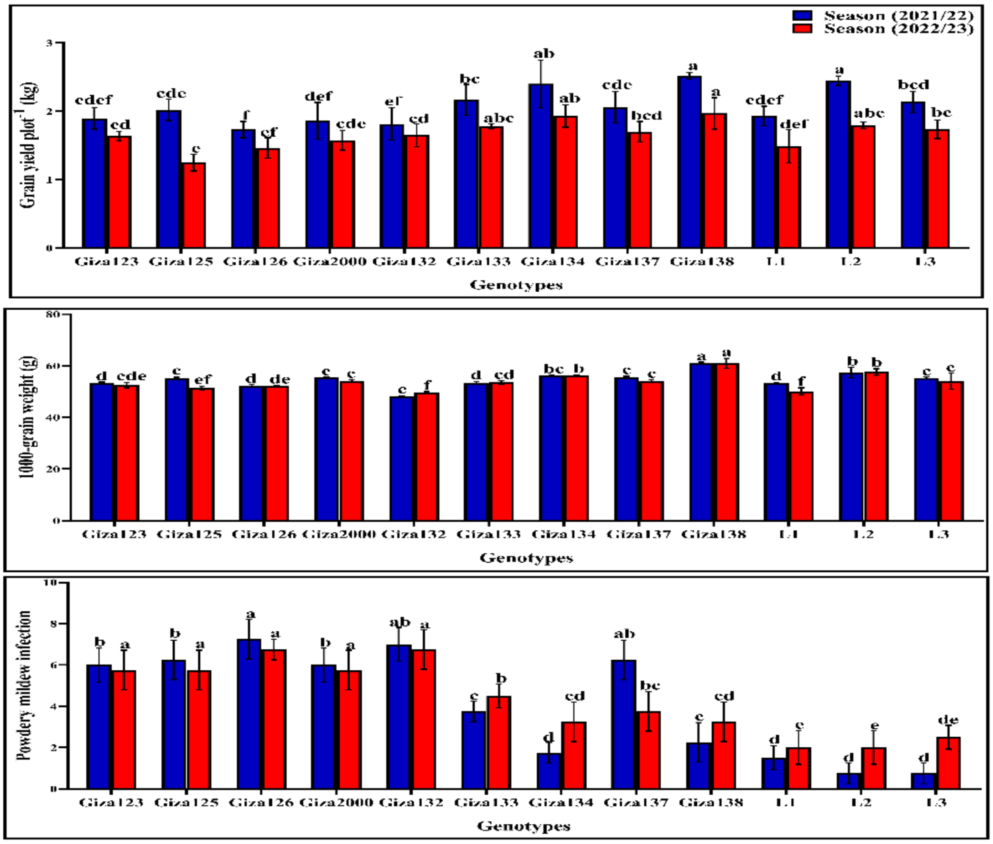


Fig 7. Biological Yield (Kg Plot -1), Number of Grains Spike-1, and Number of Spikes M-2 for the Studied Genotypes During the During the 2021/2022 And 2022/2023 Seasons

Figure 8 highlighted significant differences in Grain yield (kg plot -1), with Giza 134, Giza 138, and Line 2 achieving the highest yields in both seasons. The lowest yields were observed in Giza 126, Giza 2000, and Giza 132 in the first season, and Giza 125, Giza 126, and Giza 2000 in the second season. Regarding powdery mildew infection rates, as shown in Figure 4,5 Line 2 demonstrated the lowest and most favorable infection rates (0.50 and 2.00), followed by Giza 134, Giza 138, and Line 3 in the first and second seasons, respectively. Giza 132 and Giza 126 had the highest infection rates in both seasons. Giza 123 and Giza 2000 showed moderate susceptibility to powdery mildew.

Fig 8. The Grain Yield (Kg Plot -1), 1000 Grain Weight, and Powdery



Mildew Infection for the Studied Genotypes During the 2021/2022 and 2022/2023 Seasons

The cluster analysis for the 12 barley genotypes based on the nine agro-morphological traits is shown in Figure 9, which divided the twelve genotypes into two main groups A and B. Giza 134, Giza 138, Giza 133, L1, L2, and L3 as the highest resistance genotypes branched out in the first cluster (A). The second cluster (B) included the susceptible genotypes Giza 123, Giza 124, Giza 126, Giza 2000, Giza 132 and Giza 137. Additionally, in Fig. 9, the dendrogram divided the four nine studied traits into two groups. Plant height, No. of grains spike-1, No. of spikes m-2, biological yield, grain yield, and 1000 kernel weight were branched infection branched out in the same cluster.

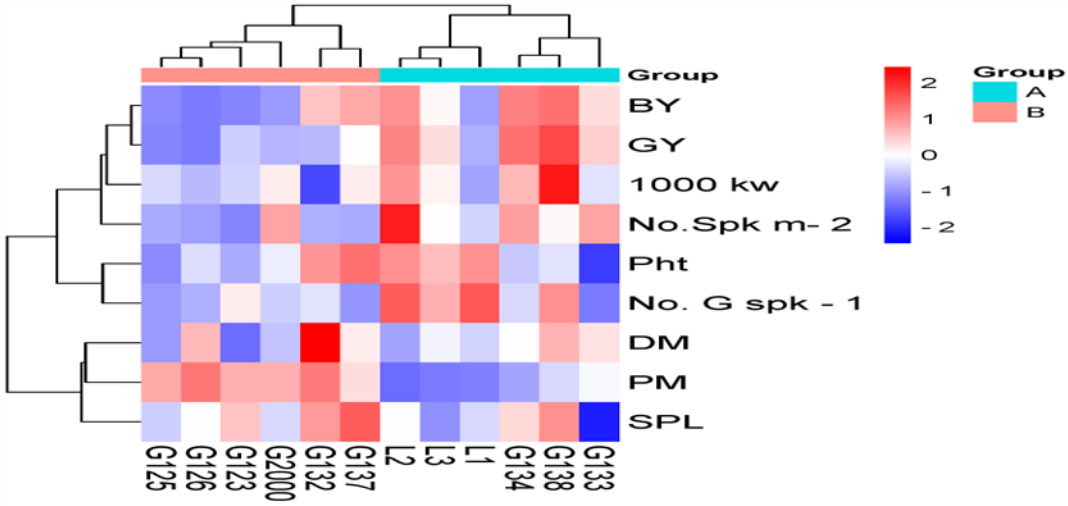


Fig 9. Cluster Analysis for the 12 Barley Genotypes Based on the Nine Agro-Morphological Traits

The analysis of principal components (PCs) revealed that the first four PCs accounted for 90.57% of the total variance observed across the nine assessed traits. Principal Component 1 (PC1) alone explained 41.96% of the total variance. The contributions to PC1 were substantial from several traits: number of grains per spike (0.381), number of spikes per square meter (0.401), biological yield (0.473), grain yield (0.406), and 1000-kernel weight. Spike length contributed less significantly to the variance in PC1. Days to maturity and powdery mildew infection did not significantly influence PC1 but were instrumental in balancing variations observed in other principal components. Thus, the predominant variance among genotypes in PC1 can be attributed primarily to the four major traits specified above.

Table 4. Eigenvalues and Eigenvectors of the First Four Principal Components

	PC1	**PC2**	**PC3**	**PC4**
Eigenvalue	3.78	1.99	1.45	0.94
Variability (%)	41.96	22.11	16.10	10.41
Cumulative %	41.96	64.07	80.16	90.57
Eigenvectors				
MD	-0.057	0.486	-0.342	-0.533

Plht	0.135	0.487	0.470	-0.181
SPL	0.028	0.585	0.001	0.546
No. G spk $^{-1}$	0.315	0.076	0.565	-0.003
No.Spk m^{-2}	0.381	-0.269	-0.101	-0.282
BY	0.401	0.277	-0.353	-0.144
GY	0.473	0.021	-0.283	0.073
1000 kw	0.406	-0.083	-0.190	0.487
PM	-0.428	0.150	-0.303	0.205

Figure 10 demonstrated that Principal Component 2 (PC2) accounted for 22.11% of the total variation observed in the study, with the major contributing traits being days to maturity (0.486), plant height (0.487), spike length (0.585), and biological yield (0.277), in descending order of their contributions. Principal Component 3 (PC3) explained 16.10% of the total variance, with plant height (0.470) and number of grains per spike (0.565) as the principal contributors. Principal Component 4 (PC4) contributed 10.41% to the total variance, with significant contributions from spike length (0.546), 1000 kernel weight (0.487), and powdery mildew infection (0.205).

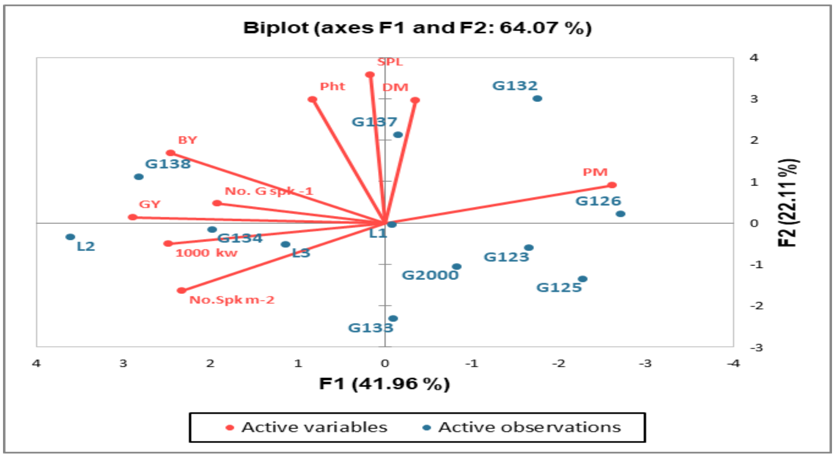


Fig 10. Principal Component Analysis Based on Agro-Morphological Traits for 12 Barley Genotypes

Molecular Diversity Assessment

Polymorphism as Detected by SSR Analysis

Data in Table (5) were obtained from six microsatellite primer pairs which were screened against 12 barley genotypes to detect polymorphic markers.

Table 5. Numbers of the Amplified DNA Bands as Well as the Polymorphism Percentage Generated by the Six SSR Primers

Primer name	*Chr. No	Amplified bands		% of polymorphism	Size of allels (bp)	RP	PIC
		Total	Polymorphic				
BMAG0872	2 H	2	2	100.00	118-147	2.79	0.29
Bmac0213	2 H	5	5	100.00	129 – 403	4.28	0.40

UMB050	2 H	2	2	100.00	137-147	2.59	0.32
GBM1187	2 H	1	1	100.00	167-197	3.15	0.35
Bmac0218	2 H	2	1	50.00	100- 108	3.19	0.38
bamac0134	2 H	2	2	100.00	137-173	2.89	0.31
Total		14	13			16	1.74
Average		2.33	2.16			2.66	0.29

RP: resolving power, PIC: polymorphic information content
*The information for chromosome assignments was obtained from www. Graingenes.com

In this study, six simple sequence repeat (SSR) primers were employed, generating a total of 14 major SSR alleles, of which 13 exhibited polymorphism, demonstrating a diversity among the alleles as depicted in Figures 11, 12, and Table 5,6. The count of alleles produced by each primer varied considerably, ranging from a minimum of one allele with primer GBM1187 to a maximum of five alleles with primer Bmac0213. This variability extended to the number of polymorphic alleles detected per primer pair, with figures ranging from one (GBM1187) to five (Bmac0213). On average, each primer yielded 2.33 alleles, with the average number of polymorphic alleles per locus being approximately 2.16. Allele sizes also displayed significant variation, extending from 100 base pairs (bp) with primer Bmac0218 to 403 bp with primer Bmac0213.

DNA Barcoding and Genotype-Specific Markers

DNA barcoding based on data of SSR is shown in Figure 11 and Table 6. For all genotypes, Line-3 revealed the lowest number of fragments (4), while, the genotype Giza 138 revealed the highest number of fragments on all genotypes (11). All genotypes gave a total of 93 fragments with an average of 9.3 fragments per genotype. Giza 123, showed only two unique fragments (120 and 170 bp as a positive specific marker), respectively (Table 6).

Table 6. Barley Genotypes Characterized by Positive and Negative Genotype-Specific Markers and their Molecular Sizes (Bp) Using SSR Analysis

Marker type	**Genotype**	**Primer**	**Positive genotype-specific marker**	**Negative genotype-specific marker**
SSR	Giza 123	Bmac0213	170bp	-----
			120bp	-----

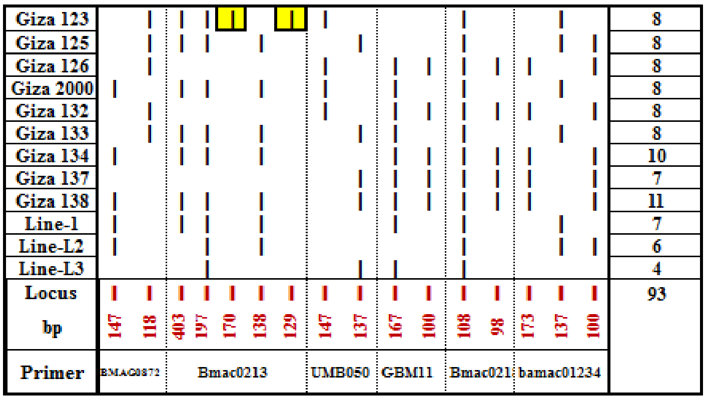


Fig 11. DNA Barcoding Based on Data of SSR Markers

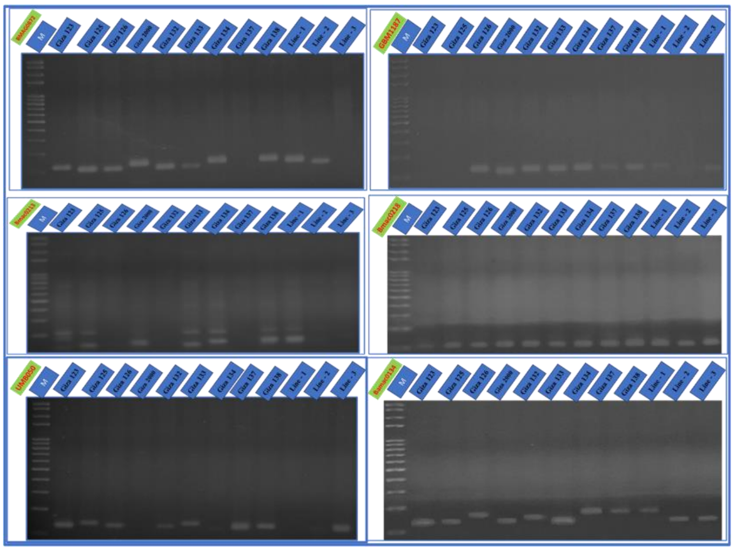


Fig 12. SSR Patterns of the 12 Barley Genotypes as Revealed by Six SSR Primers
M: 1 Kb Plus DNA Ladder

The resolving power (RP) of the used six SSR primers varied from 2.59 to 4.28. The polymorphic information content (PIC) values also showed a range, from 0.29 to 0.40, indicating a consistent rate of polymorphism across all six SSR primers, as detailed in Table 5. Notably, the SSR marker Bmac0213 displayed the highest polymorphism level, with a PIC value of 0.40. In a related study evaluating the genetic diversity of Iranian barley landraces with 17 microsatellite markers, a high average PIC value of 0.651 and an average of 8.1 alleles per locus were found, highlighting significant genetic diversity.

We created a molecular similarity matrix using a simple matching coefficient based on the data from the six SSR primers (Table 7). These primers were effective in distinguishing all the studied genotypes, allowing us to explore the genetic variation related to resistance against powdery mildew among the different barley varieties and lines.

Table 7. Genetic Similarity (GS) Matrix for 12 Barley Genotypes Obtained from Six SSR Fingerprinting

Genotypes	G123	G125	G126	G2000	G132	G 133	G 134	G 137	G 138	L1	L2	L3
G 123	1											
G 125	0.500	1										
G 126	0.300	0.200	1									
G 2000	0.454	0.500	0.300	1								
G 132	0.300	0.200	0.333	0.300	1							
G 133	0.455	0.875	0.300	0.600	0.300	1						
G 134	0.250	0.400	0.333	0.667	0.333	0.5	1					
G 137	0.290	0.222	0.500	0.200	0.500	0.333	0.375	1				
G 138	0.230	0.500	0.300	0.600	0.300	0.600	0.875	0.500	1			
L1	0.363	0.556	0.200	0.875	0.200	0.667	0.750	0.222	0.667	1		
L2	0.300	0.500	0.112	0.625	0.111	0.444	0.500	0.125	0.444	0.719	1	
L3	0.200	0.375	0.286	0.333	0.285	0.500	0.375	0.600	0.500	0.375	0.285	1

The similarity index revealed that the most resistant genotypes were more closely related to each other and more distinct from the more sensitive genotypes, with similarity values ranging between 0.111 and 0.875. Specifically, Giza 134 and Giza 138 had the highest genetic similarity (GS = 0.875), suggesting they are very similar genetically. On the other hand, Giza 132 and Line 2 had the lowest genetic similarity (GS = 0.111), indicating significant genetic differences between them.

Cluster and Principal Coordinate Analyses

The cluster analysis, shown in Figure 13 based on SSR marker data, was effectively used to explore the genetic connections among barley genotypes. This analysis indicated that the genotypes tend to cluster based on their origins. Specifically, it identified the main groups that included the cultivars most resistant to powdery mildew (G 134 and G 138) and the highly susceptible cultivars to powdery mildew (G 132 and G 126).

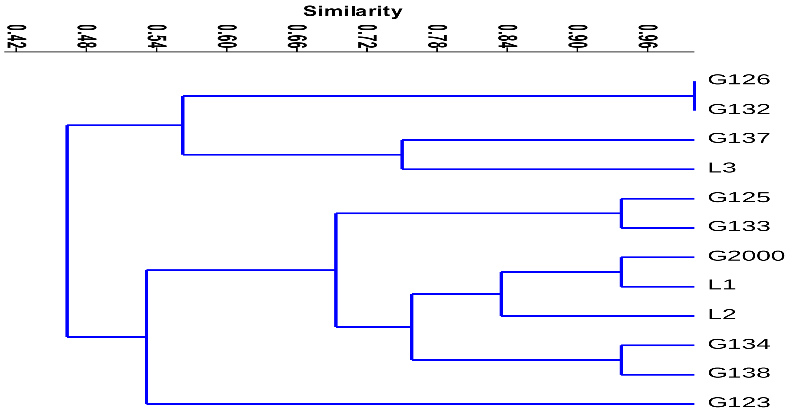


Fig 13. Dendrogram of Twelve Barley Genotypes Produced by SSR Data

DISCUSSION

Implications for Understanding and Managing Powdery Mildew Pathogenesis

The study of powdery mildew's development and impact on plant tissues, particularly barley, reveals significant insights into the disease's lifecycle and pathogenicity. Powdery mildew is a widespread fungal disease that can severely affect crop yields and quality if not properly managed. The observed development of white mycelium on various parts of the plant, including young leaflets, older leaves, and young shoots, aligns with previous findings that underscore the fungus's ability to infect plants at multiple stages of growth (Glawe, 2008). (SEM) provided detailed visual evidence of conidia formation and the structural characteristics of conidiophores. The formation of conidia, either singly or in chains, and the distinctly curved foot cells are consistent with descriptions in other studies on powdery mildew fungi (Braun, 2012). These morphological features facilitate the fungus's adhesion to the plant surface, enhancing its ability to disperse spores effectively and spread the infection.

Prolific spore production observed on infected buds, with spores adhering to glandular trichomes, suggests that the fungus may exploit these structures for nutrient uptake or other benefits. This observation is supported by studies indicating that fungal pathogens can interact with trichomes to gain an advantage during infection (Wang et al., 2014). The ability of powdery mildew to adhere to trichomes and potentially utilize their secretions could explain its success in colonizing and proliferating on host plants. The germination process, wherein spores produce a germ tube under favorable conditions, is crucial for the fungus's ability to infect deeper layers of plant tissue. This mechanism of infection has been well-documented in fungal pathogenesis, highlighting the importance of germ tubes in penetrating host defenses and establishing infection (Deising et al., 2000).

Understanding this process provides insights into potential targets for disease management and control strategies. The findings on powdery mildew's development and its interaction with host plant tissues underscore the complexity of fungal pathogenicity. Effective management strategies must consider the disease's ability to infect plants at various growth stages, its reproductive structures, and its mechanisms for spreading and penetrating plant defenses. Integrating these insights with current agricultural practices could enhance the control and mitigation of powdery mildew, ultimately improving crop health and productivity.

Breeding for Mildew Resistance in Barley

The susceptibility of barley to powdery mildew, especially under foliar irrigation methods, significantly affects yield quality and increases production costs due to a heightened need for fungicide applications. Studies, such as the one conducted by Guo et al., (2024) indicate that powdery mildew can disrupt barley's growth from seeding to maturity, with early infection potentially leading to yield reductions of up to 25% in vulnerable varieties. This considerable impact drives breeders to focus on developing resistant barley strains through selective breeding, aiming to balance high yield potential with favorable agronomic traits like maturation time, plant height, and grain yield metrics. A study by Wolfe et al., (2008) stated that challenges, emphasizing the critical need for breeding programs to prioritize the development of varieties resistant to diseases like powdery mildew. This strategy not only minimizes reliance on chemical treatments but also promotes sustainable farming practices.

The goal of identifying barley genotypes with enhanced resistance to powdery mildew aligns with who showcased the application of high-throughput SNP genotyping in barley in which research marks a significant advancement in identifying genetic markers linked to disease resistance, highlighting how selective breeding and genetic screening are essential in improving barley's disease resilience (Close et al., 2009). Current findings, reveal substantial genetic diversity among the examined genotypes for these traits, underscore the importance of genetic variability in breeding program success. This is supported by Patnaik & Khurana, (2003) who mapped resistance genes against powdery mildew (Blumeria graminis f.sp. hordei) and stem rust (Puccinia graminis) in barley, and their identification of quantitative trait loci (QTLs) for partial resistance showcases the utility of genetic diversity in enhancing disease resistance as well as exemplifies the benefits of understanding genetic variance for developing more robust barley cultivars (Patnaik & Khurana, 2003).

Our results demonstrate significant genetic variability among different genotypes for the studied traits, which could prove invaluable for breeding efforts aimed at improving these characteristics. This is in agreement with Abdel-Azeem et al., (2023) who reported significant variances for the traits under investigation. Similarly, the genotypes with higher yields also exhibited superior estimates for yield-contributing traits (e.g., number of grains per spike, number of spikes per square meter, and 1000-grain weight) (Dora et al., 2021). Notably, genotypes with higher yield potentials often showed enhanced performance in yield-contributing traits, establishing a direct link between yield efficiency and genetic resilience against powdery mildew. This integrated approach demonstrates the synergy between genetic diversity and selective breeding in bolstering barley's resistance to powdery mildew, thereby facilitating more sustainable agricultural methodologies.

Barley Genotype Diversity and Mildew Resistance

The current investigation unveils considerable genotypic variation within twelve barley genotypes under study for powdery mildew resistance, underscoring the vast potential this diversity holds for augmenting breeding programs. By providing a wide array of genetic resources, this variability aids in the selection of genotypes that not only demonstrate desirable traits but also exhibit enhanced disease resistance. Studies have emphasized the significance of genetic markers in detecting DNA polymorphism and identifying genotypes, which is fundamental for delineating phylogenetic relationships among barley cultivars (Noor-ul-Huda Ghori et al., 2020), especially those with resistance to powdery mildew which highlights how genetic diversity serves as a cornerstone for breeding efforts aimed at enhancing disease resistance in barley (Noor-ul-Huda Ghori et al., 2020). Our observations are in concordance with the findings of Dora et al., (2017) who identified similar patterns of molecular diversity among barley genotypes through SCoT and SSR analyses. The noted discrepancies in dendrogram patterns, especially those delineated by SSR markers, can be attributed to the variation in the number of PCR products analyzed. This highlights the critical importance of incorporating an ample number of loci, strategically distributed across the genome, to derive accurate genetic relationships among barley cultivars as a principle that resonates with the insights provided by Fernandez et al., (2002).

Furthermore, our genetic similarity analyses have identified that resistant genotypes, particularly Giza 134 and Giza 138, share a high degree of genetic similarity, likely contributing to their resistance to powdery mildew. This genetic linkage underscores the potential of leveraging this shared genetic base as a cornerstone for breeding programs aimed at developing barley varieties with elevated resistance profiles. Such an approach is reinforced by the findings of Silvar et al., (2010) who conducted screenings within the Spanish Barley Core Collection for disease resistance, including powdery mildew. Their work highlights the value of genetic similarity and linkage analysis in identifying and utilizing resistant genotypes for the strategic breeding of barley, offering new avenues to combat powdery mildew. Collectively, these insights echo and build upon the foundational work of previous researchers, underscoring the potential of genetic analyses in the development of disease-resistant barley cultivars.

CONCLUSIONS AND RECOMMENDATIONS

This study highlights the pivotal role of genetic diversity in breeding barley varieties resistant to powdery mildew, demonstrating the significance of leveraging genotypic variation for improved disease resistance. Through detailed genetic and molecular analyses, we identified substantial variability among barley genotypes, underscoring the potential of this diversity in selecting genotypes with both favorable agronomic traits and enhanced resistance. Genetic markers and DNA polymorphism were crucial in distinguishing superior genotypes, offering insights into phylogenetic relationships, and pinpointing candidates with inherent resistance capabilities. Key findings include the identification of genotypes, such as Giza 134 and Giza 138, which share a high degree of genetic similarity, likely contributing to their resistance to powdery mildew. This genetic linkage suggests a promising strategy for breeding programs to develop barley varieties with elevated resistance profiles, thereby minimizing reliance on chemical treatments and promoting sustainable agricultural practices. Moreover, the observed polymorphism among the genotypes, as revealed through SSR analysis, highlights the extensive genetic base available for enhancing barley's disease resilience. The study confirms the critical importance of genetic diversity and the strategic use of molecular markers in the continuous effort to improve barley's resistance to powdery mildew. The integration of genetic diversity and selective breeding emerges as a key approach in developing barley varieties that are resilient to powdery mildew. This research not only contributes to our understanding of the genetic underpinnings of disease resistance but also lays the groundwork for future breeding efforts aimed at ensuring the sustainability and productivity of barley cultivation in the face of disease challenges.

FURTHER STUDY

This study still has limitations, so further research is needed related to the topic of Identification and Genetic Differentiation of Powdery Mildew Resistance of some Egyptian Barley Genotypes in order to perfect this study and increase insight for readers.

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