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Evaluation of Egyptian wheat (*Triticum aestivum* L.) genotypes for leaf rust resistance, yield losses and chemical properties

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Abstract

This study was performed during two successive growing seasons at Nubaria province, Beheira Governorate, Egypt to evaluate nine genotypes under two investigation factors for diseases and yield traits against leaf rust disease caused by *Puccinia triticina* (*P. triticina*) and their effects on biochemical parameters of the plant, quantitative and grain composition yield. The susceptible check genotype *Triticum spelta saharinsis* (TSS), as well as the two susceptible Sids-1 and Gemmeiza-7 showed higher percentages of FRS% and values of AUDPC during the two seasons. Meanwhile, the partially resistant genotypes Line Shandweel-2(Shandweel-2), Line Nubaria-2(Nubaria-2), and Misr-3 demonstrated lower disease severity. The final rust severity (FRS %) values ranged from 16.67% to 97% across both seasons. Data was subjected analysis of variance (ANOVA) of randomized complete block design for each season. Genetic similarity and clustering dendrogram analysis results showed distinct differences between wheat genotypes based on the percentage of infection accumulated by the different accessions at the two study years. Leaf rust disease had an effect on chlorophyll and carotenoid contents in wheat genotypes. Protected genotypes showed higher chlorophyll contents than infected ones. Nubaria 2 had the highest chlorophyll and carotenoid contents in infected plants, with variations noted across all genotypes, while Nubaria 2 and Shandaweel 2 had higher phenolic content. The levels of oxidative enzyme (PO and PPO) were higher in most infected wheat genotypes, especially Nubaria 2, which had the highest PPO activity. The analysis of variance revealed significant differences in yield composition, and biochemical traits among wheat genotypes under infected and protected conditions. Genotypes Shandweel 2, Nubaria 2 and Sids 1 displayed lower losses in 1000 kernel weight and grain yield. This indicated that Sids 1 genotypes have a high level of tolerance to leaf rust infection. The second season showed higher losses in 1000 kernel weight and grain yield compared to the first season. Infected grains had higher dry matter, ash, and crude fiber, and changes in protein, gluten, and carbohydrate contents compared with protected genotypes. Principal component (PCs) measured traits in infected and protected treatment such as gluten and carbohydrate mg/g have negative correlation among them.

Keywords: Wheat, leaf rust, *Puccinia tritician*, yield losses, grain composition, biochemical, genotype.

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1. Introduction

Wheat is crucial cereal crops globally (Kayim & Alsalmo, 2022; Igrejas et al. 2020). Wheat production in Egypt in 2022 reached an above-average level of 9.62 million tons, there remains a gap between production and consumption (FAO, 2022). However, wheat production faces threats by various biotic stresses, among which are fungal diseases, particularly rust diseases caused by the genus Puccinia (Chai et al., 2022; Kumar et al., 2022; Meyer et al., 2021; El-Orabey et al., 2019). Leaf rust, specifically caused by *P. triticina* is one of the most prevalent and economically damaging diseases affecting wheat globally. These diseases are a threat to wheat production in Egypt and all over the world (Hassan et al., 2022; Bhavani et al., 2021; El-Orabey et al., 2020). Epidemics of widespread leaf rust can have profound consequences, threatening global food security (Kumar et al., 2022; Chai et al., 2020; Prasad et al., 2020; Li et al., 2018). In temperate regions, it can cause severe epidemics, compromising grain quality, necessitating effective management strategies to mitigate its impact (El-Orabey et al., 2020). The extent of yield losses depends on the timing and severity of the infection (prevalence of aggressive and/or virulent races of the pathogen), as well as the susceptibility of the wheat cultivar (Naseri & Farzad, 2021; Naseri & Sasani, 2020; Pathan & Park, 2006). In addition to yield losses, the interplay between leaf rust infection and various biochemical parameters such as chlorophyll content, phenolic compounds, enzymatic activities (PPO, PO, cellulase, protease), and proline accumulation underscores the complexity of plant-pathogen interactions (Mafa et al., 2023; Elsharkawy et al., 2022; Yahya et al., 2020; Draz et al., 2019; Fan et al., 2016; Robert et al., 2004**)**. Also, it affects the quality of wheat grain, reducing test weight, protein content, overall milling and baking characteristics. Understanding these impacts is crucial for developing resistant cultivars and implementing effective management strategies. Moreover, a number of suitable statistical techniques such as PCs and cluster analysis have been usually applied for studying the genetic diversity and character associations aimed at the selection of genotype resistant to biotic. Thus, the main objective of this study was to evaluate wheat (*Triticum aestivum* L.) genotypes for leaf rust resistance, yield losses, and chemical properties, and PCs and cluster analysis have been used as an efficient procedure to determine the structural relationships.

2. Materials and methods

Field experiments were done during 2021/2022 and 2022/2023 seasons. A field experiment was carried out at Nubaria Agricultural Research Station, Beheira, Egypt using nine genotypes *i.e.* Sids 1, Sids 12, Gemmeiza 7, Gemmeiza 11, Misr 3, Line Shandweel 2 (Shandweel 2), Line Nubaria 2 (Nubaria 2), Giza 171 and check genotype *Triticum Spelta Saharinsis* (TSS) under two investigation factors for yield and diseases traits. In both seasons 2021/2022 and 2022/2023 wheat cereals were planted in November. All genotypes were randomly distributed as well as planned in a randomized complete block design in three replicates. Each genotype was sown in plots. The plot size was 6×7 m = 42 m^2. All other cultural practices were applied as recommended. The recommended cultural practices were adopted throughout the growing season. Each genotype was sown under protected conditions by the application of 3 foliar sprays, starting from disease onset

and 15 days thereafter. The fungicide Tilt 25% EC at the rate of 75 cm /300 L water was used. On the other hand, the infected plots were left for artificial infection with a mixture of physiological races during the late tillering and late elongation stages. The main treatments were infected and protected plots. All plants were surrounded by a susceptible spreader (*Triticum spelta saharinsis*).

2.1 Disease assessment

Adult-plant reactions were scored as rust severity (%) for each genotype, at the time when rust first appeared until the early dough stage (Large, 1954). Rust infection severity of each variety was recorded every seven days after the initial infection occurred there by using the modified Cobb's scale (Peterson et al., 1948). Adult plant resistance response and severity for Leaf rust disease was recorded following the descriptions of Roelfs et al. (1992), and Singh et al. (2013).

2.2 The final rust severity (FRS)

The final rust severity (FRS) was recorded as outlined by Das et al. (1993) as the disease severity (%) when the highly susceptible check genotypes was severely rusted, and the disease rate reached the highest and final level of leaf rust severity.

2.3 Area under disease progress curve (AUDPC)

Area under disease progress curve (AUDPC); It was estimated to compare different responses of the tested genotype using the following equation (Pandey et al., 1989):

$$AUDPC = D\,[1/2\,(Y_1+Y_K) + (Y_2 + Y_3 + \ldots\ldots + Y_{(k-1)})]$$

Where: D = days between readings, Y_1 = first disease recording, Y_K = last disease recording.

2.4 The impact of Leaf rust infection on yield parameters of wheat

The effect of leaf rust infection on grain yield and 1000-kernel weight were determined in an experiment of split-plot design with three replicates for two seasons. Losses (%) were determined as the difference among the protected and infected plots using simple equation adopted by Calpouzos et al. (1976):

$$\text{Loss (\%)} = (1- Y_d / Y_h) \times 100$$

Where: Y_d = yield of diseases plants, Y_h= yield of healthy plants.

2.5 Analysis of biochemical parameters

Total chlorophyll, chlorophyll a and chlorophyll b were determined according to Palta 1990. Total and free phenolic compounds were determined using Folin-Ciocalteu reagent according to Gomaa et al. (2016). The reaction measured using a spectrophotometer at 520 nm wavelength. Total free amino acids were determined by the colorimetric ninhydrin method modified by Rosen (1957). Proline content was determined by the colorimetric ninhydrin method by Bates et al. (1973).

2.6 Assay of enzymes activity

The activity of peroxidase (POX) was determined according to modified method of Chance and Maehly (1955) described by Falade et al. (2019). Polyphenoloxidase (PPO) activity was determined according to the method described by Malik and Singh (1980). CMCase activity was determined by measuring the release of reducing sugars during CMC degradation Miller (1959).

2.7 Quantitative determination of yield composition

The determination of yield composition (dry matter %, carbohydrate mg/g, protein mg/g,

crude fiber %, Ash content %, dry gluten mg/g) of grains in nine wheat genotypes were determined in protected and infected plants. The dry gluten content was determined by drying the wet gluten obtained according to AACC (2000) and Kassegn (2018). The mass of dry gluten had been taken after drying as wet gluten for 24 h at 100 °C. Also, carbohydrates were determined by Nielsen (2010). The Bradford protein assay was used for determined protein according to Kruger (2002). Crude fiber and ash content were estimated as standard procedure stated by methods of analysis (AOAC, 2005) and value was expressed as percentage. Dry matter was estimated by measuring the weight of the samples then value was expressed as percentage (Astaoui et al., 2021).

2.8 Statistical analysis

Data collected from studied genotypes were averaged and subjected to analysis of variance (ANOVA) using "Genstat" by randomized complete block design with three replications for each season (Steel et al., 1997). Principal component analysis (PCs) was carried out by Sneath and Sokal (1973) using a correlation matrix to define the pattern of variation among the genotypes. The PCs made a significant contribution to genetic variability when eigenvalues were greater than one. The genetic distance of 9 wheat accessions was estimated using clustering of genotypes. Euclidean distance (ED) was calculated from quantitative traits after standardization (Kovach, 1995). Using Minitab v-17 software, used to calculate the distance matrix from phenotype traits was used to construct a dendrogram. The results of cluster analysis were presented in the form of a dendrogram.

3. Results

3.1 Evaluation of different wheat genotypes against leaf rust compared with susceptible check genotype (TSS) depending on two epidemiology parameters

Two epidemiology parameters (FRS and AUDPC) were evaluated against leaf rust of different wheat genotypes during two seasons 2021/2022 and 2022/2023 at Nubaria province, Beheira, Egypt (Table 1 and 2). Data in Table (1) showed the analysis of variance (ANOVA) for randomize complete block design of leaf rust disease parameters under field condition during in two seasons. Final rust severity (FRS) and area under disease progress curve (AUDPC), in both seasons were recorded. The genotypes showed significant in all studied disease traits of both seasons.

Table 1: Mean squares of the AUDPC and Final during seasons 2021/2022 and 2022/2023.

Traits		AUDPC				FRS			
		2021/2022		2022/2023		2021/2022		2022/2023	
S.O.V	DF	M.S	F pr.	M.S	F pr.	M.S	F pr.	M.S	F pr.
Rep	2	1960.9		2068.		92.59		403.70	
Genotype	8	359318.8	<.001	320393.	<.001	1820.37	<.001	1739.81	<.001
Residual	16	663.5		1206.		25.93		53.70	

Data in Table (2) showed that FRS (%) and AUDPC values during seasons 2021/2022 and 2022/2023. Final rust severity (FRS) was scored as disease severity (%), when the highly susceptible check genotype (TSS) was severely infected with rust and the disease rate reached the highest infection and final levels during the two growing seasons of the

study. The tested genotypes showed variation values of FRS and AUDPC to wheat leaf rust characterized compared with susceptible check genotype TSS. It was noticed that FRS values were run in a parallel line with the values of AUDPC during the growing seasons. Two epidemiology parameters in the second season were higher than the first season. Different levels of FRS % ranged from 16.67 to 86.67 and 30 to 97 in two seasons. The wheat genotypes Shandweel 2, Nubaria 2, and Misr 3 exhibited the lowest percentages of disease severity as reached 16.67, 23.33 and 26.67% in the first season, while, ranged 30, 37 and 40 in the second seasons as compared with the other tested wheat genotypes. The TSS genotype exhibited high percentages of rust infection which reached to 86.67 and 97 % in both seasons, respectively, followed by Sids-1 and Gemmeiza-7. In the second season, the same trend was noticed between genotype infection response but showed higher levels of FRS % compared with the first season. The tested wheat genotypes were sorted into three groups based on their slow rusting resistance, using the AUDPC values. The first group included wheat genotypes with AUDPC values less than 360, designated as resistance genotype or partially resistant. This group consisted of three wheat genotypes: Shandweel 2, Nubaria 2, and Misr 3. The second group included genotypes with intermediate AUDPC values ranging from 361 to 649. The third group consisted of genotypes with high AUDPC values exceeding 650. During two growing seasons, the obtained values of AUDPC were estimated in the first group, which included Shandweel 2, Nubaria 2, and Misr 3 *i.e.* 150.5, 199.5 and 211.17 in the first season and 213.5, 341.83, and 353.5 in the second season, respectively. The second group included Sids-12, Gemmeiza-11, and Giza-171. Whereas genotypes Gemmeiza-7, Sids-1, and T.S.S exhibited the highest AUDPC *i.e.*, 822.5, 939.17 and 1085.0 in the first season as recorded 875, 1050 and 1149.17 in the second season, respectively. The third group included Gemmeiza-7, Sids-1, and TSS, while the second growing season gave the same categorized trends: low, intermediate, and high AUDPC values.

Table 2: Mean performance for nine genotypes in disease character during 2021/2022 and 2022/2023.

Genotypes	*FRS (%)		**AUDPC	
	2021/2022	2022/2023	2021/2022	2022/2023
Sids 1	77.33 F	90 EF	939.17 F	1050.00 F
Sids12	36.67 C	50 BC	353.50 C	528.50 C
Gemmeiza 7	66.67 E	80 E	822.50 E	875.00 E
Gemmeiza 11	46.67 D	67 D	456.17 D	639.33 D
Misr 3	26.67 B	40 AB	211.17 B	353.50 B
Nubaria2	23.33 AB	37 A	199.50 B	341.83 B
Shandawil2	16.67 A	30 A	150.50 A	213.50 A
Giza 171	43.33 CD	57 CD	446.83 D	557.67 C
TSS	86.67 G	97 F	1085.0 G	1149.17 G

Means followed by different letter (s) in column are significantly different according to Dunkan's multiple range test at p = 0.05. *FRS (%) = final rust severity, **AUDPC = area under disease progress curve.

3.2 Effect of leaf rust of wheat genotypes on biochemical parameters

In this study, effects due to the biochemical characterization of protected and infected genotypes were shown in Tables (3, 4 and 5). The effects of leaf rust disease on chlorophyll contents a, b and total were determined by

observing these parameters under infected and protected with fungicide treated plots (Table 3). Data showed that chlorophyll contents a, b and total were higher in all genotypes compared with TSS under infected and protected genotypes. Data exhibited higher chlorophyll contents in protected genotypes than in infected genotypes. TSS, which recorded the lowest chlorophyll contents (a, b, and total) in infected and protected wheat genotypes, reached (0.18, 0.17, and 0.35) and (0.54, 0.36, and 0.89), respectively. The mean photosynthetic rate differed between protected and infected plots, with chlorophyll contents and also showing significant variation across all samples. Nubaria 2 had the highest chlorophyll a, b, and total content in infected plots (0.74, 0.54, and 1.28). Additionally, Nubaria 2 recorded the highest carotenoid content in infected plants. Data indicated that carotenoids were higher across all genotypes compared to TSS under both infected conditions, with variations observed between infected and protected plants for all genotypes, except Nubaria 2.

Table 3: Photosynthetic pigments fractions (mg/g) in leaves of nine wheat genotypes infection by *Puccinia triticina* or protected during the second season growing season.

Genotypes	Chlorophyll mg g-1						Carotenoids mg g-1	
	Chl (a)		Chl (b)		Total (a+b)			
	Infected	Protected	Infected	Protected	Infected	Protected	Infected	Protected
Sids 1	0.33	0.72	0.27	0.59	0.6	1.31	0.19	0.32
Sids 12	0.39	0.63	0.29	0.4	0.68	1.02	0.21	0.29
Gemmeiza 7	0.38	0.75	0.25	0.67	0.63	1.41	0.18	0.34
Gemmeiza 11	0.47	0.58	0.29	0.43	0.75	1.02	0.23	0.3
Misr 3	0.43	0.76	0.36	0.94	0.79	1.69	0.25	0.36
Shandaweel 2	0.55	0.73	0.5	0.93	1.05	1.66	0.29	0.33
Nubaria 2	0.74	0.75	0.54	0.64	1.28	1.38	0.35	0.34
Giza171	0.36	0.68	0.2	0.37	0.57	1.05	0.19	0.31
TSS	0.18	0.54	0.17	0.36	0.35	0.89	0.11	0.33

3.3 Phenolic compounds

Data in Table (4) indicated that infected wheat genotypes have lower levels of total and free phenolic compounds compared to protected ones. Additionally, proline accumulation showed variable responses between infected and protected genotypes. Nubaria 2 and Shandaweel 2 genotypes exhibited higher total and free phenolic content values, while Nubaria 2 and Misr 3 had elevated proline content in infected plots.

3.4 Oxidative and cellulolytic enzyme activity

Data in Table (5) indicated that the oxidative enzyme levels (PO and PPO) were higher in most infected genotypes. On contrast, the susceptible wheat genotype (TSS) exhibited lower concentrations of these enzymes compared to the resistant genotypes (Nubaria 2, Shandaweel 2, and Misr 3). Additionally, PPO enzyme activity was the highest in the infected Nubaria 2 genotype. Additionally, cellulase enzyme levels were higher in protected plots, except for Sids 1, Gemmeiza 11, and Giza 171.

Table 4: Phenolic contents and proline in leaves of nine wheat genotypes infection by *P. triticina* or protected during the second growing season.

Genotypes	Phenolic compounds content (mg g-1)				Proline µmol g-1 FW	
	Total		Free			
	Infected	Protected	Infected	Protected	Infected	Protected
Sids 1	31.73	25.77	14.46	7.28	36.8	24.86
Sids 12	37.16	19.64	15.23	14.53	33.42	39.48
Gemmeiza 7	21.69	20.13	12.48	9.77	38.97	12.91
Gemmeiza 11	37.19	23.88	10.25	8.67	35.27	44.12
Misr 3	34.15	22.41	10.11	8.2	43.34	39
Shandaweel 2	41.58	25.22	11.89	11.28	37.85	43.16
Nubaria 2	45.63	40.11	13.79	7.86	39.48	58.61
Giza171	35.16	34.6	17.59	9.57	31.33	24.05
TSS	30.38	26.31	13.54	7.93	38.06	17.4

Table 5: Oxidative enzyme (peroxidase (PO), polyphenoloxidase (PPO)) and cellulolytic enzyme (CMCase) activities in leaves of nine wheat genotypes infection by *P. triticina* or protected during the second growing season.

Genotypes	PO Specific activity		PPO Specific activity		CMCase mg g-1	
	Infected	Protected	Infected	Protected	Infected	Protected
Sids 1	49.04	82.74	2.78	1.44	83.05	44.11
Sids 12	68.05	105.66	2.25	3.53	80.95	138.18
Gemmeiza 7	136.98	120.24	3.22	1.55	38.05	63.99
Gemmeiza 11	96.86	49.42	3.53	2.78	75.34	48.57
Misr 3	156.65	122.67	3.79	0.5	45.06	57.36
Shandaweel 2	188.1	86.97	0.69	0.6	55.83	58.38
Nubaria 2	181.01	97.99	5.55	2.18	69.54	102.24
Giza171	120.13	182.66	4.73	0.88	73.55	73.04
TSS	103.06	84.14	0.71	1.2	63.48	78.08

3.5 Quantitative of yield component parameters

In this study, effects due to genotypes during two seasons were significant ($p \leq 0.05$) for yield component like 1000-kernel weight and grain yield /plot (kg) infected, protected and losses% under field conditions, at Nubaria province Table (6, 7 and 8). Data in Table (6) showed the analysis of variance (ANOVA) of yield component parameters in 1000 kernel weight (gm) and grain yield /plot in infected, protected and losses % under field conditions. Genotypes recorded highly significant variance in losses % of 1000 kernel weight (gm) in both seasons compared with check genotype TSS. The genotype showed a highly significant grain yield /plot in both seasons compared with check genotype TSS. Wheat genotype Shandweel 2, Nubaria 2, and Giza 171 recorded the highest 1000 kernel weight (g) value in case genotype infected at two seasons (48.6 and 47.97), (45.16 and 44.12) and (44.09 and 41.44), respectively. While, the protected genotypes showed different values in case genotype protected. It was not parallel with the genotype infected. Gemmeiza 7 genotype production remained less than the rest of the genotypes in both cases infected or protected but it was higher than TSS (Table 7). The loss of 1000 kernel weight (g) in the second season was higher than in the first season. In addition to the highest loss (%) of 1000 kernel weight (g) in two seasons have been recorded with susceptible genotype TSS (51.41 and 56.64) followed by Gemmeiza-7 (20.59 and 26.28),

and Gemmeiza-11 (15.56 and 20.23), respectively. On the other hand, genotype *i.e*. Sids 1 (4.74 and 8.31), Nubaria 2 (5.57 and 8.54), and Shandweel 2 (6.65 and 9.37) exhibited the lowest loss (%) of 1000 kernel weight (g) (Table 7).

Table 6: Analysis of variance of mean squares of yield component parameters in 1000 kernel weight (gm) and grain yield /plot (kg) infected, protected and losses % under field condition, at Research stations, El-Nubaria province, Egypt during the two growing seasons (2021/2022 and 2022/2023).

Traits		1000 Kernel weight (gm)											
		Infected				Protected				Losses (%)			
		First season		Second season		First season		Second season		First season		Second season	
S.O.V.	DF	M.S	F pr.	M.S	F pr.	M.S	F pr.	M.S	F pr.	M.S	F pr.	M.S	F pr.
Rep	2	3.554		6.16		5.463		6.228		0.0044		0.188	
Genotype	8	219.3	<.001	239.17	<.001	40.142	<.001	35.843	<.001	642.857	<.001	703.77	<.001
Residual	16			0.65		0.8234		1.093		0.072		0.043	
Traits		Grain yield /plot (kg)											
		Infected				Protected				Losses (%)			
Rep	2	0.003		0.001		0.002		0.0003		0.049		0.075	
Genotype	8	1.166	<.001	1.301	<.001	0.811	<.001	0.509	<.001	1292	<.001	1629	<.001
Residual	16	0.004		0.005		0.006		0.0097		0.035		0.059	

Table 7: Mean performance for nine genotypes in 1000 kernel weight across two growing seasons (2021/2022 and 2022/2023).

Genotype	1000 Kernel weight (gm)					
	Infected		Protected		Losses (%)	
	2021/2022	2022/2023	2021/2022	2022/2023	2021/2022	2022/2023
Sids 1	42.09 C	44.04 E	44.19 B	48.03 CD	4.74 A	8.31 A
Sids 12	41.52 C	40.09 C	46.27 CD	46.80 BC	10.25 E	14.35 D
Gemmeiza 7	35.79 B	33.96 B	45.08 BC	46.07 B	20.59 H	26.28 G
Gemmeiza 11	41.09 C	40.07 C	48.66 EF	50.22 E	15.56 G	20.23 F
Misr 3	42.49 C	42.23 D	45.85 C	47.49 BC	7.34 D	11.08 C
Nubaria 2	45.16 D	44.12 e	47.82 DE	48.23 CD	5.57 B	8.54 A
Shandawil 2	48.60 E	47.97 F	52.07 G	52.93 F	6.65 C	9.37 B
Giza 171	44.09 D	41.44 CD	49.82 F	49.81 DE	11.51 F	16.82 E
T.S.S	19.13 A	17.51 A	39.37 A	40.38 A	51.41 i	56.64 H

Means followed by different letter (s) in the column are significantly different according to Duncan's multiple range test at $p = 0.05$.

Data in Table (8) revealed that grain yield per plot (kg) showed the difference between protected and infected wheat plants of the tested genotype. These differences were, in fact, due to the differences in disease severity percentages, expressed in the tested genotypes. In the first season, the loss % of the grain yield ranged from 71.56 to 5.39%. The check genotype TSS, as well as susceptible cvs. Gemmeiza 7, Gemmeiza 11, Giza 171 and Sids 12, showed higher values of loss (%) in grain yield (30.27, 25.13, 22.66 and 20.39%) and (39.17, 35.24, 30.41 and 27.56) in two seasons respectively compared to the other genotypes. While the genotypes Sida 1, Nubaria 2, Shandawil 2, and Misr 3 gave the lowest values of loss (%) of grain yield *i.e* 5.39, 6.05, 7.66 and 8.88% in the first seasons, respectively. On the other hand, these results were confirmed throughout the second season. The highest loss (%) of grain yield was also recorded with the highly

susceptible genotypes Gemmeiza-7, Gemmeiza-11, Giza-171, and Sids-12. The genotypes Sids 1, Nubaria 2, Shandawil, and Misr 3 gave the lowest values of loss (%) of grain yield.

Table 8: Mean performance for nine genotypes in grain yield across two growing seasons (2021/2022 and 2022/2023).

Genotype	Grain yield /plot (kg)					
	Infected		Protected		Losses (%)	
	2021/2022	2022/2023	2021/2022	2022/2023	2021/2022	2022/2023
Sids 1	1.52 C	1.87 C	1.60 B	2.05 B	5.39 A	8.53 A
Sids12	1.44 C	1.55 B	1.81 C	2.14 BC	20.39 E	27.56 E
Gemmeiza 7	1.25 B	1.47 B	1.80 C	2.42 D	30.27 H	39.17 H
Gemmeiza 11	1.40 C	1.56 B	1.87 CD	2.41 D	25.13 G	35.24 G
Misr 3	2.30 E	2.43 E	2.53 E	2.76 E	8.88 D	12.15 C
Nubaria2	1.84 D	2.03 D	1.96 d	2.29 CD	6.05 B	11.26 B
Shandawil2	2.29 E	2.47 E	2.48 E	2.86 E	7.66 C	13.38 D
Giza 171	1.85 D	2.01 D	2.39 E	2.89 E	22.66 F	30.41 F
T.S.S	0.24 A	0.28 A	0.86 A	1.64 A	71.56 i	83.19 i

Means followed by different letter (s) in the column are significantly different according to Duncan's multiple range test at $p = 0.05$.

3.6 Grain compositions

Grain compositions were evaluated on different wheat genotypes at Nubaria province (Table 9 and 10). In this study, effects due to genotypes were significant ($p \leq 0.05$) for grain compositions (Table 9 and 10). Data presented in Table (9) showed chemical properties of wheat flour extracted from protected and infected of nine wheat genotypes. The values of dry matter, ash, crude lipid and crude fiber contents of protected grains ranged from (94.02 - 90.56%), (1.24 - 2.15%), (5.02 - 10.43%) and (6.87 - 8.59%), respectively. Meanwhile, the same parameters of the infected one were ranged from (95.06 - 96.67%), (1.83 - 4.07%), (1.16 - 4.7%), and (7.73 - 8.83%), respectively. Notably, the dry matter, ash, and crude fiber contents in infected wheat grains were higher than those in protected grains, except for the TSS genotype. Additionally, protected wheat flour had a higher crude lipid content compared to that from infected grains.

Table 9: Mean performance of the chemical properties in the second season.

Genotype	Dry matter (%)			ASH content dry (%)			Crude lipid (%)			Crude fiber (%)		
	Infected	Protected	Mean	Infected	Protected	Mean	Infected	Protected	Mean	Infected	Protected	Mean
Giza171	96.31abc	90.56 d	93.44	1.83 C	1.24 c	1.54	2.18 AB	10.43 a	6.31	8.39 A	7.75 ab	8.07
Gemmeiza 11	96.51 ab	93.07 bc	94.80	2.06 C	2.15 a	2.10	1.16 B	5.02 a	3.09	8.83 A	8.59 a	8.71
Gemmeiza 7	96.35abc	93.33abc	94.84	2.77 BC	1.87 ab	2.32	4.69 A	5.13 a	4.91	7.96 A	8.26 ab	8.11
Misr 3	96.53 ab	93.05 bc	94.79	1.89 C	1.82 b	1.85	1.22 B	5.21 a	3.22	8.63 A	8.17 ab	8.4
Nubaria2	96.30abc	93.05 bc	94.68	1.99 C	1.88 ab	1.94	2.25AB	5.05 a	3.65	8.55 A	8.34 ab	8.45
Shandawil2	95.95 c	92.6 c	94.28	1.95 C	1.94 ab	1.94	1.41 B	10.31 a	5.86	8.73 A	6.87 b	7.8
Sids 1	96.67 a	94.02 a	95.35	3.76 AB	1.87 ab	2.81	4.70 A	5.93 a	5.32	7.73 A	7.95 ab	7.84
Sids12	96.01bc	93.57 ab	94.79	4.07 A	1.86 ab	2.96	1.96 B	5.02 a	3.49	8.62 A	8.46 ab	8.54
T.S.S	95.06 d	92.82 bc	93.94	1.89 C	2.00 ab	1.95	2.22AB	5.30 a	3.76	8.18 A	7.64 ab	7.91

Means followed by different letter (s) in the column are significantly different according to Duncan's multiple range test at $p = 0.05$.

Table 10: Mean performance of the chemical characters during the second season.

Genotype	Protein (mg/g)			Gluten (mg/g)			Carbohydrates (mg/g)		
	Infected	Protected	Mean	infected	Protected	Mean	infected	Protected	Mean
Sids 1	3.72 B	6.83 A	5.27	28.93 A	41.75 a	35.34	16.12 E	22.63 ef	19.38
Sids12	4.79 AB	5.52 AB	5.16	25.16AB	24.90 cd	25.03	22.58 B	22.82 e	22.70
Gemmeiza7	4.68 AB	4.14 BC	4.41	21.78AB	25.39 bcd	23.59	22.32 B	24.28 d	23.30
Gemmeiza11	5.54 AB	5.49 AB	5.52	22.89AB	29.46abcd	26.17	20.81 C	31.29 a	26.05
Misr3	4.96 AB	4.81 B	4.88	29.58 A	36.95 abc	33.27	15.96 E	26.01 c	20.99
Nubaria2	5.93 A	4.98 AB	5.46	25.15AB	38.41 abc	31.78	18.95 D	22.96 de	20.95
Shandawil 2	5.08 AB	4.89 B	4.98	22.60AB	28.05abcd	25.33	19.11 D	30.04 a	24.57
Giza171	4.73 AB	2.62 C	3.68	21.52AB	39.18 ab	30.35	20.03 C	21.32 f	20.67
T.S.S	5.70 AB	5.91 AB	5.80	20.37 B	21.59 d	20.98	25.97 A	28.48 b	27.22

Means followed by different letter (s) in the column are significantly different according to Duncan's multiple range test at $p = 0.05$.

It impacts not only quantity of grain yield but also the grain composition of wheat by altering its chemical composition, including protein, gluten, and carbohydrates. Data presented in Table (10) show the effect of wheat leaf rust disease on protein, gluten and carbohydrate of wheat flour of grains for nine wheat genotypes. Furthermore, the increased protein content in the infected plots compared with protected. While gluten and carbohydrate value reduced.

3.7 Principal component analysis and clustering of genotypes based on response to disease

Principal component analysis was used to identify the small number of linear combinations for assessing grain composition traits. These combinations effectively capture the majority of the variation within the data. Results in Figure (1A) revealed that principal component measured variables/traits in the infected genotypes, like gluten and dry matter %, ash content %, and crude lipid mg/g located in the cute angle. It indicated that there was a strength relationship between them. On the other hand, there was a near-zero correlation between crude fiber and gluten, crude fiber, and carbohydrates because of their near-perpendicular vectors. In contrast, the crude fiber % and crude lipid were located at a straight angle and a negative correlation between them.

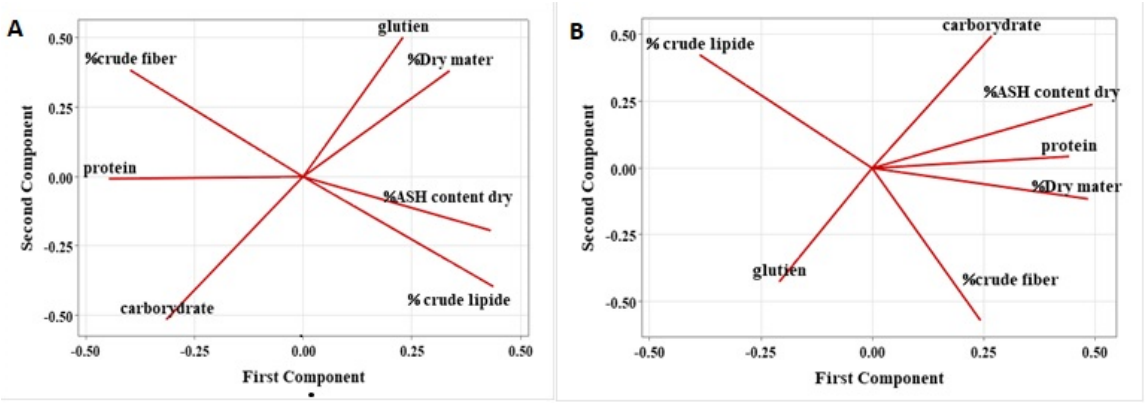
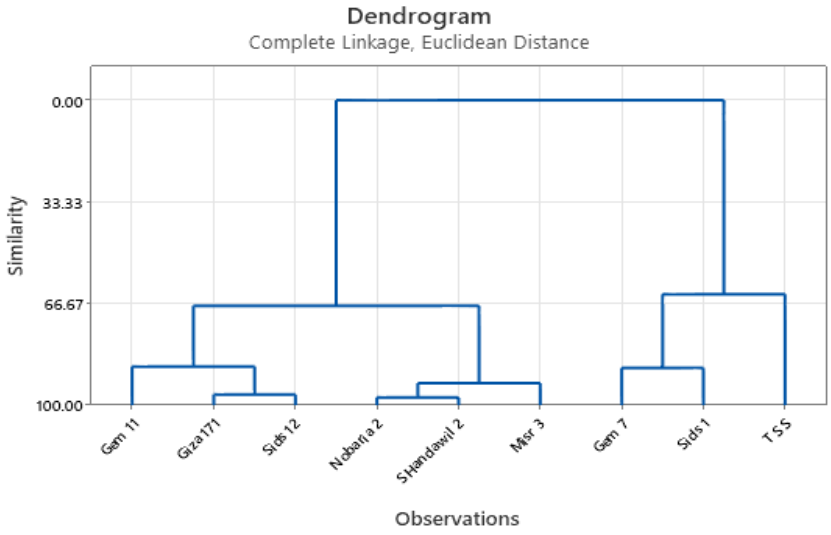


Figure 1: Principal component analysis (PCs) of 9 wheat genotype and the measured variables/traits in A: infected treatment B: protected treatment.

Traits that exhibited ash content %, protein mg/g, and dry matter % were located at a cute angle; it indicated the strength of the relationship between them in the protected treatment in Figure (1B). On the other hand, there was a near-zero correlation between crude lipid and gluten, carbohydrate mg/g, because their near-perpendicular vectors (r = cos 90 = 0), while gluten and carbohydrate mg/g, indicated by the straight angle among them. It suggested that there was a negative relationship between them. Gluten and carbohydrate mg/g have the same relationship under infected and protected conditions. In contrast, Ash content %, protein mg/g, and crude fiber have different relationships when the condition is rust infection and protected. The cluster dendrogram's prior results demonstrated the application of nine genotypes to the investigation treatment. Genetic similarity and clustering dendrogram analysis results showed distinct differences between wheat genotypes (Figure 2). Results revealed that Gemmeiza 11, Giza 171, and Sids 12 were closely related, which were located in cluster 1 depending on AUDPC value and losses in grain yield. Shandwel 2, Misr 3, and Nubaria 2 had the same characteristics and were located in cluster 2, exhibiting the lowest percentages of final rust severity and low grain yield losses. This result was confirmed by the mean performance of wheat genotypes. Additionally, Gemmeiza 7 and Sids 1 were located in cluster 3, whereas genotypes Gemmeiza-7 and Sids-1 exhibited the highest AUDPC and the final rust disease. Finally, cluster 5 was located in TSS alone, which exhibited high percentages of rust infection, considering previous results of the cluster dendrogram genetic diversity.



Gem11 = Gemmeiza 11, Gem 7 = Gemmeiza 7.

Figure 2: Dendrogram based on the percentage of infection accumulated by the different accessions at the two study years.

4. Discussion

Leaf rust caused by the fungus *Puccinia triticina*, is a sever disease affecting different wheat genotypes. It was lead to substantial yield losses and affects grain composition. Two critical epidemiological parameters used to evaluate the effect of leaf rust on wheat are

final rust severity (FRS) and area under the disease progress curve (AUDPC). These parameters were evaluated and their effects on biochemical contents and grain composition under protected and infected genotypes. The tested genotypes showed variation values of FRS and AUDPC to wheat leaf rust compared with the susceptible check genotype TSS. This study recorded that Misr-3, Shandweel-2, and Nubaria-2 were resistance genotypes or partially resistant against *Puccinia triticina* while sids1 and gemmeiza 7 were susceptible wheat genotypes. Partially resistant genotypes showed lower rust severity, and reduced AUDPC compared to susceptible ones. These results were confirmed by Boulat et al. (2014), Ali et al. (2016), and El-Orabey et al. (2017). In addition, Fahmi et al. (2015) and El-Orabey et al. (2017) classified the tested wheat varieties into three groups according to the values of FRS (%) and AUDPC. Other the other hand, Gorash et al. (2014), Mahto and Baidya (2012) and Zaman et al. (2017) revealed that resistance genes play a role in developing effective management strategies for rust-resistant wheat varieties. These studies have shown that FRS can vary among different wheat genotypes due to factors such as genetic resistance, environmental conditions, and pathogen virulence (Hassan et al., 2022; Kolmer and Fajolu, 2022; Gultyaeva et al., 2021; Ali et al., 2016). Genotypes resistance factors significantly affected FRS and AUDPC (Naseri and Jalilian, 2021; Thabet and Najeeb, 2017). FRS and AUDPC were lead to substantial yield losses. 1000 kernel weight and yield losses increased with increased FRS and AUDPC except Sids 1. These results were confirmed by Thabet and Najeeb (2017), and Yahya et al. (2020). The yield losses due to leaf rust mainly depending on the level of FRS (%) (El-Orabey et al., 2017; Sallam et al., 2016; Shahin and El-Orabey, 2016). The genotype Sids 1 had higher value for FRS and AUDPC while low losses value for 1000 kernel weight and yield losses (Thabet and Najeeb, 2017). Hierarchical cluster analysis enabled us to categorize the genotypes according to infection accumulated by the different accessions at the two study years (Derbew and Tejada, 2020). The results of the present study showed that leaf rust disease lead to changes in biochemical content, such as chlorophyll content, phenolic compounds, proline accumulation, and the activity of oxidative and cellulolytic enzymes. This study indicated that leaf rust infection leads to decrease in chlorophyll contents the results confirmed by Din et al. (2017) and Zakaria (2023). Phenolic compounds, along as well as total and free fractions, and also proline, play a major impact in plant stress responses. The results indicate that total and free phenolic compounds are important biomarkers of tolerance. These results were confirmed by Kumar et al. (2020) and Baka and El-Zahed (2023). Research indicates that leaf rust infection can lead to changes in phenolic compound content. For instance, studies have shown that resistant wheat genotypes may contain higher quantity of total phenolics upon infection compared to susceptible ones. This increase is part of the plant's defense strategy to restrict pathogen growth by accumulating phenols at the infection site (Sharma et al., 2023; Eisa and El-Naggar, 2015). On the other hand, Menden et al. (1994) suggested that alterations in free soluble or bound insoluble phenolic acids are not directly involved in resistance mechanisms. Other factors such as lignification might play more important roles. Additionally, proline accumulation showed variable responses between infected and protected genotypes. Nubaria 2 and Misr 3 as the partially resistant genotypes had elevated proline content in infected plots. Studies

show proline is a multi-functional molecule that accumulates in plants due to abiotic stress, protecting cells and preserving growth. Additionally, proline controls plant development and osmotic stress by stabilizing cellular structures, enzyme and reactive oxygen species (Meena et al., 2019; Ahmed et al., 2017; Kavi-Kishor and Sreenivasulu, 2014). Its accumulation is often associated with enhanced tolerance to abiotic stress. However, the direct impact of leaf rust infection on proline contents remains a need to further studies. In this study, it recorded that the higher activity of oxidative enzymes (PPO and PO) in the infected plants suggests defensive response from the resistant genotypes, while converse trend with some the susceptible genotype was recorded which appears less capable of mounting an effective defense mechanism against infection. These findings were consistent with those of subsequent researchers. Eisa and El-Naggar (2015) recorded that resistant cultivar against leaf rust showed higher peroxidase activity. Zhou et al., 2012 and Zhang et al. (2021) suggests a correlation between antioxidant enzyme activity and rust resistance. Susceptible genotypes had higher contents of reactive oxygen species (ROS), indicating greater oxidative stress. Peroxidase (POX) is a necessary enzyme in plant defense, involved in lignification, suberification, cell wall elongation, reactive oxygen species (ROS) regulation, polymerization, wound healing, and resistance against pathogens (Dos-Santos and Franco, 2023; Ujjainkar *et al*., 2022). Polyphenol oxidase (PPO) plays a role in plants' defense mechanisms, especially in responding to pathogen attacks. Ujjainkar et al. (2022) and Zhang (2023**)** reported that PPO contributions to disease resistance due to multiple mechanisms of action such as phenolic oxidation, cell wall reinforcement, unfavorable environment for pathogens, melanin formation and hypersensitive response. Also, rust infection affects cellulase activity compared with protected genotypes. This result recorded that increased activity of enzymes was observed in most protected genotypes compared with infected. Kubicek et al. (2014) demonstrated that plant infection elicits complex changes in cellulase activity. The observed patterns vary depending on the pathogen species, the stage of infection, and the host genotype. Also, Chaurasia et al. (2014) revealed that decreased cellulase enzyme could be due to phenolic compounds present in experiment on semi ripe tomato fruit. The combined effects of these biochemical changes and different genotypes have important impacts for nutritional value and grain composition. In the light of present study, it recorded that leaf rust infection affects the dry matter accumulation, increased ash, crude fiber percentage and protein in grain yield compared with the flour of the same genotypes protected ones while decreased carbohydrate, lipid and gluten in grain yield. These results were confirmed by Ali et al. (2016) and Surovy et al. (2020). In the light of present study, indicated the strength of the relationship between ash content %, protein mg/g, and dry matter % crude fiber % over all protected genotypes depend on PCs**.** Jabran et al. (2021) recorded those multivariate analyses, including principal component analysis were employed to study morpho-physiological traits. Surovy et al. (2020) revealed that ash content undergoes changes during leaf rust infection, altered mineral distribution patterns and enhanced accumulation of specific elements, especially calcium, manganese, and iron. Additionally, the relationship between leaf rust infection and crude fiber content is complex and often depends on the growth stage at which infection occurs. Previous studies indicate that rust infection can lead to

early infection effects reduced cellulose synthesis, decreased hemicellulose content and altered lignification patterns. While late infection effects increased fiber content due to premature tissue senescence, enhanced lignification as a defense response and modified cell wall composition. Moisture (%) related to slightly decreased dry matter percentage in infected genotypes. Previous study demonstrated that infection alters respiratory pathways and causes significant changes in carbohydrate levels which diseased tissues often show higher respiration rates and a preference for releasing carbon-1 from glucose (Daly et al., 1962). Also, Ali et al. (2016) recorded that protected wheat flour exhibited greater contents of gluten compared with flour derived from infected genotypes. This difference may be attributed to the rust's consumption of carbohydrates, resulting in a slight increase in protein content.

5. Conclusion

Leaf rust disease affects plant health by reducing chlorophyll contents and exhibited high contents of phenolic compounds and proline accumulation as well as increasing enzyme activities. This complex interaction is important for understanding plant responses to biotic stressors, developing effective disease management strategies, predicting crop quality impacts, optimizing harvesting and processing procedures, and breeding programs. Future research should focus on breeding programs or agronomic strategies to enhance leaf rust disease resistance in wheat crop, aiming to develop resilient varieties, while maintaining grain quality. This will help sustain wheat production and develop targeted interventions.

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