International Journal of Plant & Soil Science

34(23): 1488-1501, 2022; Article no.IJPSS.93978
ISSN: 2320-7035

Identification of New Four Races of Barley Stem Rust (*Puccinia graminis f. sp. tritici*) for the First Time in Egypt

B. R. Mohdly [a], A. E. Khalil [a], Mohamed Mansour [b*], Sally E. El-Wakeel [b] and S. D. Ibrehim [c]

[a] Plant Pathology Research Institute – A.R.C, Giza, Egypt.
[b] Barley Research Department, Field Crops Research Institute – A.R.C, Giza, Egypt.
[c] Agricultural Genetic Engineering Research Institute – A.R.C, Giza, Egypt.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2022/v34i232567

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/93978

Original Research Article

Received 22 September 2022
Accepted 25 November 2022
Published 30 November 2022

ABSTRACT

Four pathogenic races belonging to *Puccinia graminis* f. sp. *tritici* Eriks and Henn, were genetically identified in Egypt for the first time on barley (*Hordeum vulgare* L.), using the molecular biology method, using specific ITS primers (PCR) and Fingerprinting using (RAPD) markers, these races were recorded, during the present study, in the gene bank under accession numbers MW 931757, 931758, 931759 and 931760. Also, five Egyptian barley varieties, *i.e.* Giza 123, Giza 124, Giza 125, Giza 126 and Giza 2000 were evaluated for their resistance to stem rust and some vegetative traits under field conditions in Sids, Giza, Nubariya and Sakha agricultural research stations during growing season 2020/2021. All tested barely cultivars were resistant to the pathogen races. Cultivar Giza 125 exhibited an earliness for heading and maturity, while cv. 2000 exhibited the highest values for spike length, grain numbers/spike, 1000 grain weight, biological and grain yield.

Keywords: Barley; stem rust; PCR; RAPD markers; yield components.

**Corresponding author: E-mail: barley_breeder@yahoo.com;*

1. INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the most abundantly utilized cereal crops over the world. It accounts for 12% of total global cereal production, ranking fourth after wheat, rice, and maize. In Egypt, barley grows in the Northern Coastal Regions and new reclaimed lands.

Barley stem rust disease caused by *Puccinia graminis f.sp. tritici* Eriks and can affect also wheat, triticale and many other related grasses. It is found wherever temperate climate that favors growing of cereals [1]. The alternate hosts, out of Egypt, are *Berberis* and *Mahonia* species [2].

Rusts, are the most important common diseases of barley and wheat because of the ability to spread for long distance, in addition to their ability to produce new races that can attack resistant varieties and their potential to develop rusts rapidly high under optimal environmental conditions and cause serious losses [3,4]. Stem rust considered as one of the most dangerous diseases among the other three barley rust diseases. The causal organism under the suitable conditions may destroy the whole plants with no yield seeds. In Egypt, high losses in wheat grain yield can be expected due to the suitable environmental conditions for disease infection. On the contrary, barley varieties have a high degree of resistance [5]. Stem rust epidemics in Minnesota, North Dakota and South Dakota caused average yield losses over 20% [6,7]. A dangers epidemic of stem rust disease damaged wheat crops in the Southern states of Australia in 1974 [8]. In this study, identifying the races of stem rust caused by *Puccinia graminis* f.sp. *tritici* that infects barley using molecular biology methods is of great importance. Also, definition of appropriate locations for the pathogen spread, definition of barley resistant varieties and using them in breeding programs with excluding the susceptible lines reduces the losses and spread of the disease in the future.

2. MATERIALS AND METHODS

2.1 Isolation, Purification and Identification of the Physiological Races

Symptoms of stem rust disease were observed on barley plants (*Hordeum vulgare* L.) grown in El-Alameen County–Marsa Matrouh Governorate, during March/2019 growing season, specifically, on line which its pedigree is (Lignee 527/Gerbel/3/Boyb*2/Surb//C 11225.2D/4/M104) (Fig. 1).

Stem rust fungus isolation was carried out from a single uredial pustules (Four isolates), multiplied and the resulted spores were used to carry out the pathogenicity test on the same barley line under greenhouse conditions at Plant Pathology Research Institute, A.R.C, Giza, Egypt.

2.2 Establishment of Single –Pustule Isolates

Stem rust urediospores were transferred from infected samples with a sterile scalpel and transferred to upper leaf-surface of 7-days old seedlings on line which its pedigree is "Lignee 527/Gerbel/3/Boyb*2/Surb//C11225.2D/4/M104" [9,10]. The inoculated barley plants were directly incubated in dew chambers at 100% relative humidity for 20-24 h in the dark chambers. Inoculated seedlings returned back to the greenhouse benches. Developed individual pustules were sub cultured on leaves of healthy seedlings to propagate and generate sufficient inocula of each single-pustule [11]. Urediniospores of *P. graminis* f.sp. *tritici* of each isolate were collected and DNA was extracted from 0.50 mg urediniospores.

2.3 Molecular Identification

2.3.1 Extraction and purification of genomic DNA

Genomic DNA of the fungal was extracted from each four samples using a DNeasy Mini Kit (Qiagen, CA, USA), according to [12].

2.3.2 ITS rRNA analysis

a) PCR Reactions:

The PCR amplification performed in a total volume of 50 µl, containing 1X reaction buffer, 1.5 mM $MgCl_2$, 1U *Taq* DNA polymerase (promega), 2.5mM dNTPs, 30 poml of each primer and 30 ng genomic DNA (Table 1) [13].

b) Thermo-cycling PCR program

PCR amplification performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94ºC. Each cycle consisted of a denaturation step at 94ºC for 30 sec., an annealing step at 45ºC for 30 sec. and an elongation step at 72ºC for 1 min. The primer extension segment was extended to 7 min at 72ºC in the final cycle.

Fig. 1. Symptoms of barley stem rust caused by *Puccinia graminis* f. sp. *tritici* on barley LBYT line showing the uredial pustules on the stem

Table 1. Primer ITS forward and reverse, sequence and product size

Primer code	Sequence	Product Size
(ITS-1) F	5′- TCCGTAGGTGAACCTGCGG -3′	600bp
(ITS-4) R	5′- TCCTCCGCTTATTGATATGC-3′	

c) Detection of the PCR Products

The intensification items settled by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 volts. A 100bp DNA step was utilized as an atomic measure standard. PCR items were visualized on UV light and captured employing a Gel Documentation Framework (BIO-RAD 2000).

Purification of PCR products:

Amplified for all PCR products were purified using EZ-10 spin column PCR products purification PCR reaction mixture was transferred to 1.5 ml microfuge tube and three volumes were added of binding buffer 1 after that the mixture solution was transferred to the EZ-10 column and let it stand at room temperature for 2 minutes after that centrifuge, 750 µl of wash solution were added to the column and centrifuged at 10.000rpm for two minutes, repeated washing, 10.000 rpm was spine for an additional minute to remove any residual wash solution. The column was transferred into a clean 1.5 ml microfuge tube and 50 ul of elution buffer were added, incubated at room temperature for 2 minutes and then store purified DNA at -20°C [14].

ITS sequencing analysis:

The sequencing of the product PCR was conducted in an automatic sequencer ABI PRISM 3730XL analyzer by using big dye TM terminator cycle sequencing kits following supplied protocols of the manufacturer. Single-pass sequencing performed on each template using Rbcl Forward primer. The fluorescent-labeled fragments were purified from the unincorporated terminators with an ethanol precipitation protocol. The samples were resuspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer (Microgen Company).

Computational analysis (BLASTn) ITS:

The sequences were analyzed using BLAST program (http://www.ncbi.nlm.nih.gov/BLAST). Sequences were aligned using Align Sequences Nucleotide BLAST.

RAPD fingerprinting:

RAPD has been successfully used for the fingerprint of four fungal isolates. This method uses random sequence primers of about 10 bases in length that hybridize with chromosomal DNA. The amplification reaction was carried out in 25 µl reaction volume containing 1X PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 25pmol primer, 1 U Taq DNA polymerase and 30ng templates DNA. PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) programmed to fulfill 35 cycles after an initial denaturation cycle for 5 min at 94ºC. Each cycle consisted of a denaturation step at 94ºC for 45s, an annealing step at 36ºC for 50s, and an elongation step at 72ºC for 1min. The primer extension segment was extended to 7 min at 72ºC in the final cycle. The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 volts.

The test materials:

Five commercial barley varieties, *i.e*. Giza 123, Giza 124, Giza 125, Giza 126, Giza 2000 and the check line (LBYT, highly susceptible) (Table 2) were tested to determine their resistance to stem rust. The field trials were conducted under natural infection at Sids, Giza, Nubariya and Sakha Agricultural Research Stations, during growing season 2020/2021. All experiments were carried out in a randomized complete block design with three replicates, each was 3m x 3.5m = 10.5 m^2 plot size, the grains of the tested barley varieties were sown in 6-rows/ plot.

Data of rust reactions were scored as response and severity of disease infection (%). Disease severity (%) was recorded weekly from the first rust appearance on any test cultivar along with the stage of the through growth season.

Final rust severity (FRS %):

Rust severity (%) of each test cultivar was recorded weekly after the initial infection was occurred, using the modified Cobb´s scale (Fig. 2) [15]. Adult plant reaction scored as the percentage of rust severity (%) for each cultivar, at the time when rust was first appeared until the early dough stage [16]. Also, final rust severity (FRS%) was estimated on each cultivar under study as disease severity (%), when rust severity% reached its maximum and final level in the control plants of the highly susceptible check line LBYT28 [17]. Also, the studied field traits were days to heading, days to maturity, plant height (cm), spike length (cm), number of grains/spike, number of spikes/m^2, 1000-grain weight (g), biological yield (ton/feddan) and grain yield (ardab/feddan). [feddan = 4200m^2 and ardab = 120kg]

Table 2. The Egyptian barley cultivars evaluated throughout growing season 2020-2021 and their pedigree

No.	Barley cultivars	Pedigree
1	Giza 123	Giza 117//FAO86
2	Giza 124	Giza 117/Bahteem52// Giza 118/FAO 86
3	Giza 125	Giza117/Bahteem52//Giza118/FAO86
4	Giza 126	Baladi Bahteem/SD729-por12762-Bc
5	Giza 2000	Cr366-13-1/Giza121
6	Line (LBYT)	(Lignee527/Gerbel/3/Boyb*2/Surb//C 11225.2D/4/M104)

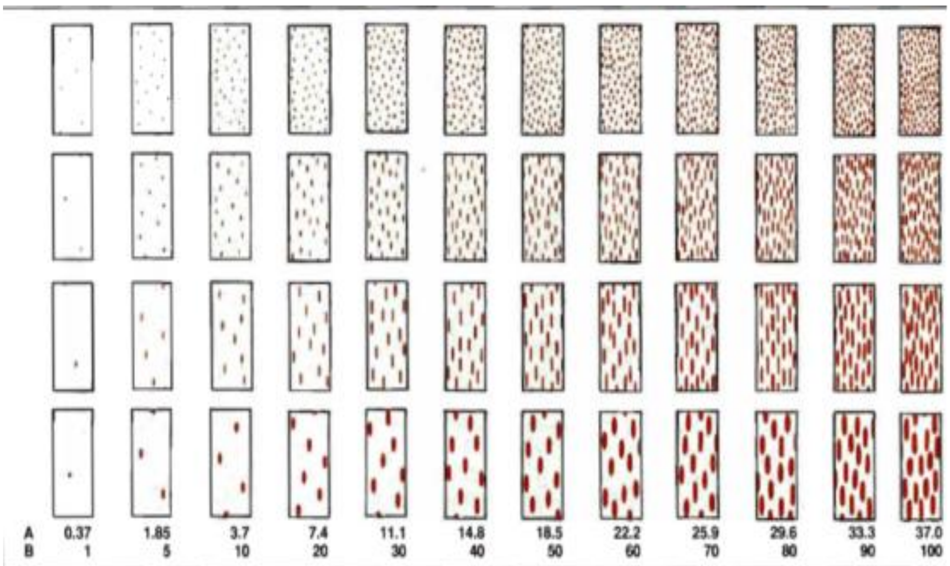

Fig. 2. The modified Cobb´s scale: A: Actual rust percentage, B: Visual rust severities [15]

3. RESULTS

Rust fungi are biotrophic pathogens that attack many plant species but are particularly destructive on cereal crops. The stem rust caused by *Puccinia graminis* f. sp. *tritici* has historically caused severe crop losses and continue to threaten production today.

Stem rust race (Ug99) poses a serious threat to both wheat and barley worldwide [18]. Barley (*Hordeum vulgare* L.) breeders have controlled major stem rust epidemics. Several barley landraces were found to possess a high level of resistance at both the adult plant stages.

3.1 Molecular Identification

3.1.1 PCR ITS amplification

PCR products of approximately 600bp amplified with the ITS-1 F and ITS-4R primers and corresponding to the ribosomal RNA gene were obtained from the tested four isolates (Fig. 3). After purification of PCR products and sequencing, the BLAST-n alignments results showed that the four sequences were associated with high levels of sequence similarity with the ribosomal RNA gene sequences for *Puccinia graminis* f. sp. *tritici*. Sequences were deposited in the GenBank database of four accession numbers, *i.e*. MW931757, MW931758, MW931759 and MW931760 (Badawy 1, Badawy 3, Badawy 6 and Badawy 9, respectively).

3.1.2 Similarity and differences among isolates

Genetic dice similarity showed differences among the races under study and the species *Puccinia graminis* f. sp. *tritici* in the GenBank database a midst of (99.36 to 98.48 %), according to the top genetic similarity (99.36 %) between the two races, (isolate Badawy_3 and (isolate JQ688990.1, JQ688957.1, and JQ688952.1) as shown in Table (4) and Fig. (5). While, the lowest value of genetic similarity (98.48 %) was observed between (isolate Badawy_1) and (isolates KC853409.1, JQ688984.1, JQ688977.1, JQ688964.1, JQ688954., JQ688947.1, JQ688945.1, JQ688934.1, and JQ688902.1) as shown in Table (3) and Fig. (4).

3.1.3 Analyzing pathotypes obtained from the two main clusters

The first cluster included isolate Badawy 6, while the second cluster included isolate Badawy 9, from the individual primer analysis, it seems that isolate Badawy 6 belongs to the cluster in tested similarly, by a percentage (99.01), (Table 5 and Fig. 6), while isolate Badawy 9 which belongs to the cluster in tested similarly by a percentage (99.19) as shown in Table 6 and Fig. 7.

It is therefore more likely these isolates (two members of the cluster) shared the majority of their genetic materials and will probably meg have originated from similar source.

Watched the degree of difference and kinship between the races that are defined in Fig. 8 which shows Phylogenetic tree using (MEGA5) of four *Puccinia graminis tritici* strains using *ITS rRNA,* showing names of fungus species and accession numbers.

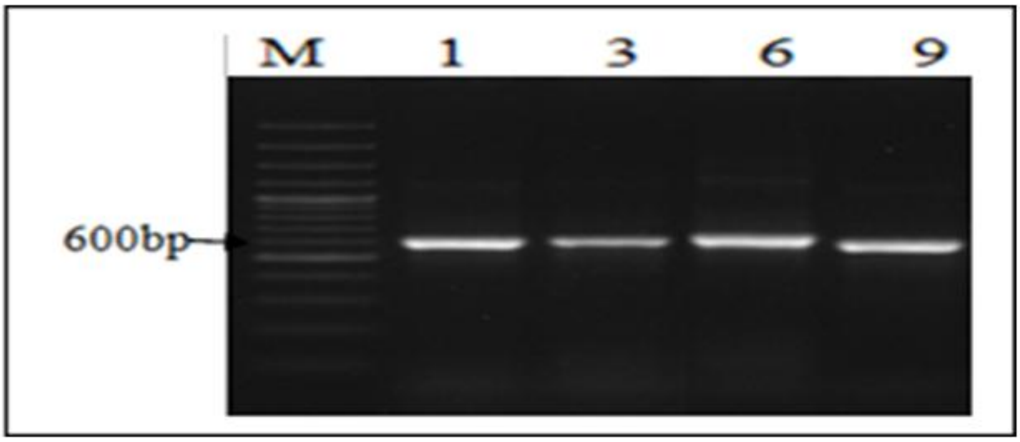

Fig. 3. Profile of ribosomal RNA-internal transcribed spacer (rRNA-ITS) amplified by primers ITS1 and ITS4 from the physiologic races. Lanes: M Marker (100 bp DNA), 1, 3, 6 and 9

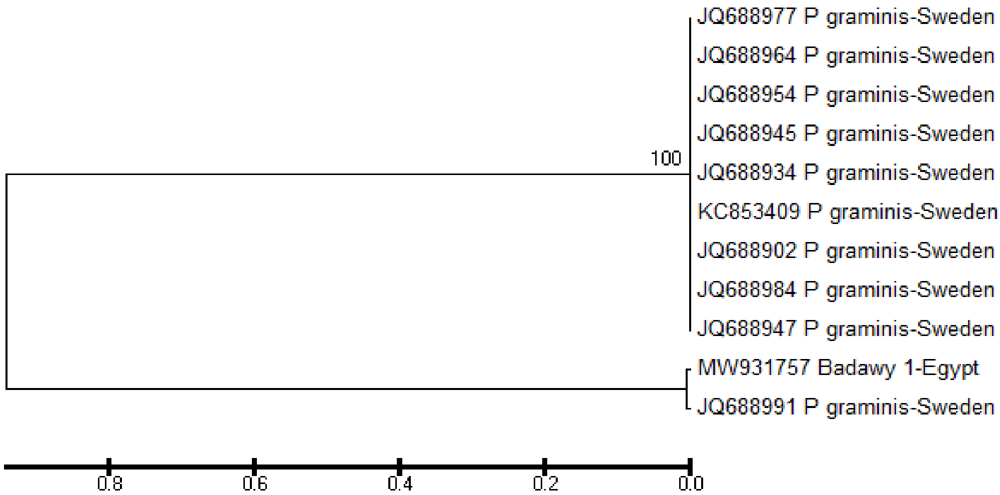

Fig. 4. Phylogenetic tree using (MEGA5) of *P. graminis* f. sp. *tritici* race Badawy_1 using *ITS rRNA,* showing names of fungus species and accession numbers

Table 3. *ITS rRNA* of *Puccinia graminis tritici* isolate Badawy1and related fungus species with the similarity percentage of more than 99%, downloaded from GenBank database

Accession No.	E-value	Query coverage (%)	Similarity (%)
JQ688991.1	0.0	100	99.32
KC853409.1	0.0	100	98.48
JQ688984.1	0.0	100	98.48
JQ688977.1	0.0	100	98.48
JQ688964.1	0.0	100	98.48
JQ688954.1	0.0	100	98.48
JQ688947.1	0.0	100	98.48
JQ688945.1	0.0	100	98.48
JQ688934.1	0.0	100	98.48
JQ688902.1	0.0	100	98.48

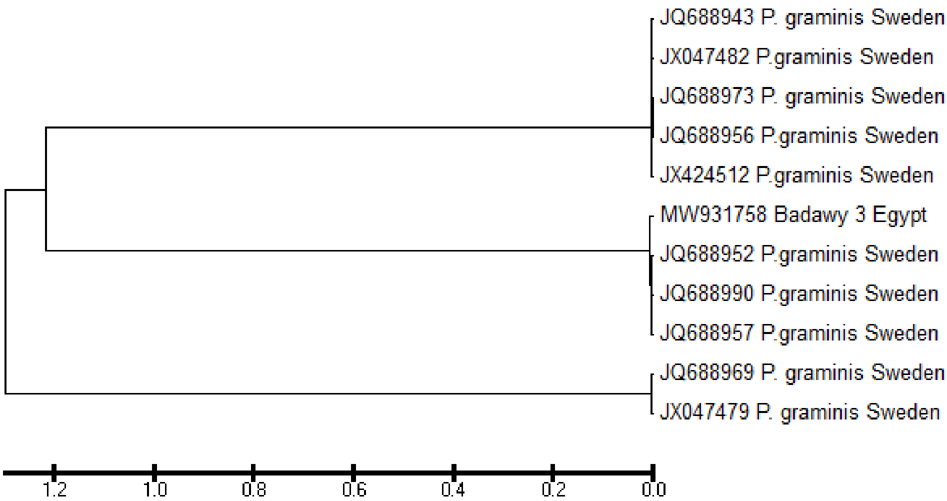

Fig. 5. Phylogenetic tree using (MEGA5) of *P. graminis* f. sp. *tritici* race Badawy_3 using ITS rRNA, showing names of fungus species and accession numbers

Table 4. *ITS rRNA* of *Puccinia graminis tritici* isolate Badawy 3 and related fungus species with the similarity percentage downloaded from GenBank database

Accession No.	E-value	Query coverage (%)	Similarity (%)
JQ688990.1	0.0	100	99.36
JQ688943.1	0.0	100	98.70
JQ688957.1	0.0	100	99.36
JQ688973.1	0.0	100	98.70
JQ688956.1	0.0	100	98.70
JQ688969.1	0.0	100	98.54
JQ688952.1	0.0	100	99.36
JX047479.1	0.0	100	98.54
JX424512.1	0.0	100	98.70
JX047482.1	0.0	100	98.70

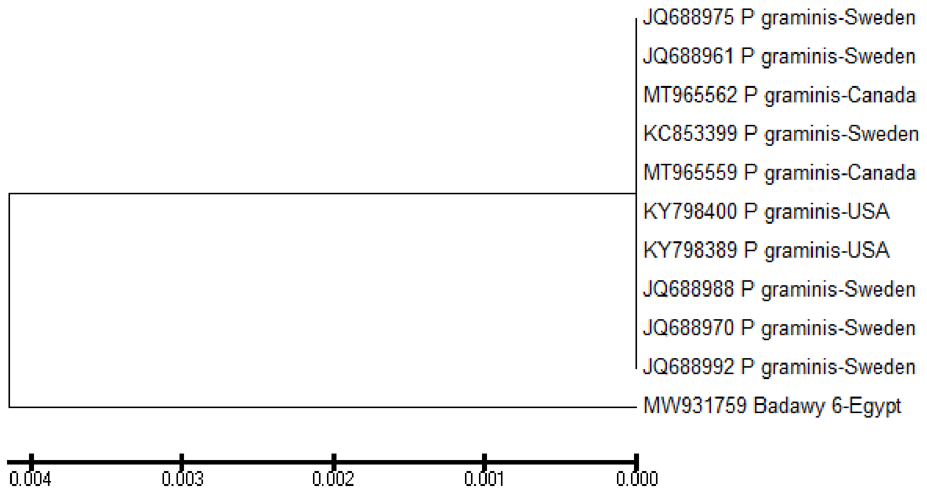

Fig. 6. Phylogenetic tree using (MEGA5) of *P. graminis* f. sp. *tritici* race Badawy_6 using *ITS rRNA,* showing names of fungus species and accession numbers

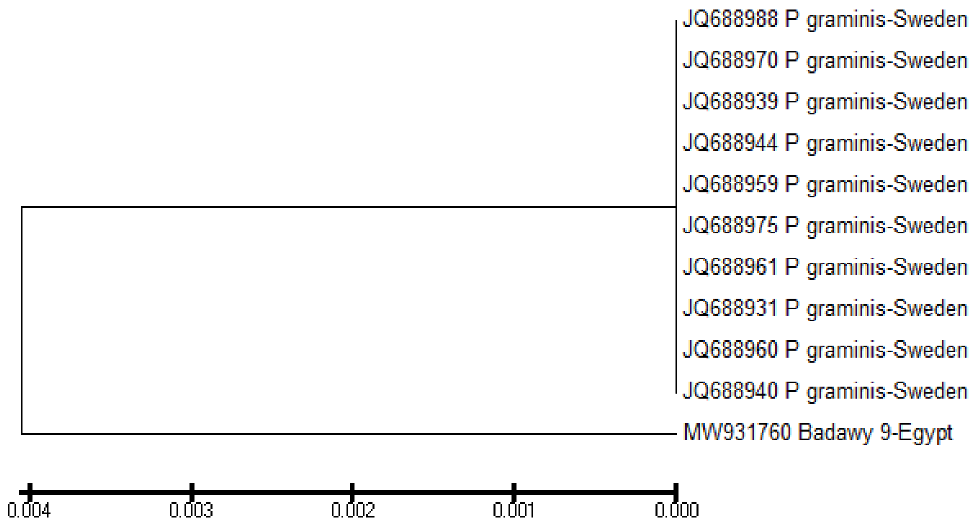

Fig. 7. Phylogenetic tree using (MEGA5) of *P. graminis* f. sp. *tritici* race Badawy 9 using *ITS rRNA,* showing names of fungus species and accession numbers

Table 5. ITS rRNA of *Puccinia graminis tritici* isolate Badawy 6 and related fungus species with the similarity percentage downloaded from GenBank database

Accession No.	E-value	Query coverage (%)	Similarity (%)
KY798400.1	0.0	100	99.01
KY798389.1	0.0	99	99.01
MT965562.1	0.0	99	99.01
MT965559.1	0.0	99	99.01
KC853399.1	0.0	99	99.01
JQ688992.1	0.0	99	99.01
JQ688988.1	0.0	99	99.01
JQ688975.1	0.0	99	99.01
JQ688970.1	0.0	99	99.01
JQ688961.1	0.0	99	99.01

Table 6. *ITS rRNA* of *Puccinia graminis tritici* isolate Badawy 9 and related fungus species with the similarity percentage downloaded from GenBank database

Accession No.	E-value	Query coverage (%)	Similarity (%)
JQ688988.1	0.0	100	99.19
JQ688975.1	0.0	100	99.19
JQ688970.1	0.0	100	99.19
JQ688961.1	0.0	100	99.19
JQ688960.1	0.0	100	99.19
JQ688959.1	0.0	100	99.19
JQ688944.1	0.0	100	99.19
JQ688940.1	0.0	100	99.19
JQ688939.1	0.0	100	99.19
JQ688931.1	0.0	100	99.19

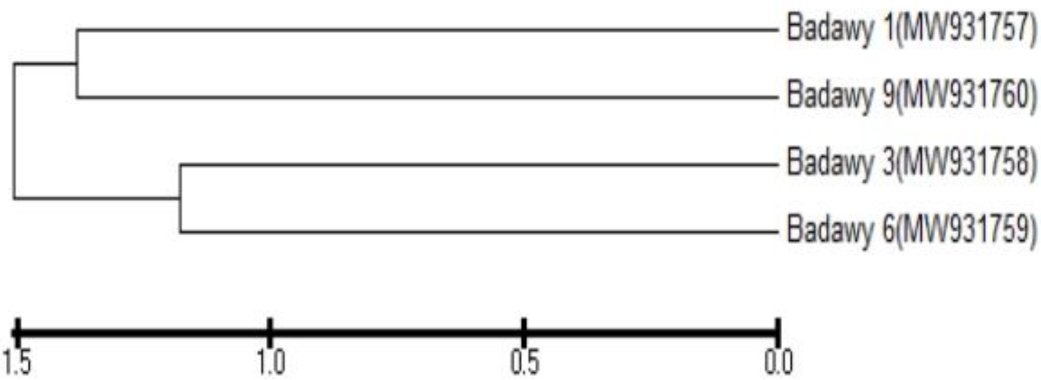

Fig. 8. Phylogenetic tree using (MEGA5) of four *Puccinia graminis tritici* races using *ITS rRNA,* and accession numbers

3.1.4 RAPD finger printing

This study at this perspective indicated differences between the *P. graminis* f. sp. *tritici* isolates, which have been collected and studied, using RAPD analysis (Fig. 8). These results are similar to those reported by [19] who found in a study on a worldwide basis collections of *P. graminis* f. sp. *tritici* differences for their virulence in addition to molecular back grounds.

Contrarily, [20] studied a sample of 115 *P. graminis tritici* isolates from the United States and identified six pathotypes and five random amplified polymorphic DNA (RAPD) groups. They found a low correlation coefficient between pathotypes and RAPD groups.

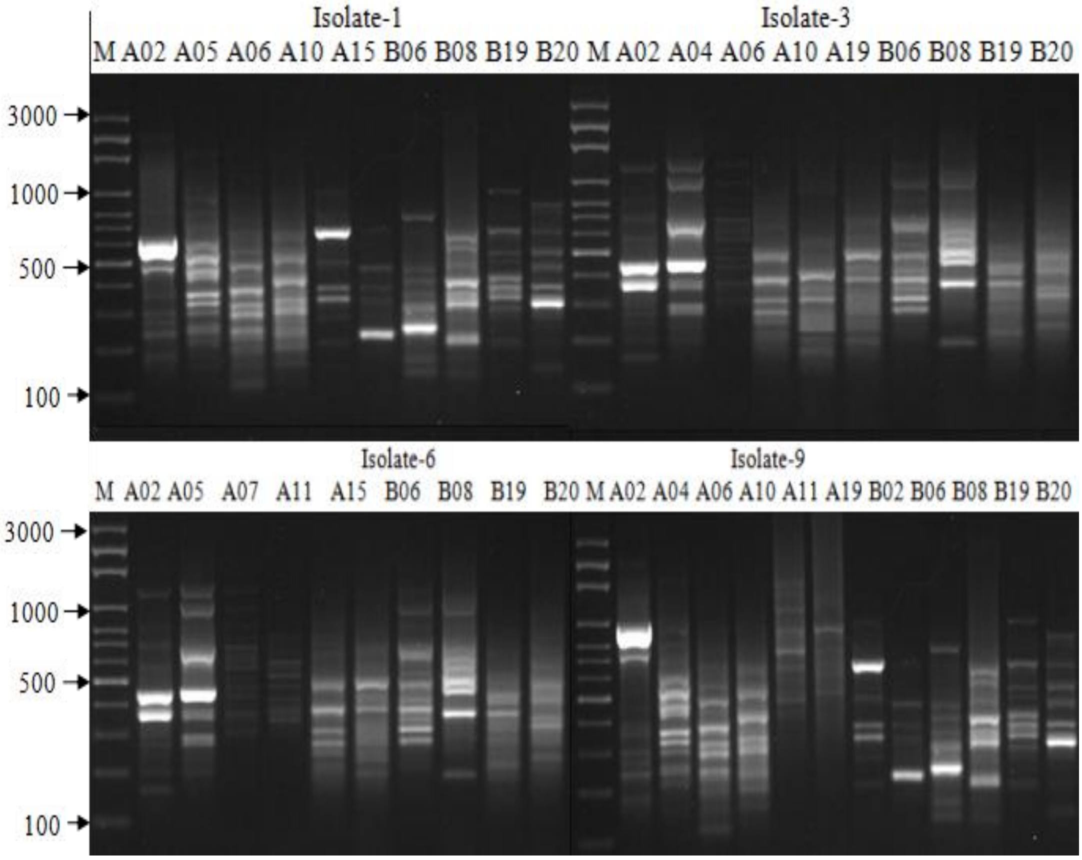

Fig. 9. RAPD fingerprinting profiles generated by arbitrarily primed PCR, show results for the four isolates of *Puccinia graminis f. sp. tritici*, isolate 1, isolate 3, isolate 6 and isolate 9, respectively

3.1.5 Pathogenicity test

Data presented in Table (7) show that typical symptoms of rust infection, *Puccinia graminis f. sp. tritici* were observed on the susceptible barely line (LBYT) after 14 days from inoculation and were similar to those previously observed on the collected infected barley samples, The Egyptian barley varieties, i.e. Giza 123, Giza 124, Giza 125, Giza 126, and Giza 2000, showed high levels of resistance to stem rust by estimate final rust severity (FRS %), compared to the control line (LBYT, the highly susceptible), where all the cultivars under study in the four locations recorded a (zero) Final rust severity (FRS %), Infection rate of the disease, however, control (line LBYT) recorded the highest rate of infection with stem rust in Nubariya, Sakha, Sids and Giza locations (76.67, 66.22, 63.31and 60.00 %), respectively.

Table 7. Effect of infection by stem rust on barley cultivars grown under field conditions in four Egyptian locations during 2020/2021 growing season

No.	Barley cultivar	Location			
		Sids	**Giza**	**Nubariya**	**Sakha**
1	Giza 123	0.00	0.00	0.00	0.00
2	Giza 124	0.00	0.00	0.00	0.00
3	Giza 125	0.00	0.00	0.00	0.00
4	Giza 126	0.00	0.00	0.00	0.00
5	Giza 2000	0.00	0.00	0.00	0.00
6	Control (Line LBYT)	63.31	60.00	76.67	66.22

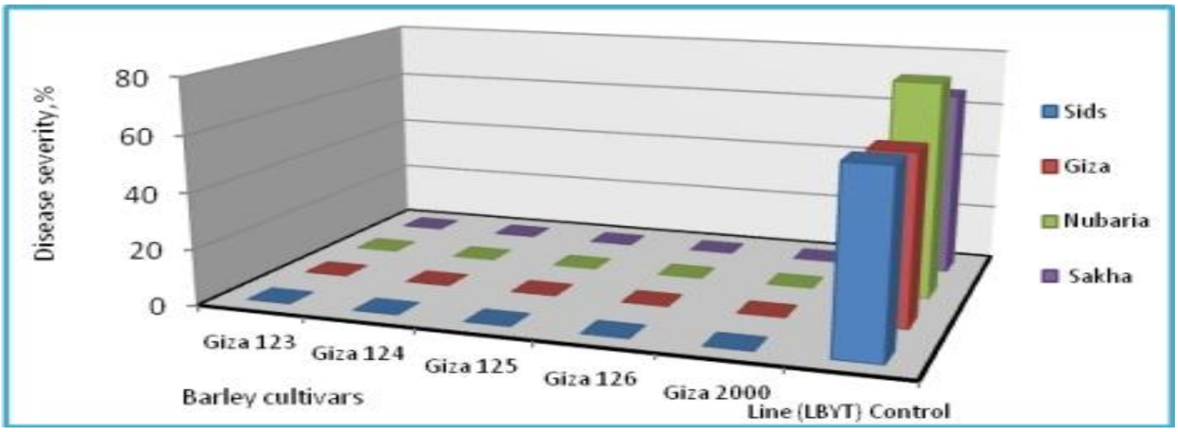

Fig. 10. Effect of stem rust on barley cultivars grown in four locations during 2020/2021 growing season

3.1.6 Agronomic traits

The results pointed out that the mean squares due to genotypes were significant for all based on average of the four locations (Table 8).

Overall mean values for days to heading and for days of maturity showed that the most desirable mean values towards the earliness were exhibited by the cv. Giza 125 with average values of (85.85 and 123.22 days), respectively, concerning plant height, cv. Giza 125 plant were the tallest with value (115.22 cm). Meanwhile, cv. Giza 2000 had the highest mean values for spike length (8.69cm), number of grains/spike (68.68 grain), number of spikes/m2 (513.1 spike), 1000-grain weight (54.34g), biological (7.45 ton) and grain yield (18.96 ardab) (Table 9).

4. DISCUSSION

Stem rust of barley and wheat caused by *Puccinia graminis* f. sp. *tritici* Eriks., and E. Henn. is historically one of the most important plant diseases, stem rust epidemics often result in major grain losses [21]. All things considered, due to the pathogen's tall potential for changeability by change and sexual or agamic recombination and its capacity to duplicate quickly and spread over awesome separations, it remains a genuine risk that cannot be disregarded within the brief or long term. This was painfully outlined in 1999 and 2001 a new race Ug99 or TTKSK with virulence to stem rust disease tolerant cultivars was detected in Uganda and Kenya, respectively [18] but has since spread throughout East Africa and is now in the Middle East [22]. Race TTKSK is predicted to spread to the world's most important wheat and barley growing regions in the near future [23,24] it is the most dangerous threat to cereal crop production in more than 50 yr because it is virulent to most barley crop and wheat cultivars grown in the major barley and barley producing areas worldwide [23,25].

Rusts disease are biotrophic fungal pathogens (phylum: Basidiomycota) that cause disease on almost every major family of plants [26]. Puccinia is by distant the biggest class of rust parasites with more than 5000 portrayed species [27,28]. The cereal rusts, especially those attacking the major food crops such as barley and wheat caused famines throughout history and epidemics have been documented in the literature since the time of Aristotle [29].

Barley are hosts to leaf, stem, and stripe rusts disease. The stem rust pathogen *Puccinia graminis* is composed of a number of different *formae speciales* or “special forms” that principally attack one or a few hosts [30,31].

That *Puccinia g*. f. sp. *tritici* is one of the most important studied fungal plant pathogen systems [32]. After Eriksson sent the concept of the *formae speciales* of *P. graminis* [26], Stakman detailed an indeed better level of have specialization: the capacity of *P. graminis f. sp. tritici* confines to particularly assault fair a few wheat genotypes and not others. This concept of physiological specialization in rust organisms driven to the classification of races of *P. graminis f. sp. tritici* and improvement of wheat differentials to distinguish them [33].

Table 8. Analysis of variance of different agronomic traits for barley genotypes as average of the four locations during 2020/ 2021 growing season

SOV	*df*	Days to heading	Days to maturity	Plant height	Spike length	Number of grains/spike
Rep	2	1.49	3.13	4.24*	0.01	0.92
Genotypes	2	21.97**	22.10**	30.85**	1.34**	93.47**
Error	4	1.54	3.29	0.63	0.01	0.65
SOV	*df*	Number of spikes/ m^2	1000-grain weight	Biological yield	Grains yield	Stem rust infection
Rep	2	78.76**	0.95*	0.01*	0.08*	0.003
Genotypes	2	3955.76**	16.05**	1.10**	5.37**	13.180**
Error	4	10.13	0.13	0.02	0.02	0.009

(*) And (**) significant at 0.05 and 0.01 levels of probability, respectively.

Table 9. Mean performance estimates of the studied traits for barley genotypes in four locations 2019/ 2020 growing season

Genotypes	Days to heading	Days to maturity	Plant height (cm)	Spike length (cm)	Number of grains/ spike	Number of spikes/m^2	1000-grain weight (g)	Biological yield (ton/ feddan)	Grains yield (ardab/ feddan)
Giza-123	86.86	126.25	109.08	8.08	66.66	440.37	52.52	6.85	17.96
Giza-124	87.85	127.26	112.11	8.08	66.66	436.33	53.94	7.06	18.08
Giza-125	85.85	123.22	115.14	7.58	63.63	484.82	50.30	6.58	17.28
Giza-126	87.86	125.24	112.11	7.47	60.60	492.90	50.10	6.47	16.54
Giza-2000	87.87	131.30	106.05	8.69	68.68	513.10	54.34	7.45	18.96
Line-1	87.12	127.71	113.00	6.73	53.46	424.00	48.6	5.63	14.75
Mean	87.24	126.83	111.25	7.77	63.28	465.25	51.63	6.68	17.26
$L.S.D._{0.05}$	1.84	2.70	1.17	0.15	1.19	4.73	0.53	0.08	0.20
$L.S.D._{0.01}$	2.62	3.83	1.67	0.21	1.70	6.72	0.75	0.11	0.28

Hence the importance of defining the races of the diseased causative agent *Puccinia graminis* f. sp. *tritici* in this study using molecular biology, where it is more accurate by using (RAPD) genetic fingerprinting and recording the isolates in the gene bank and determining the degree of similarity between the races that were defined under the study that were deposited in the gene bank and other races of the diseased causative *Puccinia graminis* f. sp. *tritici* agent the registered in the gene bank from all countries of the world, where the degree of similarity between them reached (99%).

Induction of rust resistant disease cultivars considered as the primary strategy for combating the rusts [34,35]. Since the 1960s, in wheat incorporating multiple resistance genes into cultivars has effectively controlled stem rust. On the other hand, barley stem rust disease has been kept in check since the 1940s by breeding varieties with one major durable gene, *Rpg1*; however, other factors such as a largely resistant barley and wheat crops shorter maturation period may contribute to the long-lasting disease control [35].

The Egyptian barley varieties, i.e. Giza 123, Giza 124, Giza 125, Giza 126, and Giza 2000, showed high levels of resistance to stem rust *Puccinia graminis* f. sp. *Tritici*. A similar study on barley leaf rust, confirmed, the ability of barley varieties resistance to leaf rust (*Puccinia hordei*) by [36,37,38], this is the first evaluation of these varieties against stem rust disease, and this requires further research on the resistance genes present in these varieties, and their inclusion in the breeding programs for resistance, especially in the transfer of resistance genes found in barley varieties to wheat, whereas, barley, being a true diploid compared to the closely related hexaploid wheat, is the best model in which to study host–pathogen interactions with the expectation that knowledge will lead to effective control measures.

The findings of agronomic traits reveled that mean square due to genotypes of all traits were significant, such results indicated that the tested genotypes varied from each other and ranked differently from season to another [39,40,41,42].

5. CONCLUSION

The genotypes which exhibited desirable values for high productivity and resistance to stem rust disease such as Giza 124 and Giza 2000 could be used in breeding program for improving barley production and resistance to stem rust disease.

DISCLAIMER

This paper is an extended version of a preprint document of the same author. The preprint document is available in this link: https://papers.ssrn.com/sol3/papers.cfm?abstract_id=4216664.
[As per journal policy, pre-print article can be published as a journal article, provided it is not published in any other journal].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Leonard KJ, Szabo LJ. Stem rust of small grains and grasses caused by Puccinia graminis. Mol Plant Pathol. 2005;6(2):99-111.
2. Wang MN, Wan AM, Chen XM. Barberry as alternate host is important for Puccinia graminis f. sp. tritici but not for Puccinia striiformis f. sp. tritici in the U.S. Pacific Northwest. Plant Dis. 2015;99(11):1507-16.
3. El-Daoudi Y, Nazim M, Sherif S, Shafik I, Khalifa M. Genes conditioning resistance to wheat leaf and stem rust in Egypt. In: Proceedings of the 5th congress of the Egypt. Phytopathological Soc. Giza.1987;387-404.
4. El-Daoudi Y, Mamluk O, Bekele E, Ghanem HE, Solf M, Shafik I. Preliminary results for leaf and stem rust of wheat, their prevalence and resistance in the Nile valley countries and Yemen. Fifth Arab Congr Pi Prot Fez Morocco; 1994,
5. Mohdly BR, Sallam M, El-Banoby F, Boulot O. First record of stem rust (Puccinia graminis) on barley growing in Egypt. J Phytopathol. 2013;41(1):219-20.
6. Leonard K. Stem rust—future enemy. In: (Stem Rust of Wheat) from Ancient Enemy to Modern Enemy Peterson PD, editor. St. Paul, MN: APS Publishing.2001;119-46.
7. Bengham L, Watters D, Foulkes H, Paveley N. Crop trial and the tolerance of wheat and barley to foliar disease. Ann Appl Biol. 2008;54:159-73.
8. Watson. Wheat and its rust parasites in Australia. In: (Wheat Science – Today and

Tomorrow) Evans, L.T. and Peacock. W.J.; 1981, eds). London: Cambridge University Press, :129–147.
9. Roelfs P. Races of Puccinia graminis f. sp. tritici in the USA during 1970. P1ant disease Reptr. 1971;55:986-90.
10. Harder DE, Dunsmore KM. Incidence and virlence of Puccinia graminis f. sp. tritici on wheat and barley in Canada in 1989. Can J Plant Pathol. 1990;12(4):424-7.
11. Abd El-Hak TM, El-Sherif N, Shafik I, Bassioni AA, Keddis SE, El-Daoudi YH. Studies on wheat stem rus virulence and resistance genes in Egypt and neighboring countries, Egypt. J Phytopathol. 1982;14(1-2):1-10.
12. Nilsson RH, Abarenkov K, Larsson K-H, Kõljalg U. Molecular identification of fungi: rationale, Philosophical Concerns, and the UNITE Database. The Open Appl Inform J. 2011;5(81)(Suppl 1-M9):81-6.
13. White T, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: A guide to methods and applications. 1990;18:315-22.
14. Brown JKM. The choice of molecular marker methods for population genetic studies of plant pathogens. New Phytol. 1996;133(1):183-95.
15. Peterson RF, Campbell AB, Hannah AE. A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. Can J Res. 1948;26c(5):496-500.
16. Large EC. Growth stages in cereals. Illustration of the Feek,s scale. Plant Pathol. 1954;3(4):128-9.
17. Das MK, Rajaram S, Kronstad WE, Mundt CC, Singh RP. Associations and genetics of three components of slow rusting in leaf rust of wheat. Euphytica. 1993;68(1-2):99-109.
18. Pretorius ZA, Singh RP, Wagoire WW, Payne TS. Detection of virulence to wheat stem rust resistance gene Sr 31 in Puccinia graminis f. sp. tritici in Uganda. Plant Dis. 2000;84(2):203.
19. Kolmer JA, Liu J, Sies M. Virulence and molecular polymorphism in Puccinia recondita f. sp. tritici in Canada. Phytopathology. 1995;85(3):276-85.
20. Chen X, Lin R, Leung H. Relationship between virulence variation and DNA polymorphism in Puccinia striiformis. Phytopathology. 1993;83(12):1489-97.
21. Wanyera R, Kinyua MG, Jin Y, Singh RP. The spread of stem rust caused by a. Puccinia graminis f. sp. tritici, with virulence on Sr31 in wheat in eastern Africa. Plant Dis. 2006;90(1):113.
22. Nazari K, Mafi M, Yahyaoui A, Singh RP, Park RF. Detection of wheat stem rust (Puccinia graminis f. sp. tritici) race TTKSK (Ug99) in Iran. Plant Dis. 2009;93(3): 317.
23. Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Njau P, Wanyera R et al. Will stem rust destroy the world's wheat crop? Adv Agron. 2008;98:271-309.
24. CIMMYT. Dangerous wheat disease jumps Red Sea: devastating fungal pathogen spreads from eastern Africa to Yemen, following path scientists predicted. México D.F., Mexico: International Maize and Wheat Improvement Center; 2007. Available:http://www.seedquest.com/News/releases/2007/january/18117.htm (verified 8 June 2009).
25. Raloff J. Food for thought: wheat warning—new rust could spread like wildfire. Science News. Available from: http://www.sciencenews.org/view/generic/id/6601/ (verified 4 June 2009); 2005.
26. Agrios GN. Plant pathology. 5th ed. New York: Elsevier Academic Press; 2005.
27. Cummins GB, Hiratsuka Y. Illustrated genera of rust fungi. 3rd ed. Am. Phytopathological Soc. St. Paul, MN; 2003.
28. Swann EC, Frieders EM, McLaughlin DJ. Urediniomycetes. New York: Springer; 2001.
29. Bushnell WR, Roelfs AP 1984. The cereal rusts. Vol. 1. Origins, specificity, structure, and physiology. Orlando, FL: Academic Press.
30. Farr DF, Bills GF, Chamuris GP, Rossman AY. Fungi on plants and plant products in the United States. St. Paul, MN: APS Publishing; 1995.
31. Mohdly BR, Khalil AE, Amer KA. First record of an isolate (pathotype) of Puccinia striiformis f. Sp. hordei the Causative of strip rust on barley in Egypt. Egypt. J Phytopathol. 2019;47(1):367-9.
32. Roelfs P. Wheat and rye stem rust. In: Roelfs AP, Bushnell WR, editors. The cereal rusts. Vol. 2. New York: Harcourt Brace Jovanovich. 1985;3-37.
33. Stakman EC, Piemeisel FJ. Biological forms of Puccinia graminis. J Agric Res. 1917;10:429-95.

34. Kolmer JA. Early research on the genetics of Puccinia graminis and stem rust resistance in wheat in Canada and the United States. 2001;51-82.
35. Steffenson BJ. Analysis of durable resistance to stem rust in barley. Euphytica. 1992;63(1-2):153-67.
36. Ghobrial E, Morsi L, Sabet T. Sources of resistance to leaf rust of barley Puccinia hordei Otth. In: Are. Proceedings of the 2nd phytopathol conf. 1976;671-82.
37. Ghanem E, Ghobrial E, Rizk R, Abdel-shafi A, Sherif S, Ageez A. Sources of resistance to leaf rust of barley (Puccinia hordei). Proceedings of the EMCIP Sympostium. 1984;2:220-6.
38. Niks RE, Walther U, Jaiser H, Martines F, Rubiales D, Anderso O et al. Resistance against barley leaf rust (Puccinia hordei) in West-European spring barley germplasm. Agronomie. 2000;20(7):769-82.
39. Hartleb H, Meyer U, Lehmann C. Resistance behaviour of common barleys to different isolates of *Drechslera teres* (Sacc.) Shoem. Archiv für Phytopathology und Pflanzenschutz. 1990;26(3):257-64.
40. Zaki K, Al-Masry A. Detection of biochemical genetic markers for net blotch disease resistance and barley grain yield. Egypt J Phytopathol. 2008;36:1-17.
41. Amal Mahmoud Hassan Abdel-Haleem and Mohamed Mansour Abdel-Aty. The Relationship between Varieties and Acrylamide Formation in Roasted Barley. Egypt. J. Chem. 2021;64(9):5357-5372.
42. Mansour M, Aboulila AA. Molecular variability and salinity effects on growth characters and antioxidant enzymes activity in Egyptian barley genotypes. Physiol Mol Plant Pathol. 2021;116:10173.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/93978