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Uses of Quenchers Methodology in the Management of Loose Smut Disease (*Ustilago nuda*) of Barley (*Hordeum vulgare*) through Seed Dressing and SRAP Markers Associated with Resistance in Some Egyptian Barley Genotypes

Sherin Ph. Mikhail[1], Samah, A. Mariey[2] and Ismeal A. Khatab[3]

1. Department of Barley Diseases, Plant Pathology Institute, Agricultural Research Center, Cairo 12619, Egypt

2. Barley Field Crops Research Institute, Agricultural Research Center, Cairo 12619, Egypt

3. Department of Genetics, Faculty of Agriculture, Kafrelsheikh University, Kafrelsheikh 33516, Egypt

Abstract: Barley production has been constrained by various factors, of which loose smut (*Ustilago nuda*) is the main biotic factor. Molecular and agronomical screening analyses were evaluated to study the similarity levels and marker assisted selection associated with resistance to loose smut among eight barley genotypes using sequence-related amplified polymorphism (SRAP). Agronomical parameters during two consecutive 2021 and 2022 seasons were studied to identify their reaction to loose smut. The results exhibited significant differences among all studied genotypes for all studied characters, and the highest mean values for all studied traits were detected in Giza 136 and Giza 137. Six SRAP selected primer combinations were amplified and gave 56 total fragments, where primer combination me1+em3 gave the highest polymorphism (100%) and the highest polymorphic information content of PIC was 0.96. The dendrogram of SRAP markers had clustered all studied genotypes into two main clusters. Cluster I includes all the resistance genotypes Giza 136, Giza 137, Giza 123, Giza 132, Giza 138 and Line 2. However, cluster II includes only Line 1 and Line 3 as susceptible genotypes. Thus, SRAP marker could be efficiently used to assess genetic variation among barley genotypes and useful for barley germplasm management in terms of biodiversity protection and design of new crosses for loose smut breeding programs, and seed dressings are commonly used to prevent infected seed from developing smutted heads. Some effective seed dressings include Triticonazole, Black seed oil and gamma rays 150, 200, 250 Gy. The tested fungicide was highly effective in controlling the disease and gave more than 99% disease control with high grain yield while nature product as black seed oil and utilizing radiation gamma ray 250 GY was the most effective. Utilizing radiation and natural ingredients to manage loose barley smut is economical.

Key words: *Hordeum vulgare*, loose smut (*Ustilago nuda*), SRAP, UPGMA cluster analysis.

1. Introduction

This study investigated the possible use of a foliar fungicide, black seed oil, utilizing radiation Gymma 250, 200 and 150 to control loose smut in barley.

Barley (*Hordeum vulgare* L.) is a cereal crop that is grown throughout the world and is ranked fifth in world crop production. Barley can be grown in many different climatic regions due to its adaptability to diverse conditions. These climatic conditions include variable growing seasons, temperatures, and precipitation rates [1].

Barley, like most crops, is attacked by many disease-causing organisms. Some cause only minor damage while others can completely destroy the crops. One of the most devastating diseases is loose smut which is

Corresponding author: Sherin Ph. Mikhail, Ph.D., Mikhail researcher, research fields: plant pathology.

caused by a fungus (*Ustilago nuda*). This fungus can drastically reduce both the yield and quality of crops which has been considered as a common and world-wide seed-transmitted pathogen [2]. The mycelium, that is localized in the embryo, spreads systemically and asymptotically in the developing plant; during flowering, the inflorescence is largely replaced by sari containing the black teliospores of the fungus.

Seeds infected by loose smut produce normal-looking tillers up until the time of ear emergence. Thus, the affected plants compete for light, water and nutrients alongside healthy plants [3]. In a diseased plant spikelet and kernels of each spike there is smut infected mass instead of grains enveloped in greyish membrane which later on ruptures and black powdery mass i.e. teliospores emerged out [4]. Infected seeds are the primary means of dispersing loose smut; airborne transmission is also possible, albeit not very far [5]. Since all of the grains from a smutted spike are often lost, the reduction in yield is about equal to the percentage of smutted heads [6, 7]. Because the loose smut disease is internally seed-borne, meaning that the spores stay in the seed embryo, it can subsequently infect the newly formed plant from the same seed under the right circumstances. Contaminated seeds are those that have been exposed to harmful pathogen (*Ustilago nuda*). Higher elevations are typical where the disease manifests itself, and cool, damp weather is conducive to the illness [8]. Contaminated seeds are the only source of perpetuation and loose smut causes yield losses up to 5%-7% where farmers recycle their own seed [9, 10]. Presence of loose smut infection cannot be predicted until plant impregnated with the inoculum produces a spike characteristic symptom i.e. early emergence and blackening of emerging spike. In Egypt the control of loose smut in barley can be effectively achieved by utilizing resistant barley varieties and applying. pre-sowing fungicidal treatments which have been shown to significantly reduce the incidence of this disease. Therefore, is only viable, a popular method for its effective management. The systemic fungicides like Raxil (tebuconazole), Premis, Sumi-8, were recommended in past decades [11]. Azole group is the largest group of antifungal agents and extensively used in a wide range of crops in many countries for its good control of fungi diseases like powdery mildew, rusts, Septoria leaf blotch. Azoles work by targeting the sterol 14α-demethylase CYP51 (a member of the cytochrome P450 family), which is an important regulatory enzyme in the ergosterol biosynthetic pathway. Azole fungicides bind through direct coordination of the triazole N-4 or the imidazole N-3 nitrogen as the sixth ligand of the haem iron [12]. Other treated seeds were exposed to 150 Gy, 200 Gy and 250 Gy of gamma rays. Irradiation was achieved at the National Center for Research and Radiation Technology, Atomic Energy Authority, Nasr City, Egypt. The untreated seeds served as check. The experiments were laid out in randomized block design, with three replications during 2022/2023 growing season.

A sick plant's spikelet and kernels contain an infected mass instead of grains, covered in a greyish membrane that eventually bursts and releases a black, powdery material that is actually teliospores [4]. When farmers recycle their own seed, loose smut causes yield losses of up to 5%-7%, and contaminated seeds are the only source of perpetuation [9, 10]. Egypt is resistant to this disease; however, the presence of loose smut infection cannot be predicted until the plant impregnated with the inoculum produces a spike distinctive characteristics, i.e., early emergence and blackening of emerging spike. Given these details, the only practical and widely used approach for its efficient management is the application of fungicidal seed treatment before sowing. The basidiomycete pathogen *Ustilago nuda* (Jens.) Rostr. (hereafter referred to as *U. nuda*) is known to infect barley across all global cultivation regions [13, 14]. During periods of epidemic outbreaks, *U. nuda* has been responsible for yield reductions exceeding 30% [14]. Presently, the majority of cultivated barley varieties exhibit susceptibility to loose smut. Disease

management primarily involves the utilization of uninfected seed or seeds treated with systematic fungicides [13, 14]. The application of fungicide on infected seeds can adversely affect both the quality and quantity of the crop.

Improvement of resistant cultivars is one of the most effective and economical means of controlling barley loose smut. Identification and incorporation of new and effective sources of resistance are key to the success of barley breeding programs. Molecular markers display an important role and are considered as a tool in parallels with conventional breeding for barley improvement. The first step to design breeding program for useful trait is choosing parental genotypes based on its genetic dissimilarity [15].

Sequence related amplified polymorphism (SRAP) is a PCR based marker system as described by Li and Quiros [16]. It is simple, discloses numerous co-dominant markers, targets open reading frames (ORFs), and allows easy isolation of bands for sequencing. SRAP markers have been successfully used to measure the genetic diversity and relationships in barley [15, 17]. Nonetheless no much works for using SRAP as a marker for genetic diversity in barley for loose smut.

The objectives of the present study were to fingerprint and to determine the relationships among some barley genotypes based on the SRAP markers as the first work for loose smut associated with loose smut resistance to use them in barley breeding programmers.

2. Materials and Methods

2.1 Field Experiments and Plant Materials

Eight Egyptian barley genotypes were used in this study. Their names and pedigree are shown in Table 1. Barley genotypes were grown in the Experimental Research Station of Sakha (Egypt) during two growing seasons 2020/2021 and 2021/2022 with the aim to evaluate the yield and its related traits and loose smut reaction. Randomized complete block design with three replications was used. Plot size was 1.8 m^2 (6 rows × 0.2 m × 1.5 m). Studied characteristics determined in this study included plant height (cm), number of spike m^{-2}, number of grains $spike^{-1}$, grain yield (t/ha). Barley genotypes were inoculated at the growth stage "59" according to the Zadoks scale [18] with loose smut teliospores utilizing a modified "go-go" method [19] during the growing season of 2021/2022. The seeds harvested from the preceding year were subjected to treatment 24 h prior to sowing in the subsequent year 2022/2023 through a slurry treatment methodology involving Premis® 25% FS (Triticonazole), a natural product (black seed oil), while additional treated seeds were exposed to gamma radiation at doses of 150 Gy, 200 Gy, and 250 Gy. The irradiation process was conducted at the National Center for Research and Radiation Technology, Atomic Energy Authority, located in Nasr City, Egypt. Untreated seeds functioned as the control group. The experimental design employed was a randomized block layout, comprising three replications during the 2021/2022 growing season. Standard agronomic practices were adhered to for the cultivation of barley genotypes. Observations regarding the total number of smutted tillers were documented after 75 and 90 days post-sowing. The percentage of disease control relative to the check was computed based on the count of infected tillers. A statistical analysis of the smutted tillers and grain yield per replicate was performed.

2.2 Inoculation and Disease Evaluation

Smutted heads (SMT) (disease incidence): Assessment of 100 randomly selected plants from three central rows of each plot at the time of heading and calculated as percentage of plants showing symptoms**.**

Loose smut incidence (LSI) was assessed in each cultivar according to the method described by Menzies et al. [20], and was calculated as follows:

$$\text{LSI (\%)} = \frac{\text{Number of smutted plants}}{\text{Total number of plant}} \times 100$$

Table 1 Names, type and pedigree for the studied barley genotypes.

No.	Name	Type	Pedigree
1	Giza 123	Hulled	Giza 117/FAO 86
2	Giza 132	Hulled	Rihane-05//AS 46/Aths*2Athe/Lignee 686
3	Giza 136	Hulless	Plaisant/7/cln-b/ligee640/3/s.p-b//gloriaar/come b/5/falconbar/6/linocln-b/a/s.p-/lignee640/3/s.p-b//gloria-bar/come b/5/falconbar/6/lino
4	Giza 137	Hulled	Giza 118 /4/Rhn-03/3/Mr25-//Att//Mari/Aths*3-02
5	Giza 138	Hulled	Acsad1164/3/Mari/Aths*2//M-Att-73-337-1/5/Aths/lignee686/3/Deir Alla 106//Sv.Asa/Attiki /4/Cen/Bglo.“S”)
6	Line1	Hulled	Giza 117/3/Alanda/Hamra//Alanda-01
7	Line2	Hulled	Giza 118/3/Alanda/Hamra//Alanda-01
8	Line3	Hulled	Giza 117/6/Alanda//Lignee527/Arar/5/Ager//Api/CM67/3/Cel/WI2269//Ore/4/Hamra-01

Table 2 Name and dose for all treatments for the studied barley genotypes.

No.	Treatments	Dosage per kg of seed (rate of application)
1	Premis® 25% FS (Triticonazole)	2 cm^3
2	Black seed oil	8 h soaking (15 cm^3)
3	Gamma Rays	150 Gy
4	Gamma Rays	200 Gy
5	Gamma Rays	250 Gy

2.3 Molecular Markers Assay

The molecular assay was carried out in the Genetics Laboratory of Genetics Department, Faculty of Agriculture, Kaferelshikh University, Egypt. Genomic DNA was isolated using CTAB method from fresh leaves of the used eight genotypes of barley according to J. J. Doyle and J. L. Doyle [21]. The PCR reactions using nine SRAP combinations were used in this study as shown in Table 2. The reactions for SRAP was optimized and mixtures were prepared (in total volume of 25 μL). PCR cycling was carried out as the following program: initial denaturation at 94 ℃ for 4 min, followed by five cycles comprising 1-min denaturation at 94 ℃, 1-min annealing at 35 ℃, and 30 s of elongation at 72 ℃. In the following 30 cycles, denaturation at 94 ℃ for 1 min, annealing at 50 ℃ for 1 min, and elongation at 72 ℃ for 30 s were carried out, ending with an elongation step for 10 min at 72 ℃. The amplified products were stored at 4 ℃. The PCR products were separated by electrophoresis using 2% agarose gel in 1 × TBE buffer against 100 bp DNA Ladder as a size marker. Bands were detected with ethidium bromide staining and visualized under UV light, then photographed on Gel Documentation.

2.4 Data Analysis

2.4.1 Agro-Morphological Traits Analysis.

Data collected from field experiment were statistically analyzed as a randomized complete block design (RCBD) using analysis of variance (ANOVA) as a combined analysis [22].

2.4.2 Molecular Marker Analysis

The amplified bands from SRAP were scored as a binary data under the heading of total scorable fragments, which was determined for each cultivar. The data were used to estimate the genetic similarity on the basis of number of shared amplification products [23]. Polymorphism information content (PIC) values were done according to Anderson et al. [24]. Cluster analysis was performed to produce a dendrogram using PAST program adapted by Hammer et al. [25].

Table 3 SRAP primers name and sequences.

No.	Name	Sequences SRAP 5'----3'	
1	me1+em1	TGAGTCCAAACCGGATA	GACTGCGTACGAATTAAT
2	me1+em2	TGAGTCCAAACCGGATA	GACTGCGTACGAATTTGC
3	me1+em3	TGAGTCCAAACCGGATA	GACTGCGTACGAATTGAC
4	me2+em1	TGAGTCCAAACCGGAGC	GACTGCGTACGAATTAAT
5	me2+em2	TGAGTCCAAACCGGAGC	GACTGCGTACGAATTTGC
6	me2+em3	TGAGTCCAAACCGGAGC	GACTGCGTACGAATTGAC

3. Results and Discussion

3.1 Agro-Morphological Traits Analysis

The results exhibited significant differences among the genotypes for all studied characteristics. This provides an evidence for the possibility to carry out a sufficient selection program on the basis of these traits using the studied cultivars in Tables 4-6. The results significantly varied in plant height clearly indicating that line 3 was the tallest cultivar in all treatments than line 1. However, Giza 132 was the shortest cultivar. To explain that loose smut infected barley with loose smut tends to be higher than heathy plants. Loose smut infects barley during flowering. The fungus survives inside the seed and when the seed germinates the mycelium grows upward into the plant. The infection remains invisible until head emergence. Infected heads contain masses of olive-brown smut spores infecting neighboring healthy seeds perpetuating the cycle. Infected plants tend to grow taller and mature earlier than healthy plants, the fungus replaces developing grain with masses of spores, which are then planted in subsequent seasons [26].

3.2 Assessment of Disease and Yield Components

The mean performance of number of grains spike^{-1} indicted that Giza 137 gave the highest no. of grains spike^{-1} in all treatments. Fungicide (513.13 h) black seed oil (511.2 h) G250 had the highest (495.3 h) grains spike^{-1}). However, the lowest no. of grains spike^{-1} was produced by Giza 123, which gave 61.5 grains spike^{-1}. Concerning number of spikes m^{-2}, the means of the cultivars showed that Giza 137 gave the highest number of spikes m^{-2} (513.2 spikes/m^2), while the lowest number of spikes m^{-2} was obtained by Giza 138 with value of 450.8 spikes m^{-2}. Regarding grain yield, Giza 137 had maximum value (3.7 t/fed). However, Giza 132 had the lowest grain yield (2.3a fungicide, 2.1a black seed oil, 2.4ab GY250 th^{-1}). The results in Tables 4-6 were in close agreement with those obtained by Mariey et al. [15] who found high genetic variation for most of agro-morphological traits among barley genotypes under normal condition.

High loose smut incidence (LSI) was observed in both line 1 and Line 3 of this study.

Loose smut was common in all districts surveyed [3]. Infected barley seeds used for planting purposes in repeated cycles, have led to the multiplication and distribution of the fungus across large areas of the region. Line 1 and line 3 did not act as a potential activator of plant defense responses to biotic stresses. The genetic also helped the plant to become susceptible.

The data in Table 4 show that the fungicide treatment generally leads to better growth and higher yields compared to the control treatment. For instance, the first cultivar has a significant increase in height, new growth, and grain yield when treated with the fungicide and the number of infected plants is also zero for the fungicide treatment, indicating that the treatment effectively prevents the disease.

Data in Table 5 show that Giza 137 showed the best performance in terms of grains per spike and had a relatively low number of infected plants, indicating it might be a strong candidate for resistance to loose smut and Giza 136 and Giza 132 also performed well in terms of height and yield, compared with control.

Table 4 Efficiency of fungicide (Triticonazole) on eight Egyptian barley genotypes for agro-morphological traits and loose smut reaction during 2022/2023 growing season.

Cultivar	Plant height (cm)		No. grains spike^{-1}		No. of spikes m^2		Grain yield (t/h)		Total Number of infected plant	
	Tri	Control	Tri	Control	Tri	Control	Tri	Control	Tri	Control
Giza 123	97.4b	99.7b	491.6f	305.8d	60.97abc	39b	3.9d	1.2f	0a	140c
Giza 132	85.8a	85.8a	486e	335.8e	61.7bcd	41.1c	2.3a	1.1e	0a	136b
Giza 136	108.3d	102.3c	508g	365.8g	62.5cd	45d	3.4c	1d	0a	133ab
Giza 137	114.2e	108.7e	513.13h	374h	63.5d	48.7e	3.7d	0.9c	0a	130a
Giza 138	106.7d	106.7d	450.4a	350.7f	63.1d	49.1e	3.1b	1.1e	0a	135b
Line 1	99bc	115.9f	465.47c	245.6b	60.5ab	20.5a	2.5a	0.5b	10 c	250d
Line 2	100bc	100b	474d	249.67c	61abc	45.4d	3.3bc	1.1e	0a	135b
Line 3	101.8c	118.9g	462.2b	240.3a	59.8a	20.1a	3.4c	0.4a	8b	258e

Table 5 Efficiency of black seed oil on eight Egyptian barley genotypes for agro-morphological traits and loose smut reaction during 2022/2023 growing season.

Cultivar	Plant height (cm)		No. grains spike^{-1}		No. of spikes m^2		Grain yield (t/h)		Total number of infected plant	
	Black	Control	Black	Control	Black	Control	Black	Control	Black	Control
Giza 123	98.2b	99.7b	490.2f	305.8d	63.33b	39b	3.7d	1.2f	55e	140c
Giza 132	85.2a	85.8a	480e	335.8e	59.77ab	41.1c	2.1a	1.1e	50a	136b
Giza 136	108.03e	102.3c	499g	365.8g	60.87ab	45d	3.1c	1d	51b	133ab
Giza 137	113.3f	108.7e	511.2h	374h	60.5ab	48.7e	3.2c	0.9c	52c	130a
Giza 138	106.17d	106.7d	448.2a	350.7f	62.1ab	49.1e	2.9b	1.1e	53d	135b
Line 1	98b	115.9f	462.2c	245.6b	60.1ab	20.5a	2.1a	0.5b	75g	250d
Line 2	100c	100b	470.67d	249.67c	60.33ab	45.4d	3.1c	1.1e	53d	135b
Line 3	101.23c	118.9g	454.23b	240.3a	59.2a	20.1a	3.1c	0.4a	73f	258e

The data in Table 6 are vital for understanding how different treatments can help manage loose smut in barley and Gamma Ray 250 is more effective than Gamma Ray 200 and Gamma Ray150.

3.3 Molecular Markers Analysis

The SRAP marker system is becoming the marker of choice for characterization and genetic diversity studies in a wide range of plants. The study described in the present paper shows that SRAP analysis is a powerful tool also for the characterization of barley genotypes. In our study, the SRAP markers were used for the first time for assessment of the genetic diversity among Egyptian barley genotypes for loose smut resistance. The results obtained in this study showed that there were high levels of polymorphism in genotypes under study especially when the genotypes were compared for the loose smut reaction. A total of 56 fragments were amplified with six primer combinations. Results in Table 4 showed the average percentage of polymorphic loci for all primer combinations was 77.67% and the average band number amplified from each pair of primers was 9.33% bands, which included 7.5% polymorphic bands, among which the maximum band number among the six primers combinations was 13 obtained by primer combination me1+em3 giving the highest polymorphism (100%) and highest polymorphic information content (PIC) (0.96), which generated specific band associated to loose smut with size 550 bp (Fig. 1) found in the susceptible genotypes (line 1 and line 3). However, primer me1+em2 had the lowest polymorphism (57.4.0%) and lowest PIC value (0.58).

Table 6 Effective gamma rays on eight Egyptian barley genotypes for traits: plant height, No. grains spike^{-1}, No. of spikes m^2, grain yield and total number of infected plant on loose smut reaction during 2022/2023 growing season.

Cultivar	Plant height (cm)				No. grains spike^{-1}				No. of spikes m^2				Grain yield (t/h)				Total number of infected plant			
	150GY	200gy	250gy	Con.	150GY	200gy	250GY	Con.	150GY	200gy	250GY	Con.	150GY	200GY	250GY	Con.	150GY	200GY	250GY	Con.
Giza 123	97.2b	91.33b	98.2b	99.7b	470.3f	473.2f	480.1e	305.8d	54ab	55.67a	58b	39b	1.9b	2.4c	2.5b	1.2f	1.9b	78c	1.2f	140c
Giza 132	84.1a	84.3a	85.1a	85.8a	461.2e	463.87d	475.3d	335.8e	52.2a	54.3a	56.2a	41.1c	2.1bc	2.1abc	2.4ab	1.1e	2.1bc	70ab	1.1e	136b
Giza 136	107.8e	108.1ef	108.23e	102.3c	470.33f	473.3f	483.4f	365.8g	54.4ab	56.2a	61.2de	45d	2.3c	2.2bc	2.4ab	1d	2.3c	71b	1d	133ab
Giza 137	112.3f	112.8f	113f	108.7e	480.2g	486.4g	495.3h	374h	54.2ab	55.5a	58.2b	48.7e	2.1bc	2.3c	2.5b	0.9c	2.1bc	68a	0.9c	130a
Giza 138	105.9d	106.2def	106.8de	106.7d	430.3a	435a	440.3a	350.7f	56.1b	56.8a	62.1e	49.1e	2.3c	2.1abc	2.6b	1.1e	2.3c	70ab	1.1e	135b
Line 1	97.3b	97.8c	98.3b	115.9f	435.7b	438.7b	443b	245.6b	54.1ab	54.6a	60.1cd	20.5a	1.5a	1.9ab	2.1a	0.5b	1.5a	80c	0.5b	250d
Line 2	100c	102cde	105d	100b	452d	465.7e	485.23g	249.67c	55.1b	55.8a	61de	45.4d	2.3c	2.1abc	2.5b	1.1e	2.3c	70ab	1.1e	135b
Line 3	100.1c	100.2cd	100.77c	118.9g	442.3c	445.7c	447.8c	240.3a	54.8ab	55.3a	59.2bc	20.1a	1.4a	1.8a	2.4ab	0.4a	1.4a	78.67c	0.4a	258e

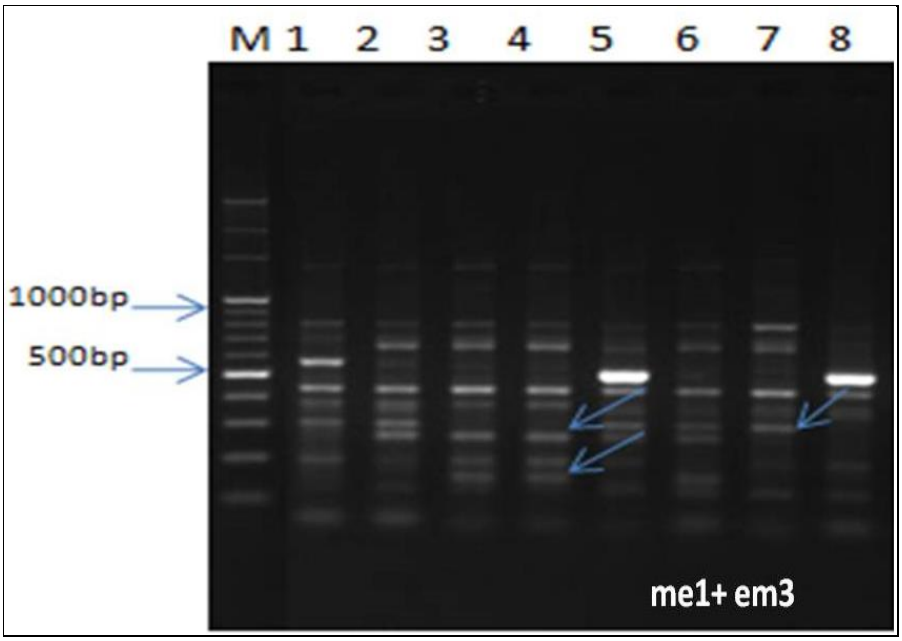

Fig. 1 Agarose gel electrophoresis using SRAP primer combinations me1+em3 (A) and me2+em2 (B) in eight barley genotypes. (M) marker, (1) Giza 123, (2) Giza 132, (3) line 1, (4) line 2, (5) line 1, (6) Giza 136, (7) Giza 137 and (8) Line 3.

Table 7 Polymorphism number and rate for six SRAP primer pairs used to amplify genomic DNA templates from eight Egyptian barley genotypes.

Primer combination	Number of total fragments	Number of polymorphic fragments	Percentage of polymorphic fragments	Polymorphic information content (PIC)
me1+em2	7	4	57.14	0.58
me1+em3	14	14	100.00	0.96
me2+em1	10	7	70.00	0.71
me2+em3	8	6	75.00	0.68
me1+em2	9	8	88.89	0.83
me1+em1	8	6	75.00	0.76
Average	9.33	7.5	77.67	
Total	56	45		

A dendrogram (Fig. 2) based on the genetic similarity coefficient was constructed using the six SRAP primers. In this dendrogram, all the eight barley genotypes divided into two main clusters. Cluster I includes all the resistance six barley genotypes Giza 136, Giza 137, Giza 123, Giza 132, Giza 138 and Line 2. However, cluster II includes the genotypes Line 1 and line 3 which are susceptible genotypes with genetics similarity (0.82).

In present study, genetic diversity level of Egyptian barley genotypes for loose smut is higher than other genetic diversity studies using different marker systems for loose smut such as RFLP [27], SSR [28] and SCAR marker for loose smut in wheat [29]. SRAP markers mainly targets exons which are expected to be evenly distributed along all chromosomes with GC-rich regions and introns with AT-rich regions [16]. With small and simple barley genome taken into consideration, many intron and exon regions may have influenced the number of excess polymorphic bands. Therefore, using SRAP marker to assess the genetic diversity among barley genotypes for environment stress will be useful for barley germplasm management in terms of biodiversity protection and design of new crosses for disease resistance to loose smut breeding programs. These results were in good harmony with

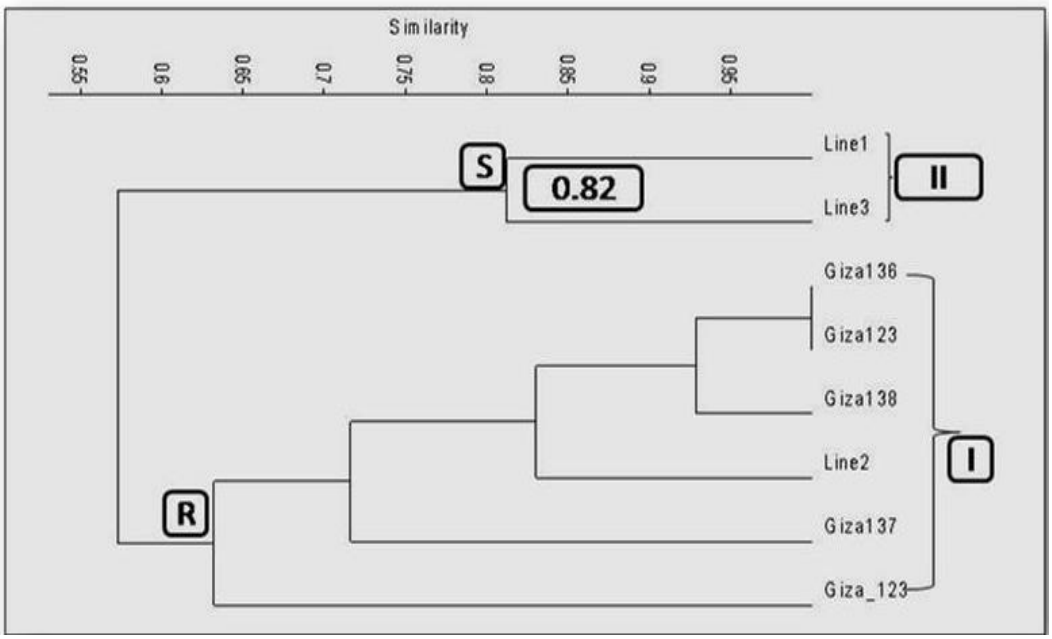

Fig. 2 SRAP based dendrogram of the eight barley genotypes constructed using Unweighted Pair-GroupMethod with Arithmetic (UPGMA).

Mariey et al. [15]. They reported that the SRAP marker will be efficiently used to assess genetic variation among barley genotypes and would be useful for barley germplasm management in terms of biodiversity protection and design of new crosses for environment stress breeding program.

4. Conclusions

4.1 Genetic diversity Assessment

This marks the first attempt to evaluate genetic diversity and resistance to loose smut in Egyptian barley genotypes using SRAP markers. The findings indicate that SRAP analysis is a powerful tool for characterizing barley genotypes and assessing their genetic diversity.

4.2 Effectiveness of Seed Dressings

All tested seed dressings were found to reduce the incidence of loose smut. Among these, the most effective treatments included fungicides, black seed oil, and gamma rays at doses of 150, 200, and 250 Gy. The tested fungicide achieved over 99% disease control, significantly enhancing grain yield.

4.3 Polymorphism in Genotypes

The study revealed high levels of polymorphism among the barley genotypes, particularly in relation to their reaction to loose smut. A total of 56 fragments were amplified using six primer combinations, with the combination me1+em3 showing the highest polymorphism (100%) and the highest polymorphic information content (PIC) of 0.96.

4.4 Cluster Analysis

The dendrogram analysis based on genetic similarity coefficients categorized the eight barley genotypes into two main clusters. Cluster I included six resistant genotypes, while Cluster II comprised the two susceptible genotypes, indicating a clear genetic distinction based on resistance to loose smut.

4.5 Implication for Breeding Programs

The results suggest that SRAP markers can be effectively utilized for assessing genetic variation among barley genotypes. This information is crucial for barley germplasm management, biodiversity protection, and the design of new breeding programs aimed at

enhancing resistance to loose smut.

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