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Evaluation of Leaf Rust Resistant by Detection of *Lr* Genes in New Egyptian Wheat Lines.

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ABSTRACT

Leaf rust caused by *Puccinia triticina* is one of the most damaging diseases of wheat in Egypt and worldwide. Lack of durable resistance in local wheat varieties is the main reason to leaf rust epidemic which could limit yields. The use of genetically resistance is the most economical method of reducing yield losses due to leaf rust. The aim of this study was to screen leaf rust resistant lines at the seedling stage by using Sequence tagged site (STS) marker for four *Lr* resistance genes against *Puccinia triticina*. The new tested lines may have genes can be used to further manipulation in wheat breeding programs. The obtained results showed that the new lines No. 2, 3 and 9 produced the highest grain yield; while the lines No. 1 and 8 gave the lowest grain yield during both seasons. On the other hand, some lines showed resistance at the seedling and at adult stages such as lines No. 3, 7 and 8. The remaining the lines were resistance at the seedling stage and susceptible at the adult plant i.e. No. 1, 2, 4, 5, 6, 9 and 10. The STS marker for resistance genes *Lr1* (560 bp) and *Lr24* (700 bp) showed in all leaf rust resistance wheat lines at the seedling stage. On the other hand, the amplification product of *Lr9* (1,100 bp) occurred in resistance lines No. 8 and 9. In addition, STS marker for *Lr47* gene (282 bp) revealed only in the resistance line No. 1. Consequently, it should be taken into consideration in the future breeding programs using as recurrent parents the susceptible high-quality wheat line and leaf rust resistant ones as donor parents.

Keywords: *Triticum aestivium* L, *Puccinia triticina*, phytopathological analysis, STS marker.

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INTRODUCTION

Wheat leaf rust caused by fungal pathogen *Puccinia triticina* is a severe disease worldwide that is responsible for major crop damage and results in both yield losses and downgrading in quality [1-4]. Abdel Hak et al.[5] estimated crop losses of up to 50% due to leaf rust infection in Egypt. Leaf rust (*Lr*) resistance gene(s) have been used successfully in breeding programs to develop new wheat cultivars with improved disease resistance. However, single *R*-genes tend to be quickly overcome by changes in the *P. triticina* population. More durable resistance can be achieved by gene pyramiding e.g., the stacking of multiple *Lr* genes, such as the Canadian cultivar Pasqua [6-8]. The incorporation of effective and durable resistance is a valuable breeding strategy for wheat improvement. The 'pyramiding strategy', as to say the incorporation of more than one resistance gene to the same or different pathogens in a single genotype, could aid the breeder to maintain resistance any longer. Seedling resistance genes could be of little use when deployed alone in some regions, while they could be useful when deployed in combination with other genes [9]. Specific molecular markers closely linked with resistance genes can facilitate expeditious pyramiding of major genes into elite background, making it more cost effective. Moreover, expression of molecular markers is not affected by environment, and they can be detected at all stages of plant growth [10].

To date, 50 leaf rust resistance genes have been designated and mapped in wheat [11]. Half of which originate from wheat relatives, both wild and cultivated. Recent virulence surveys [12-14] have shown *Lr19* to be one of the most effective across time and space. Resistance gene expression is dependent on the genetics of host-parasite interaction, temperature conditions, plant developmental stage, and interaction between resistance genes with suppressors or other resistance genes in the wheat genomes. Genes expressed in seedling plants have not provided long-lasting effective leaf rust resistance. Adult-plant resistance genes *Lr13* and *Lr34* singly and together have provided the most durable resistance to leaf rust in wheat throughout the world. Continued efforts to isolate, characterize, and map leaf rust resistance genes is essential given the ability of the leaf rust fungus to overcome deployed resistance genes [15].

Lr19 still provides valuable resistance to wheat leaf rust in many parts of the world [16-18]. *Lr19* carriers respond to pathogen attack by generating a hypersensitive reaction [19, 42]. Along with the race specificity of the resistance, this reaction suggests a typical *R* gene, a number of which have been cloned in recent years [12, 20].

The aim of this study was to screen leaf rust resistance lines at the seedling stage by using Sequence tagged site (STS) marker for four *Lr* resistance genes against *Puccinia triticina*. The tested lines may have genes can be used to further manipulation in wheat breeding programs.

MATERIALS AND METHODS

The Plant Materials

New nine bread wheat lines and one check variety, Gemmeza 11 used in this study. These lines derived from three way crosses between Egyptian wheat cultivars with CYMMIT and ICARDA germplasm lines.

At Adult Stage

The ten tested wheat lines were planted in non-replicated field experiments at Nubaria Agric. Res. Station, in 2012/2013 winter growing season for severity of leaf rust at the adult plant stage. The experiment was surrounded by spreader rows planted with mixtures of the highly susceptible varieties i.e. Morocco, Thatcher and *Triticum spelta* Saharinsis. Randomization was not used in planting these lines, since it seemed to be unnecessary [21] because of the high proportion of the infection reaching the tested genotypes from the spreader rows. Artificial inoculation was carried out using a mixture of uredospores of the prevalent races mixed with talcum powder at a rate of 1 (spores): 25 (talcum powder) (v:v) according to the method described by Tarvit and Cassell[22]. The rust response was recorded after the heading stage by combining severity from 0 to 100 % (percent of infection) according to the modified Cobb scale [23] and reaction (type of reaction) [24].

Also, field experiments lay out in a randomized complete block design with three replications in Shebin EL-Kom, Menofiya Governorate, Egypt, during 2012/13 winter growing season to estimate grain yield.

Each progeny lines were grown in three rows three meter long. The spacing between and within rows were maintained at 30 and 10 cm, respectively. All the normal agronomic practices were followed as usual in the ordinary wheat field in the areas of study. The two characters studied are, 100-kernel weight (gm) and grain yield/plant (gm). The statistical analysis of data obtained is conducted according to Gomez and Gomez [25].

Phytopathological Analysis

At Seedling Stage

Ten wheat lines (20 plants from each line) were evaluated to leaf rust pathotypes under the greenhouse conditions at Wheat Disease Dep., Plant Pathology Institute, Agricultural Research Center. The tested lines were sown in 10 cm diameter plastic pots filled with peat moss and vermiculite in greenhouse with Randomized complete block. 7-days old wheat seedlings were artificially inoculated with mixture of leaf rust races e.g., TSTTK, PJTLS, NKTST and PTTST during season 2012/13. Inoculated seedlings were placed for 24 h in a dark, humid chamber at 19°C and then moved to a glasshouse, under a 16-h photoperiod and a 25°C (day) and 22°C (night) temperature regime. After 12 days of inoculation, the infection type descriptions still in use are based on the original scales proposed by Stakman et al. [26] to leaf rust. The rust reactions 0, 0;, 1, and 2 were considered resistant (R) response, while 3, and 4 were considered susceptible (S) response.

DNA Extraction

Genomic DNAs were extracted from leaves of ten wheat lines the resistance and healthy control at the seedling stage according to the Cetyl trimethylammonium bromide (CTAB) method of Silva and Procunier [27].

PCR Amplification

Polymerase chain reaction (PCR) was performed in 25 µl reaction volume containing: 2.5 µl 10X PCR buffer, 100 ng of genomic DNA, 1X *Taq* DNA polymerase (2.5 unit/µl), 10 *p*mol of forward and reverse primers, and 2.5 mM of each dNTPs. The sequence for each STS primer set and PCR conditions were listed in Table (1). After amplification PCR products were separated on 1.5% agarose gels according to amplicon dimension, stained with ethidium bromide and visualized under UV light.

RESULTS AND DISCUSSION

The analysis of variance of weight 100 grain weight and grain yield per plant for ten wheat lines was presented in Table (2). The tested wheat lines showed significant differences in 100 grain weight and grain yield reflect their different genetic backgrounds. The lines mean performance (Table 3) showed that the lines No., 2, 3 and 9 had the highest values of grain yield, while lines No., 1, 6 and 8 gave the lowest values of grain yield under the field conditions, comparing to check variety (Gemmeza 11). These results were agree with those obtained by Zarei et al. [28] who mentioned that grain yield was a complex polygenic quantitative trait, hence, selection based on the performance of grain yield alone, was usually not very efficient .Thus, identifying characters contributing to grain yield was important as it increase breeding efficiency; therefore, easily measurable characters along with the high heritability and having useful relationship with grain yield were of the paramount importance to practice indirect selection for the high yield [29]. Behmanesh et al. [30] evaluated 13 genotypes yield of advanced endemic Durum wheat. The variance analysis showed significant differences among the studied genotypes yield and yield components.

This study detection of leaf rust resistant genes by the method phytopathological testing in identification of four genes *Lr1* from (common wheat), *Lr9* from (*Aegilops umbellulata*), *Lr24* from (*Thinopyrum ponticum*), *Lr47* from (*Aegilops speltoides*). Resistance of the ten wheat lines to leaf rust infection at the seedling (Figure 1) and the adult plant stages are shown in Table (4). Some lines showed resistance in both stages such as lines No. 3, 7 and 8. The rest of the lines were resistance at the seedling stage and susceptible at the adult plant such i.e. lines No. 1, 2, 4, 5, 6, 9 and 10 (Table 4). These results were in an agreement with Eyal and Peterson [31] showed that the plant infected by rust parasites resistant at moderate temperatures become susceptible at significantly higher ones. Most of the authors related these changes in response to host resistance genes, which are called "temperature genes". Kharouf et al. [32] found that the variety Jpateco73S was susceptible during both stages indicating the lack of any resistance gene in its genetic composition. The

variety Sadari, on the other hand, was resistance during both stages and, consequently, the resistance gene during seedling stage gave the plant the necessary resistance during the adult stage. The variety Oxely APR was susceptible during the seedling stage but resistant during the adult stage. In contrast, the variety Avocet Yr 18 was susceptible only during the adult stage. This condition was due to the effect of temperature. In breeding for leaf rust resistance and studying its inheritance, Kaul and Shaner [33] observed that its expression seemed to be influenced by temperature.

Molecular marker STS closely linked to the *Lr* genes used in this work, were tested for their presence/absence in ten lines wheat to their application into breeding programs as suggested by Gupta et al.[10] and Korzun [34] (Table 5). The STS marker for resistance genes *Lr1* (560 bp) and *Lr24* (700 bp) [35] showed in all leaf rust resistance wheat lines at the seedling stage (Figures 2, 3). Moreover, the amplification product of *Lr9* (1,100 bp) specific to line Tc**Lr1* occurred in resistance lines No. 8 and 9 (Table 5 and Figure 2). Furthermore, a STS marker for *Lr47* gene (282 bp) revealed only in the resistant line No. 1 (Figure 3). These results were in an agreement with Schachermayer et al. [36] mentioned that the first molecular STS marker was discovered by for *Lr9* gene derived from *Aegilops umbellulata*. Resistance genes toward leaf rust resistance *Lr1*, *Lr9*, *Lr24*, *Lr28*, *Lr29* and *Lr37* were mapped on chromosomes with Random amplified polymorphic DNA (RAPD) markers; *Lr19*, *Lr26* and *Lr46* were mapped on chromosomes with Amplified fragment length polymorphisms (AFLPs) markers. However, resistance genes toward leaf rust *Lr1*, *Lr9*, *Lr10*, *Lr24*, *Lr28*, *Lr35*, *Lr37* and *Lr40* were mapped with chromosomes with STS markers and leaf rust resistance genes *Lr13*, *Lr16*, *Lr22a*, *Lr22b*, *Lr39* and *Lr50* were mapped with chromosomes with Simple sequence repeat (SSR) markers. [9, 37] observed that markers linked to *Lr9*, *Lr24* or *Lr47* were found in the respective 'Thatcher' NILs (near-isogenic lines near-isogenic lines), while no amplified products were detected in genotypes lacking *Lr9*, *Lr24* and, only by using PS10L and PS10R primers, in the genotypes lacking *Lr47*. The resistance tests performed at the seedling stage with leaf rust pathotype 03766, avirulent to all the resistance genes used, were compared with the molecular tests confirming the presence/absence of the corresponding gene.

Although a large number of molecular markers were now available, little has yet been done about their practical use in a wheat breeding [10]. Moreover, being the genome of common wheat very complex some molecular markers (STS, Sequence characterized amplified regions-SCAR) may give false-positive answers about the presence of the gene involved, especially considering the different genetic backgrounds of the cvs used either as donor or recipient parents [38]. The expression of resistance genes is known to be modified by the genetic background of a cultivar [39], especially when these genes were transferred in common wheat from related species [40, 41]. The introgression of resistance genes should be confirmed by phytopathological tests also to verify their phenotypic expression in the new genetic background, discarding modifications for the presence of modifiers or suppressors. Nocente et al. [9] found that conventional cereal breeding was time consuming and depends on environmental conditions. The utilization of molecular markers in breeding programs will allow improving the efficiency and the earliness of selection, also by detecting a single resistance gene in a complex background of other resistance genes. Novel selected genotypes will be available, useful to further breeding work. As a whole, conventional cereal breeding was time consuming and depends on environmental conditions. The utilization of molecular markers in breeding programs will allow improving the efficiency and the earliness of selection, also by detecting a single resistance gene in a complex background of other resistance genes.

Table 1: The nucleotide sequences of STS Primers, sizes of amplified marker fragments and references for leaf rust resistance gene markers used in this study.

Marker	Sequence of primers 5-3	PCR amplification conditions	Size of amplified marker fragments	Reference
Lr1	pTAG621-5: GGGTCACGT ACTACTATATA p TAG621-3: CCT TGC CAG CCC AAA AGA AG	94°C 5 min; 30 cycles (92°C-1 min., 55°C-1 min., 72°C-2 min); 72°C-10 min	560 bp	Feuillet et al.[35]
Lr9	J 13/1: TCC TTT TAT TCC GCA CGC CGG J 13/2: CCA CAC TACCCC AAA GAG ACG	94°C 5 min; 35 cycles (92°C-1 min., 58°C-1 min., 72°C-2 min); 72°C-5 min	1,100 bp	Schachermayr et al.[36]
Lr24	SC-H51: AGT CGT CCCCGA AGA CCC GCT GGA SC-H52: TCG TCC CCT GAT GCC ATG TAA TGT	94°C 3 min; 38 cycles (92°C-1 min., 68°C-2 min., 72°C-2 min); 72°C-5 min	700 bp	Dedryver et al.[43] (1996)
Lr47	PS10R: GCT GAT GACCCT GAC CGG T PS10L: GGG CAG GCG TTT ATT CCA G	94°C 3 min.; 7 cycles of touchdown (94°C-30 s, 70°-64°C-30 s, 72°C–30 s); 35 cycles (94°C-30 s, 63°C-30 s, 72°C-30 s); 72°C-7 min	282 bp	Helguera et al.[44] (2000)

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Table 2: Mean square values for 100 grain weight and grain yield/plant among the ten bread wheat lines evaluated under field conditions in 2012/2013 winter growing season.

S.O.V	D.F	100 Grain weight (g)	Grain yield /plant (g)
Reps	2	0.001	0.365
Lines	10	2.9**	26.21**
error	20	0.003	4.39

Table 3: Mean performance for 100 grain weight and grain yield/plant of ten bread wheat lines evaluated under field conditions in 2012/2013 winter growing season.

Line No.	100 grain weight (g)	Grain yield /plant (g)
1	3.66	12.6
2	3.69	16.6
3	3.33	16.0
4	3.2	14.0
5	4.73	14.6
6	4.76	12.6
7	3.41	14.6
8	3.61	12.0
9	6.35	21.3
10 (Gemmeza 11)	5.27	15.0
Average	4.19	14.9
L.S.D.	0.53	3.57

Table 4: Phytopathological testing to ten wheat lines against leaf rust resistance.

At the seedling stage				At the adult stage	
Line No.	Infection type	Host Response	Symptoms	The percentage of leaf rust infection	Leaf rust reaction
1	1	Resistant	Small uredia with necrosis	66%	Susceptible
2	1	Resistant	Small uredia with necrosis	66%	Susceptible
3	0;	Very resistant	Hypersensitive flecks	11%	highly resistance
4	0;	Very resistant	Hypersensitive flecks	66%	Susceptible
5	1	Resistant	Small uredia with necrosis	66%	Susceptible
6	1	Resistant	Small uredia with necrosis	66%	Susceptible
7	1	Resistant	Small uredia with necrosis	22%	highly resistance
8	2	Moderately Resistant	Small to medium sized uredia with green islands and surrounded by necrosis or chlorosis	22%	highly resistance
9	2	Moderately resistant	Small to medium sized uredia with green islands and surrounded by necrosis or chlorosis	66%	Susceptible
10 (Gemeza 11)	2	Moderately resistant	Small to medium sized uredia with green islands and surrounded by necrosis or chlorosis	66%	Susceptible

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Table 5: Detection of leaf rust resistance genes *Lr1*, *Lr9*, *Lr24* and *Lr47* in the ten wheat lines using molecular markers and host-pathogen interaction test at the seedling stage.

Line No.	Host-pathogen interaction	Molecular marker test			
		STS (*Lr1*) (560 bp)	STS (*Lr9*) (1,100 bp)	STS (*Lr24*) (700 bp)	STS (*Lr47*) (282bp)
1	R	+	-	+	+
2	R	+	-	+	-
3	R	+	-	+	-
4	R	+	-	+	-
5	R	+	-	+	-
6	R	+	-	+	-
7	R	+	-	+	-
8	R	+	+	+	-
9	R	+	+	+	-
10 (Gemeza 11)	R	+	-	+	-

R= resistant
(+) = presence of amplified product.
(-) = absence of amplified product.

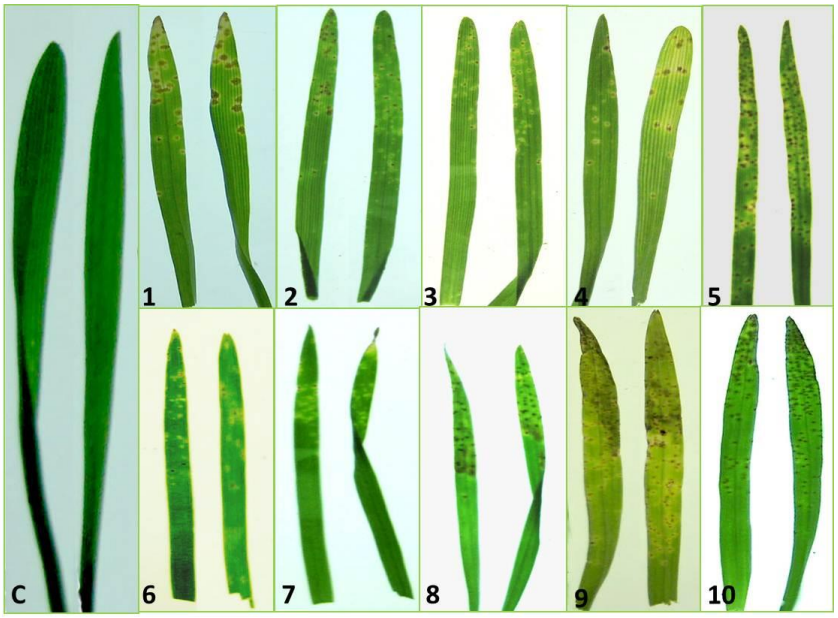
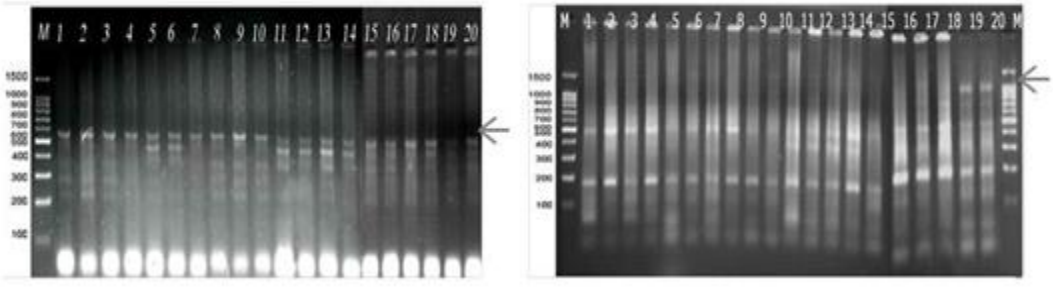


Figure 1: Manifestation of leaf rust pathotype in ten wheat lines at the seedling stage compared with the control (C).



(a) **(b)**

Figure 2: Detection of leaf rust resistance genes (a) *Lr1*, *Lr9* (b), in ten wheat lines by STS marker. Lane M: molecular size marker 100 bp.

Lanes 1, 3, 5, 7, 9, 11,13, 15, 17, 19 the healthy plants of wheat lines No. 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, respectively.
Lanes 2, 4, 6, 8, 10, 12,14, 16, 18, 20 the leaf rust resistance wheat lines No. 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, respectively.

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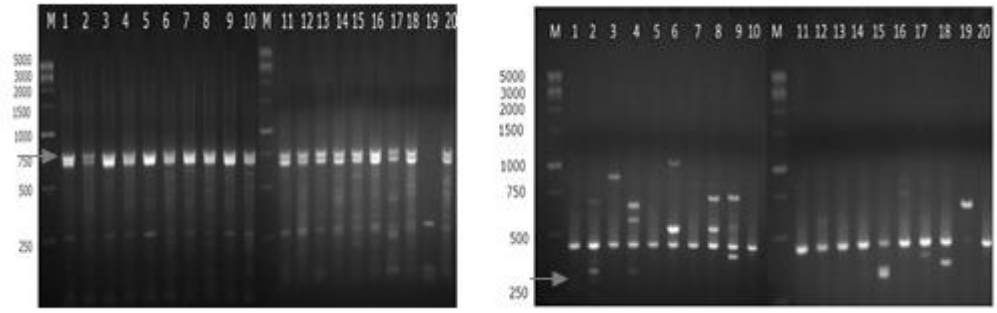

(a) (b)

Figure 3: Detection of leaf rust resistance gene *Lr24* (a) and *Lr47* (b) in ten wheat lines by STS marker. Lane M: molecular size marker 1 Kbp.

Lanes 1, 3, 5, 7, 9, 11,13, 15, 17, 19 the healthy plants of wheat lines No. 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, respectively.
Lanes 2, 4, 6, 8, 10, 12,14, 16, 18, 20 the leaf rust resistance wheat lines No. 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, respectively.

CONCLUSION

STS can be used in routine wheat breeding programs to detect of *Lr* resistance genes. Wheat cultivars continuously need incorporation of new rust resistance genes. This can be achieved by traditional hybridization and selection procedures. STS marker can be employed effectively to screen segregating populations which is basic material for development of new and improved cultivar.

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