Ascertaining of Ug99-race specific genes in wheat genotypes assigned to stem rust resistance based on phenotypic and genotypic reaction

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Abstract: Five wheat genotypes including two commercial cultivars (Mist1 and Misr2) and three Ug99-race specific genes (*Sr24*, *Sr25*, and *Sr31*) were screened for stem rust caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*) based on phenotypic and genotypic reaction. Based on phenotypic reaction, *Sr24* (Lc*Sr24*Ag) and *Sr31* (Seri-82) exhibited seeding and adult plant resistance while the wheat cultivars, Misr1 and Misr2 were susceptible. *Sr25* Agatha (CI 14048)/9*LMPG-6 DK16 exhibited seedling resistance while showed susceptibility at adult plant stage. Based on genotypic reaction, DNA markers showed that only gene *Sr25* was present in Misr1 and Misr2 while *Sr24* and *Sr31* were absent in both cultivars. Although, virulence to *Sr25* was detected. However, wheat cultivars Misr1 and Misr2 are not prone to infection by Ug99 and its variants, as both carry the *Sr25* gene. Avirulence to *Sr24* likely being best-suited gene to be incorporated into wheat genotypes for resistance to local *Pgt* population.

Keywords: Wheat, stem rust, Ug99-race, race specific genes, phenotyping, DNA markers

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1 Introduction

Stem rust caused by the basidiomycete fungus *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn. (*Pgt*), is a very destructive disease of hexaploid common wheat (*Triticum aestivum* L.) and tetraploid durum wheat (*Triticum turgidum* var. *durum*) (Roelfs et al., 1992). Stem rust has historically been a major problem in most wheat-growing areas of the world, causing devastating yield losses. In Egypt, epidemic cases of stem rust occurred in 1947 and 1968 (Gomma, 1968). Stem rust of wheat has re-emerged as a major threat to global wheat production, when severe infections were observed on CIMMYT (International Maize and Wheat Improvement Center) wheat nurseries planted in Uganda in 1998. In South Africa in early 1999, a race of *Pgt* was identified with virulence to stem rust resistance gene *Sr31*. This race, commonly known as Ug99, was characterized as race TTKSK based upon the North American *Pgt* nomenclature system **(**Roelfs and Martens 1988; Jin et al., 2008). Race TTKSK was demonstrated to be virulent on nearly all Asian cultivars and the majority of North American cultivars (Singh et al., 2008). Variants of race TTKSK have been detected with avirulence to these genes, increasing the devastative potential of the TTKSK group of races **(**Jin et al., 2008). Since its discovery, race TTKSK and related races have been detected in Kenya, Ethiopia, Sudan, Yemen, Iran, Tanzania, Zimbabwe, and South Africa **(**Singh et al., 2011).

It is better to protect the local wheat genotypes from infection by the disease, genes for resistance should be incorporated into the high yielding genotypes. During the first two-thirds of the twentieth century, an emphasis on breeding resistant varieties of wheat and monitoring the pathogen population successfully resulted in the control of wheat stem rust in Egypt. The fastest way to reduce the

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susceptibility of important wheat cultivars and the best new germplasm is to systematically incorporate diverse sources of resistance through limited or repeated backcrossing. Because most of these race specific effective genes are of alien origin, co-segregating molecular markers for some of them are already available and can aid selection (Mago et al., 2005). To avoid fast breakdown, the best strategy is to use race-specific resistance genes in combinations to transfer two or more effective resistance genes into an adapted cultivar (Singh et al., 2015).

To date, more than 50 stem rust resistance genes have been reported in wheat and its wild relatives, most of them confer resistance to at least one race in the Ug99-lineage (McIntosh et al., 2008; Singh et al. 2015). Most of these genes are specific to pathogen race except *Sr2*, which is race-nonspecific and provides durable resistance (Singh et al., 2008). Screening of breeding material for disease resistance genes using conventional approaches requires time, as some genes express only at later stages of plant growth. Another drawback of the conventional approach is that disease inoculum has to be applied to plants, which are dangerous in regions where a particular pathogenic race is not present. Gene-for-gene specificity between host resistance genes and different avirulence genes in the pathogen can be employed for postulation of resistance genes in the host plant. However, this method is best suited for seedling resistance genes because the interaction between resistance genes and stage of plant development at which these genes express can obscure the gene postulation. These problems can be overcome by using DNA based markers to identify the resistance genes that may be present.

Several stem rust resistance genes have been identified that are effective to Ug99. Genes *Sr22*, *Sr25*, *Sr26*, *Sr33*, *Sr35*, *Sr45*, and *Sr50* are, at present, possibly the most useful race-specific resistance genes, provided that they are used in combinations. *Sr24* offers resistance to Ug99 (TTKSK) but not to its variants. If proper care in their deployment is not taken, then these genes are expected to lose effectiveness in Eastern and Southern Africa, where high populations of the Ug99 race group and other races exist each year (Jin et al., 2007; Singh et al., 2011, 2015). Most of these genes were introgressed from wild relatives of wheat and some possess genetic linkage to undesirable traits. In order to achieve long-lasting resistance to stem rust, and race TTKSK in particular, combinations of multiple genes will need to be deployed in cultivars.

Identification of specific genes to Ug99 closely linked based on plant reaction in conjugation with DNA markers could provide information for Ug99-race management and combining of effective resistance genes in breeding genotypes for stem rust resistance. Molecular markers linked to at least 18 race Ug99-effective resistance genes are available for genes transferred from diploid and tetraploid wheat relatives (Mago et al., 2002, 2005, 2011; Liu et al., 2010) and two genes native to bread wheat (Hiebert et al., 2011). Markers linked to additional stem rust resistance genes native to bread wheat and effective to race TTKSK are desirable to provide breeders with several genes for combining in adapted varieties. Previous reports identified wheat cultivars Misr1 and Misr2 as possessing seedling and adult plant resistance to race Ug99 and reported to be possessed resistance genes to this race. Misr1 and Misr2 are breeding varieties developed during the last decade in Ethiopia, the hotspot of Ug99 race (Singh et al., 2011). Further ascertain of the genes conferring the plant resistance in Misr1 and Misr2 to Ug99 isolates is needed. Molecular markers provide an efficient way to address problems faced in conventional breeding methods.

Several DNA markers linked to various stem rust resistance genes in wheat, including genes we studied, *Sr24* (Mago et al., 2005), *Sr25* (Liu et al., 2010) and *Sr31* (Mago et al., 2002), have been identified and developed. Development of molecular markers has led to efficient methods of plant breeding for disease resistance. Previously studies to identify stem rust resistance genes in wheat genotypes based on phenotyping (plant reaction) and/or genotyping (DNA markers) have been achieved by Khanzada (2008), Liu et al. (2010), Kokhmetova et al. (2011), Hailu et al. (2015), Li et al. (2016), Shamanin et al. (2016) and Xu et al. 2017. The objectives of this research were to detect seedling and adult plant resistance of wheat cultivars (Misr1 and Misr2) and race specific genes (*Sr24*, *Sr25* and *Sr31*) assigned to Ug99 under influencing by local stem rust populations and to use

molecular markers linked to Ug99-race specific genes in order to determine their presence or absence in wheat cultivars tested.

2 Materials and methods

2.1 Plant materials

The wheat materials used in this study, including three Ug99-race specific genes, *Sr24*, *Sr25* and *Sr31*, and two wheat cultivars assigned to Ug99-race resistance in Egypt, Misr1 and Misr2, were provided by Wheat Disease Research Department, Plant Pathology Research Institute, Agricultural Research Centre of Egypt. Wheat cultivar "Morocco" served as the susceptible check in the plant reaction test (Nazari and Mafi, 2013) and as the negative check in the molecular marker analysis (Kokhmetova et al., 2011). The detailed description of these accessions are available in Table 1.

Table 1 Detailed description of the tested wheat materials including genotype, origin/pedigree along with chromosome location for race specific genes

Genotype	Origin/Pedigree	Chromosome location
Sr24 (Lc*Sr24*Ag)	Little Club/Agent (CI 13523)	3DL
Sr25	Agatha (CI 14048)/9*LMPG-6 DK16	*7DL*
Sr31	Seri-82	1BL.1RS
Misr1	CIMMYT advanced line/Oasis/SKauz//4 *Bcn/3/2 *Pastor	
Misr2	CIMMYT advanced line/Skauz/Bav92	
Morocco	Moroccan Land Variety	

2.2 Plant resistance evaluation

Seedling resistance of accessions was conducted in the greenhouse at Wheat Dis. Res. Dept., Sakha Agricultural Research Station, ARC, Egypt, by applying a mixture of *Pgt* populations, originated from the commercial wheat fields during two growing seasons. Urediniospores isolated from stem rust infected wheat collected from the commercial wheat fields were used in inoculation. Protocols for inoculum preparation, inoculation, incubation, and disease rating were as described by Stakman et al. (1962) and Nazari and Mafi (2013). Plants were then placed in a greenhouse at 18-22 ℃ with 16 h of supplementary light (7600 Lux) and 8 h dark. Seedling infection types (ITs) were recorded 14 days after inoculation using the 0-4 scale described by Stakman et al. (1962). In this scoring system, plants scored as infection types (ITs) 0, 0; (fleck), 1, 2, or combination thereof, are considered resistant, and plants scored as 3 or 4 are considered susceptible. For leaves exhibiting a combination of ITs, order indicated the predominant type; hence an IT 23 would have predominant IT 2 with decreasing amounts of IT 3. The plus (+) and minus (−) signs were used to indicate pustules larger or smaller, respectively, within each class. The letter C indicated that infected leaves had more chlorosis than normally observed. The letter N indicated leaf necrosis. The slash (/) symbol was used to indicate heterogeneity within an accession, with the predominant type listed first. Hence a 2/34 indicated that plants with IT 2 were predominant to plants with IT 34. For each accession–race, five to six primary leaves were scored.

Adult plant resistance was evaluated on the same set of materials at the Experimental Farm of Sakha Agricultural Research Station, ARC, Egypt, under influencing natural infection with *Pgt* populations during two growing seasons. All cultural practices recommended to be applied. The stem rust data of adult plant reaction were scored as plant response and rust severity combined together. Plant response was rated as resistant (R), moderately resistant (MR), moderately susceptible (MS), or susceptible (S) as described by Roelfs et al. (1992), and disease severity was assessed using the Modified Cobb's Scale according to Peterson et al. (1948). Virulence or avirulence key to race specific genes affected with Ug99 group identified until 2015 are shown in Table 2 cited by Singh et al. (2011, 2015).

Table 2 Virulence or avirulence key of Ug99 group to race specific genes

Race	Alias	Virulence (+) or Avirulence (-) Key				
		Sr31	*Sr21*	*Sr24*	*Sr36*	*SrTmp*
TTKSK	Ug99	+	+	-	-	
TTKSF		-	+	-	-	
TTKST	Ug99+*Sr24*	+	+	+	-	
TTTSK	Ug99+*Sr36*	+	+	-	+	
TTKSP		-	+	+	-	
PTKSK		+	-	-	-	
PTKST		+	-	+	-	
TTKTT		+		+		+
TTKTK		+				+
TTHSK		+				
PTKTK		+	-			+
TTHST		+		+		

Note: Race designation follows the North American nomenclature system described by Jin et al. (2008). Blank = uncertain.

2.3 DNA Marker analysis

Genomic DNA was extracted from leaf tissue (bulk of 3-5 seedlings per entry) following a cetyl trimethyl ammonium bromide (CTAB) protocol (Doyle and Doyle, 1987). DNA samples obtained from the tested wheat cultivars and lines were screened for polymerase chain reaction (PCR) using the dominant marker Gb for *Sr25* locus (Liu et al. 2010), the dominant STS marker *Sr24*#12 for *Sr24* locus (Mago et al., 2005) and the dominant STS marker Iag95 for *Sr31* locus (Mago et al., 2002). The PCR Master Mix (Dream Taq Green PCR Master Mix (2X), Thermo Scientific) was used for PCR reaction, containing PCR reagents (dNTPs 0.4 mM each, 2X Taq DNA polymerase and 4 mM MgCl2). A total volume of 25 µL PCR reaction contained 12.5 µL of Master Mix., 0.8 µL of forward/reverse primers, 2 µL of DNA template and 9.7 µL sterile distilled water. The PCR conditions (Techne, PROGENE Thermocycler) for the used primers sets were optimized in initial studies of Liu et al. (2010) for *Sr25*, and Mago et al. (2005) for each of *Sr24* and *Sr31*. Amplification products of PCR (10 µL each sample) along with DNA ladder (100 bp DNA ladder H3 RTU, Nippon Genetics Europe GmbH) were electrophoreses at 100 V for about 20 min on 1.5% agarose gel stained with ethidium bromide. The DNA banding patterns were visualized using UV-transilluminator (Herolab UVT 2020, Kurzwellig) and photographed. The obtained bands were scored and used to indicate the presence/absence of the tested race specific genes.

3 Results

3.1 Phenotypic reaction

Stem rust resistance of the tested wheat genotypes presented in Table 3 indicated that *Sr24* (Little Club), *Sr31* (Seri-82) exhibited seedling and adult plant resistance rating; C1, 10MR; 1N-, TrR and 1-N, TrR, respectively while the wheat cultivars, Misr1 and Misr2 exhibited susceptibility with 40S and 10S, respectively. The *Sr25* gene (Agatha) showed seedling resistance (rated 2-C) while was susceptible at adult plant stage (20MS).

3.2 Genotypic reaction

Three DNA markers were used to determine the presence/absence of Ug99-race specific genes (*Sr24*, *Sr25*, and *Sr31*) in wheat cultivars (Misr1 and Misr2). The dominant STS marker *Sr24*#12 was used to detect stem rust resistance gene *Sr24*. This marker amplified a 500-bp fragment only in the positive control 'Lc*Sr24*Ag'. All other genotypes, including negative control 'Morocco', did not yield any band associated with *Sr24*, indicating the absence of this gene (Figure 1 and Table 3). The presence/absence of *Sr25* was determined using marker Gb. This marker produced a 130-bp fragment in the positive control along with Misr1 and Misr2, indicating the presence of this gene. The negative control did not produce fragment, indicating the absence of this gene (Figure 2 and Table 3). Dominant marker Iag95 amplified the expected 1100-bp band in the positive control 'Seri-82', suggesting the presence of *Sr31* gene. Wheat cultivars, Misr1 and Misr2 along with the negative control 'Morocco' did not amplify the 1100-bp band, indicating the absence of *Sr31* (Figure 3 and Table 3).

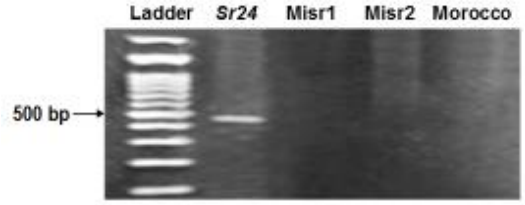

Figure 1 PCR amplification of 500 bp fragment for STS marker Sr24#12 only in the *Sr24*-positive line, while no PCR products was obtained in Misr1, Misr2 and negative check "Morocco" that lack *Sr24*

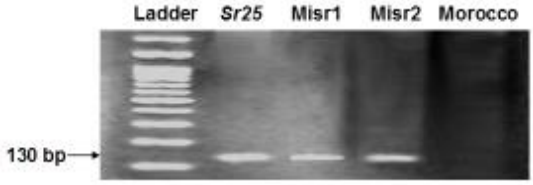

Figure 2 PCR amplification of 130 bp fragment for marker Gb in the *Sr25*-positive line along with Misr1 and Misr2, while no PCR product was obtained in negative check "Morocco" that lack *Sr25*

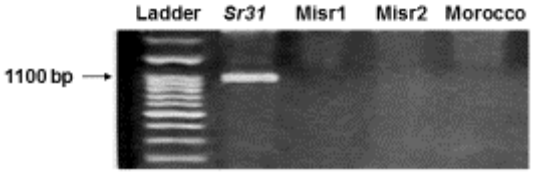

Figure 3 PCR amplification of 1100 bp fragment for STS marker Iag95 in the *Sr31*-positive line (Seri-82), while no PCR products were obtained in Misr1, Misr2 and negative check "Morocco" that lack *Sr31*

Table 3 Phenotypic and genotypic reactions of wheat genotypes refer seedling and adult plant resistance along with presence or absence of race specific genes

Genotype	Phenotypic reaction		Genotypic reaction		
	Seedling	Adult plant	*Sr24*#12	Gb	Iag95
Little Club	;C1	10 MR	*Sr24*		
Agatha	2-C	20 MS		*Sr25*	
Seri-82	;1N-	Tr R			*Sr31*
Misr1	3+	40 S	None	*Sr25*	None
Misr2	23-	10 S	None	*Sr25*	None
Morocco	4+	70 S	None	None	None

Note: ITs ((fleck), 1, 2) resistance, (3, 4) susceptible, (23) predominant 2 decreasing 3. (+) pustules larger, (−) pustules smaller, (C) chlorosis, (N) necrosis. (R) Resistance, (MR) Moderately Resistance, (S) Susceptible, (MS) Moderately Susceptible. (Blank) excluded.

4 Discussion

Global wheat production is threatened by the evolution of new races of *P. graminis* f. sp. *tritici* (*Pgt*) in North Africa and their migration to other parts of the world. The new races have broken down the resistance of widely deployed stem rust resistance genes. Development of resistant wheat varieties is one way of coping with this threat. The present study was conducted to investigate seedling and adult plant resistance of three Ug99-race specific genes and assigned cultivars along with their molecular detection to make sure the presence/absence of these genes and their effectiveness. The broad virulence spectrum present in Ug99 and its derivatives has been implicated with partial to high susceptibility of numerous important wheat varieties sown on over 80% to 95% of total wheat area, as well as breeding materials, regardless of the originating countries (Singh et al., 2008, 2011). Virulences to resistance genes *Sr24*, *Sr31*, *Sr36*, and *Sr38* were considered the most significant because these genes were providing resistance to other predominant *Pgt* races and were present at a relatively high frequency in adapted wheat backgrounds. In the present study, phenotypic reaction reveled seedling and adult plant resistance to stem rust for *Sr24* (Little Club) and *Sr31* (Seri-82) while wheat cultivars Misr1 and Misr2 assigned to Ug99 resistance were susceptible. It is worthy to notice that virulence to *Sr25* (Agatha) were detected in the current study as a first report. Jain et al. (2009) detected virulence to *Sr25* in India, this gene is effective against race Ug99 (TTKSK).

Development of successful commercial cultivars in the future will demonstrate their commercial utility (Singh et al., 2015). Phenotyping data combined with pedigree and linked molecular marker (if available) information are useful in postulating race-specific resistance genes in wheat lines distributed internationally (Singh et al. 2015). Babiker et al. (2016**)** located the TTKSK locus on chromosome 6DS to the same location as *Sr42* based on seedling phenotyping, bulk segregant analyses (BSA) and genotyping a SNP marker linked to race-specific *Sr* genes. This gene are associated with inconsistent seedling reactions and disease severities ranging from low to intermediate on wheat lines carrying them, indicating that their expression is enhanced in the presence of APR genes. This complicates their precise phenotyping in mapping populations; however, their combinations with APR genes could be developed by selecting plants with lower disease severities and reduced infection types. APR genes enhance the expression of moderately effective race-specific resistance genes such a *SrTmp* (Singh et al. 2011). Hiebert et al. (2011) reported the enhancement of field resistance conferred by *SrCad* in the presence of pleiotropic APR gene *Sr57*/*Lr34* in Canadian AC Cadillac wheat. Therefore, it is recommended that molecular marker-assisted incorporation of race-specific resistance genes should be done in conjunction with seedling and adult-based phenotyping.

Race specific gene *Sr24* (second highly utilized stem rust resistance gene after *Sr31* throughout the world) confers resistance to most races of stem rust, including the virulent race Ug99 (TTKSK) now established in East Africa and Ethiopia. *Sr24* is not effective against a recent variant of Ug99, designated TTKST. Incidents of virulence to this major resistance gene has been reported in South Africa (Le Roux and Rijkenberg, 1987) and India (Bhardwaj et al., 1990). From their examination of the rust-resistant hexaploid wheat Agent, Smith et al. (1968) first determined *Sr24* to reside on the 3DL chromosome, within a spontaneous translocation from the 3Ag chromosome of *Agropyron elongatum*. In 1973, Sears developed more recombinant lines, successfully introducing a much smaller *A. elongatum* translocation

segment containing *Sr24* into the 3DL chromosome. This truncated segment broke the linkage between *Sr24* and red grain color observed in Agent, allowing the subsequent introgression of *Sr24* into white-grained wheats. *Sr24* was also discovered in the wheat variety Amigo, located in an *A. elongatum* segment within a translocation derived from rye. In Amigo-type resistance varieties, *Sr24* is found on 1BS rather than 3DL (1BL.1BS-3Ae#1 translocation). There are different molecular markers available for *Sr24*, including an SSR (BARC71) and two STS (*Sr24*#12 and *Sr24*#50) (Mago et al., 2005). The *Sr24*#12 marker has been found completely linked to *Sr24*. The *Sr24*#50 marker failed to predict the presence of *Sr24* in some germplasm. In the current study, we used the dominant STS marker *Sr24*#12 to detect stem rust resistance gene *Sr24*. Results showed absence of this gene in wheat cultivars Misr1 and Misr2, so deployment of this gene in these cultivars should be encouraged. This will provide resistance to other prevalent *Pgt* races and may provide residual resistance to its variants in agreement with Knott (2008). Moreover, *Sr24* gene is also useful due to its linkage with *Lr24*. Validation of this marker (*Sr24*#12) have also been achieved by Kokhmetova et al. (2011) and Xu et al. (2017)

Sr25 is a race specific effective gene against Ug99 **(**Jin et al., 2007; Singh et al., 2015; Li et al., 2016). *Sr25* was transferred into wheat from *Thinopyrum ponticum* Barkworth and Dewey that was translocated onto the long arm of wheat chromosomes 7D (Friebe et al., 1994). We used STS marker Gb to detect *Sr25* gene at 130-bp fragments. This marker was validated by Liu et al. (2010) and Xu et al. (2017). Liu et al. (2010) also tested a co-dominant marker BF145935 for *Sr25*, which showed 198- and 180-bp fragments in *Sr25*-positive varieties, and 202- and 180-bp bands in *Sr25* non-carriers. We preferred using Gb, as the 4-bp difference resulting from BF145935 was relatively difficult to resolve on agarose gel. Our results showed presence of *Sr25* in wheat cultivars Misr1 and Misr2. This gene has been widely exploited in Australian and CIMMYT germplasm (Bariana et al., 2007) and needs to be incorporated into other local wheat varieties so as to broaden their genetic base against the various Ug99 races.

Before the emergence of Ug99, stem rust resistance was maintained mainly by *Sr31* in most of the countries around the world except Australia (Singh et al., 2008). *Sr31* located on translocation 1BL.1RS from rye (*Secale cereale*) (Pretorius et al., 2000) and is present in many European wheats and some Chinese and USA wheats as well as being widely used in wheats distributed by the CIMMYT program (e.g. Bobwhite and Veery selections) and continues to occur at high frequencies in CIMMYT breeding populations. We used STS marker Iag95 (Mago et al., 2002) to assay wheat cultivars Misr1 and Misr2 for this gene and was found to be absent in both cultivars. Marker Iag95 also has been validated by Pretorius et al., (2012) and Xu et al. (2017). Shamanin et al. (2016) found that the genetic basis of a set of 146 spring wheat varieties and breeding lines was limited to *Sr25*, *Sr31*, *Sr36*, *Sr6Ai*, *Sr6Ai*#2, and some unknown major genes depending on phenotyping and genotyping.

The present findings revealed that wheat cultivars Misr1 and Misr2 assigned from CIMMYT for Ug99 resistance were susceptible to local stem rust *Pgt* races. Ug99-race specific resistance gene *Sr25* was present in both cultivars, indicating that *Sr25* is ineffective against local stem rust races. *Sr24* and *Sr31* were absent in both cultivars. Moreover, susceptible genes can still provide resistance along with effective genes, a phenomenon known as ghost or residual resistance (Knott, 2008). So other stem rust resistance genes need to be incorporated into these cultivars. We suggest that *Sr24* having seedling and adult plant resistance under influencing local *Pgt* races is suitable to be incorporated into these cultivars. The obtained findings have been supported by Hailu et al. (2015) who reported that *Sr24* was an effective gene conferred resistance to *Pgt* races, TTTTH, TTKSK (Ug99), TKTTF (Digalu race), HKPPF and HKNTF, identified in the area. Unlike results by Khanzada (2008) showed that varieties Kiran-95, Tandojam-83, and 'Sarsabz-86' were susceptible to stem rust race (RRTTF) in Southern Pakistan and only Tandojam-83 showed presence of *Sr31*. Therefore, our results do not provide evidence that local *Pgt* race(s) carry virulence to *Sr31*, since the local races need to be repeatedly tested against

all stem rust resistance genes to know their virulence/avirulence patterns. Race TTTTF has complex virulence, but is not related to the Ug99 race group and it is avirulent on genes *Sr31*, *Sr24* and *Sr25*. Similar races have been detected in nearby regions (Sicily epidemic) and appear to be spreading rapidly (Alemayehu, 2017). Although, virulence to *Sr25* was detected. However, avirulence to *Sr24* likely being suitable gene to be incorporated into wheat genotypes for resistance to local *Pgt* population.

5 Conclusion

Wheat cultivars Misr1 and Misr2 are not prone to infection by Ug99 and its variants, as both carry the *Sr25* gene. Therefore, it is important to broaden the genetic base of stem rust resistance in future wheat varieties by pyramiding multiple stem rust resistance genes, especially those effective against local *Pgt* races along with Ug99 and its variants. Pyramiding several, major and minor, stem rust resistance genes into adapted varieties as opposed to breeding varieties with a single resistance gene is considered a more effective method to combat new races. Recent progress on molecular marker development and improved donor sources are accelerating the pyramiding and deployment of cultivars with more durable resistance to stem rust (Haile and Röder, 2013). Major issue is that various effective resistance genes are being tested in various countries to mitigate the immediate threat from *Pgt* races including Ug99. Should they not to be deployed until their combinations are developed is a viable option. Nevertheless, effectiveness of race-specific resistance genes in combinations has stimulated more areas of the world to use them more successfully in breeding program.

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