Identification of the Resistance Gene Sr2 in some Egyptian Wheat Hybrids

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ABSTRACT

Seven Egyptian wheat cultivars were crossed with Sr2 (the stem rust resistant gene), and their parents, F1, F2 crosses were tested to natural stem rust disease in the field of Gemmeiza Agricultural Station, ARC, during the 2019 to 2021 growing seasons. The Sr2 gene was completely resistant to stem rust, while the Egyptian cultivars showed different responses to the disease. Sakha-95 was completely resistant, Misl-1 and Misl-2 showed high susceptibility. Gemmeiza-12, Giza-171, and Sakha-94 showed moderate resistance (MR), while a trace of moderate susceptibility (Tr-MS) was recorded for the cultivar Misr-3. All the F1 crosses were resistant, indicating the dominance of resistance to stem rust disease. Chi-square goodness of fit was used to assess the independence of Sr2 resistance gene in the tested cultivars and F2 crosses. The crosses Sr2XGemmeiza-12, Sr2X Sakha-94, Sr2XSakha-95 and Sr2XGiza-171 have the expected segregation ratio of 15:1 for a duplicate gene. However, the crosses Sr2XMisl-1 and Sr2XMisl-3 fit a 3:1 ratio conditioned by one dominant gene. The resistance of the cross Sr2XMisl-2 fits complementary gene action (9:7). Three specific SSR markers (xgwm533, stms559tag, and stms598cag) were used to identify Sr2 in the Egyptian cultivars and their F1 populations. The three markers confirmed presence of Sr2 gene in the Egyptian cultivars and their F1 populations. However, the stms598cag marker could differentiate between the tested cultivars and their F1 populations by amplifying an additional band with a molecular weight of 200bp. Molecular analysis confirmed the results of chi-square test and could be serving in developing wheat programs.

Keywords: wheat, stem rust, segregating population

INTRODUCTION

Stem rust caused by *Puccinia graminis* f. sp. *tritici* is among the fungal diseases that significantly affect wheat production around the world (Bhavani et al., 2022; Guo et al., 2022). To combat stem rust, breeding for resistant cultivars remains the most efficient and reliable method to control this disease (Kumar et al., 2022b). There are different sources of resistance to stem rust, such as wheat wild relatives. *Triticum turgidum* (Yaroslav emmer) was transferred to the Hope cultivar (Mago et al., 2011). Slow rusting genes like Sr2, Sr55, Sr56, Sr57, and Sr58 confer resistance at the adult plant stage under high disease pressure (Herrera-Foessel et al., 2014; Singh et al., 2015; Kosgey et al., 2021). Previously, Sr2 was used as a parent in several breeding programs around the world and used frequently in CIMMYT breeding materials (Singh et al., 2011a). There are several sources for Sr2 gene, including Pavon, Arthur, Lancer, and Hope. When present alone, the Sr2 gene showed slow rusting under intense disease pressure, but when combined with other minor genes to form the “Sr2 complex,” it provides an appropriate level of resistance (Singh et al., 2011a; Singh et al., 2015). This gene tends to be non-specific and is currently effective against all isolates of *Puccinia graminis* f. sp. *tritici* throughout the global regions that grow wheat. In Egypt, Sr2 (Pavon76 cultivar) has shown stable adult-plant resistance (APR) to stem rust in the field from 2016–2020 seasons (El-Shamy et al., 2019; Elkot et al., 2020). Shahin et al. (2020) evaluated Sr2, Sr24, and Sr26 under field conditions during the 2014 and 2015 seasons in Kafr elsheikh, Sharqia, and Nubaria governorates and found that Sr2 was highly effective for resistance in both seasons. El-Shamy et al. (2019) found that Pavon showed complete resistance to stem rust under field conditions at recommended and late dates of cultivation. Molecular genetic analysis has been used for the evaluation of both major and minor genes in various crops. Moreover, in wheat, molecular markers linked to race-specific genes for rust resistance have been identified and utilized for marker-assisted selection (Suennaga et al., 2003; Javadi et al., 2021). Molecular markers have been developed for numerous stem rust resistance genes, including Sr2 (Spielemeyer et al., 2003; Hayden et al., 2004; Mago et al., 2011). The aims of this study are to detect gene actions of resistance against stem rust disease in seven crosses with Sr2 gene and to detect the presence of Sr2 gene in the seven Egyptian bread wheat cultivars using molecular marker technique.

MATERIALS AND METHODS

1. Plant materials

Seven of the recent Egyptian bread wheat cultivars i.e. Gemmeiza-12, Sakha-94, Sakha-95, Giza-171, Misr-2, Misr-3 and Shandweel-1 were crossed as females with the resistant cultivar Pavon76 carrying stem rust gene Sr2 as a male parent in 2018/ 2019 growing season at Gemmeiza Agricultural Station, ARC. The names and pedigree of tested materials were reported by El-Shamy et al. (2019). Seeds of Pavon (Sr2) stock were obtained from ICARDA, while the bread wheat cultivars derived from The National Wheat Dept., Crop Research Institute. In 2019/2020 growing season, the hybrid seeds were sown in 1row, 2m long, 40 cm apart and 20 cm within rows for each cross to have F1 seeds. In 2020/2021 growing season, the parents and F1 hybrid seeds

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were sown in 1 row, 2m long, 40 cm apart and 20 cm within rows while about 100 to 150 grains of F2 seeds of each cross were drilled in plots consisted of 6 rows, 7m. long, 20cm apart and 10 cm in-between seeds for evaluation to stem rust disease. The experiment was surrounded by border 1 m of a highly susceptible cultivar i.e. Morocco and left to natural stem rust infection. Fertilizer application followed recommended cultural practices 70 kg N and 100 kg P/fed.

2. Disease assessment

Disease severity was scored for the parents, F1 and F2 plants of each cross when Morocco plants showed maximum rust severity using the modified Cobb scale (Peterson et al., 1948) and the host response was classified as either 0 (immune), R (resistant), MR (moderately resistant), M (intermediate), MS (moderately susceptible) or S (susceptible) according to Roelfs (1992). The disease severity is multiplied by a constant value for each infection type of stem rust to calculate (ACI) according to (Saari and Wilcoxson, 1948).

3. Chi-square analysis

Chi-square analysis was used to evaluate the goodness of fit of observed segregation with expected ratios and to estimate the number of genes responsible for stem rust resistance of the F2 population (Snedecor and Cochran 1989).

4. Molecular study

DNA extraction

The total DNA of Sr2, wheat cultivars, and their F1 populations were extracted from 200 mg of 10 days seedlings. The extraction method was carried out according to Emara et al. (2016).

PCR amplification conditions

Three specific microsatellites (SSR) markers linked to Sr2 were used to detect its presence in the 7 bread wheat cultivars and their F1 population. The markers’ names, sequences, and annealing temperatures were listed in Table 1. The PCR was conducted following Hayden et al. (2004).

RESULTS AND DISCUSSION

Results
Evaluation at the adult stage

The Sr2 showed completely resistance to stem rust under field conditions at the adult stage during the years of the study (Fig.1). The Egyptian wheat cultivars showed varied reactions to stem rust disease under field conditions ranging from zero to susceptible reaction (30S). The wheat cultivars divided into two groups, the resistant group was Sakha-95(0), Gemmeiza-12(Tr- MR), Giza-171(Tr-MR), and Sakha-94 (5MR). While the susceptible group included Misr-3 (Tr-MS), Misr-2(20S), and Misr-1(30-S), respectively.

The Chi-square test

An analysis of adult plants resistance of F1 and F2 populations to stem rust disease is shown in Table (2). The results clear that all the F1 populations of the seven crosses showed adult plant resistance responses to natural infection of stem rust. The F2 populations showed segregation in its responses as follow. The crosses Sr2XGemmeiza-12, Sr2X Sakha-94, Sr2XSakha-95 and Sr2XGiza-171 showed 15R:1S ratio, which segregated in 150R:10S, 220R:20S, 200R:30S, and 230R:10S, respectively. However, F2 plants of the crosses Sr2XMisr-1 and Sr2XMisr-3 showed 3:1 ratio which segregated in 110R:30S and 160R:50S, respectively. The F2 plants of the cross Sr2XMisr-2 showed 9R:7S which segregated in 80R:60S.

The molecular detection of Sr2

The markers xgwm533, stm559tgag, and stm598cac were used to determine the presence of Sr2 in the Egyptian cultivars and the F1 population. Amplification with the xgwm533 primer developed for Sr2 produced two PCR amplicon with a size of 120 bp and 65 bp (Fig. 2). The amplified fragment with 120 bp was present in all of the tested cultivars and the F1 populations. While, the additional band with molecular weight from 65 bp appeared in all the Egyptian cultivars, and absent from their F1. Amplification with the stm598tgag marker developed for Sr2 produced a single PCR amplicon with a size about 80 bp in the Sr2 parent but in the Egyptian cultivars and their F1 hybrids with the molecular size 100 p (Fig. 3).

Amplification with the stm598tgag marker produced two amplicons with size from 61 to 67 bp in the Sr2, the Egyptian cultivars as parents and their F1 populations (Fig. 4). Another fragment was amplified with molecular weight 200bp in Misr-1, Misr-3 and Giza-171 and their F1 populations. Moreover, this band appears in Sakha-95 cultivar and absent from its F1 population. However, it was absent in Gemmeiza-12, Sakha-94, and Misr-2 cultivars and present in their F1 population (Table 3).
Table 2. Segregation of resistance to stem rust pathogen in the F2 populations derived from the cross Sr2 and 7 Egyptian wheat cultivars.

<table>
<thead>
<tr>
<th>Cross name</th>
<th>No. of tested plants</th>
<th>Response</th>
<th>Segregation</th>
<th>Expected ratio</th>
<th>X²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sr2XGemmeiza 12</td>
<td>F1 54, F2 160</td>
<td>R 38, S 16</td>
<td>10 150 10 15 1 0.00 1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sr2XSakha-94</td>
<td>F1 65, F2 230</td>
<td>R 44, S 21</td>
<td>20 220 20 15 1 1.778 0.1824</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sr2XSakha-95</td>
<td>F1 49, F2 230</td>
<td>R 40, S 9</td>
<td>30 200 30 15 1 18.116 0.0001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sr2XMisr-1</td>
<td>F1 52, F2 140</td>
<td>R 33, S 19</td>
<td>60 80 60 9 7 0.045 0.8314</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sr2XMisr-3</td>
<td>F1 47, F2 210</td>
<td>R 34, S 13</td>
<td>50 160 50 3 1 0.159 0.6903</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sr2XGiza-171</td>
<td>F1 50, F2 240</td>
<td>R 39, S 11</td>
<td>10 230 10 15 1 1.778 0.1824</td>
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</table>

R=Resistant, "I=Intermediate, "S=Susceptible.

Discussion

Wheat stem rust affects wheat production around the world as more than 90% of wheat cultivars have susceptible reactions to the Ug99 race group (Singh et al., 2011b). In Egypt, this pathogen causes 1.96 to 8.21% losses in the yield of most of the local wheat varieties (Shehab-Eldeen and Abou-Zeid, 2020). The deployment of diverse resistance genes especially from wild relatives is the most effective way to reduce the potential of stem rust. To date, 73 Sr genes/alleles have been identified and about 35 are effective against the Ug99 race lineages (Singh et al., 2015). Unfortunately, most of them are race specific and don't provide durable resistance to stem rust. On the other hand, adult plant resistance provides long-lasting durable resistance to multipathogenic resistance and is provided by some resistant genes including Sr2, Sr54, Sr55, and Sr57 (Kosgey et al., 2021). Of them, Sr2 has been successfully deployed in many breeding programs around the world like Australia, India, CIMMYT, and China due to its high yield and resistance to stem rust (McIntosh et al., 1995; Malik et al., 2013; Bhavani et al., 2014; Xu et al., 2017). In Egypt, Sr2 has been evaluated under field condition and provide high resistance to stem rust (El shamy et al., 2019; Shahin et al., 2020; Elkot al., 2016; El-Oraby et al., 2019). Also, Singh et al. (2006) reported that both Sonalika and Pavon 76 (Sr2) were resistant during field assessments in 2004 and 2005 in Kenya with a maximum disease score of 15MS. In our study Sr2 was crossed as a male parent with seven Egyptian cultivars, and their F1 and F2 were evaluated to stem rust under natural infection during the study. Sr2 and Sakha-95 were completely resistant to stem rust. Gemmeiza-12, Giza-171 and Sakha-94 showed moderate resistance ranged from traces to 5 MR. While cultivars Misr-3,
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Misr-2, and Misr-1 showed susceptible reaction ranged from traces to 30-S. The F1 of all crosses showed completely resistance to stem rust indicating that the resistance is dominant, but the F2 population showed segregation in their responses to the disease. A chi-square goodness-of-fit test was calculated for F2 crosses to determine the number of genes that control resistance to stem rust. The chi-square test on the observed segregation ratio was consistent with digenic Mendelian segregation for the crosses Sr/2XGemmeiza-12, Sr/2XSakha-94, Sr/2XSakha-95 and Sr/2XGiza-171 segregated in 15R:1S ratio, indicating that two dominant independent genes conditioning the resistance to stem rust in these crosses. However, the F2 plants of the crosses Sr/2XMisr-1 and Sr/2XMisr-3 segregated in 3:1 ratio indicating that the two crosses possess one (a single) dominant gene. The F2 plants of the cross Sr/2XMisr-2 showed 9R:7S which segregated in 160R:50S indicating that it possess a pair of dominant complementary genes. Nzuve et al. (2013) crossed five resistant wheat lines (KSL-2, 3, 5, 12 and 19) with the susceptible line Caucake stem rust to determine the stem rust resistance gene inheritance. Chi square test showed that the segregation in KSL-2 parent is conditioned by a single dominant gene of resistance. While, the stem rust inheritance in the parents KSL-5, KSL-19, KSL-12 and KSL-3 were conditioned by two genes. Previous studies conducted in Egypt showed that Sr2 provides resistance to stem rust under field conditions and the tested Egyptian cultivars contain Sr2 as confirmed by SSR markers (Elkot et al., 2020; Abu Aly et al., 2014). However, it couldn't differentiate between cultivars regardless of their response to stem rust-resistant and susceptible ones. In our study, three diagnostic-specific markers linked to Sr2 were used; gwm533, stm559tgag, and stm598cac to accurately determine the presence of the Sr2 gene in the Egyptian cultivars and their F1 population. The two additional markers stm559tgag and stm598cac were used in this study to overcome the drawbacks of the gwm533 marker. Hayden et al. (2004) stated that Sr2 gene closely linked microsatellite marker Xgwm533 and typically amplifies a 120-bp fragment from wheat lines known to carry Sr2. However, it couldn't discriminate between the Sr2 carrier and non-Sr2 carrier wheat lines. Also, Vishwakarma et al. (2019) suggested the existence of allelic homoplasies in Sr2 gene non-carriers varieties like the Aroona variety. The three tested markers indicated the presence of Sr2 in the tested cultivars and their crosses. The stm598cac marker is considered a diagnostic marker as it could differentiate between tested cultivars and their F1 populations by amplifying an additional band with a molecular weight of 200bp. This marker is considered as a diagnostic marker since it could make differences between the tested cultivars.

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CONCLUSION

Sr2 is a good source of resistance to stem rust since it confers resistance to stem rust. Moreover, the stm598cac marker is a diagnostic marker as it is the only marker that could differentiate between the tested cultivars and their F1 population by amplifying an additional band with a molecular weight of 200bp.

REFERENCES


عند تحديد عدة أنصاف من صنف الصدأ الأسود المستقلة في المحطة بحوث القمح في مركز بحوث أمراض القمح - المعهد بحوث المحاصيل الحقلية - مزارع الجزين، تم استخدام ثلاث من المعلمات الأولى والثانية لجرح صنف صدأ الساق في محطتي بحوث أمراض القمح - المعهد بحوث المحاصيل الحقلية - مزارع الجزين. تمت اختبار جميع أنصاف الصنف المصري والأسود المستقلة في جرحة الصدأ الأسود في محطة بحوث أمراض القمح - المعهد بحوث المحاصيل الحقلية - مزارع الجزين خلال الأشهر من يناير 2019 إلى بناءً على النتائج، تم اختيار أنصاف الصنف المصري المستقلة في محطة بحوث أمراض القمح - المعهد بحوث المحاصيل الحقلية - مزارع الجزين. هذه الأنصاف تم استخدام في التجارب اللاحقة لتحديد المقاومة للمصادر المصرية المستقلة.
