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# Genetic Analysis of Adult Plant Resistance Genes to Stem Rust in Some Egyptian Bread Wheat Cultivars

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**Adult** plant resistance (APR) to stem rust in seven Egyptian wheat cultivars *i.e.*, Giza 168, Misr 1, Misr 2, Sakha 94, Gemmeiza 12, Sids 13 and Sids 14 and five stem rust resistance genes *i.e.*, *Sr9e*, *Sr11*, *Sr13*, *Sr28* and *SrPl*, were characterized during 2014/2015, 2015/2016 and 2016/2017 growing seasons at Sids Research Station. Stem rust resistance in seven Egyptian wheat cultivars was experimentally measured using two epidemiological parameters; final rust severity (FRS %) and area under disease progress curve (AUDPC). All of these parameters were found to be lower in the partially resistant (PR) cultivars; Giza-168, Sids-14, Sakha-94 and Gemmeiza-12, rather than those in the highly susceptible or fast-rusting cultivars; Misr-1, Misr-2 and Sids-13 as well as the check variety; Morocco. The partially resistant (PR) cultivars were slightly affected by stem rust infection resulting in little or few grain yield loss (%), less than the highly susceptible or fast-rusting cultivars which were severely rusted and showed high loss in grain yield under field conditions. F1 plants resulted from the crosses between these cultivars and the five stem rust resistance genes were planted, during 2014/2015 growing season, to produce F2 seeds. The resulted F2 plants were tested under field conditions in 2016/2017 at adult stage against a mixture of *Puccinia graminis* f.sp. *tritici* races. Segregations of F2 plants indicated that the stem rust resistance gene; *Sr13* was identified in all the tested wheat cultivars, except Misr 2. While *SrPl* was detected in the three wheat cultivars *i.e.*, Sids 14, Sakha 94 and Gemmeiza 12, Also the three wheat cultivars; Giza 168, Sids 13 and Gemmeiza 12 proved to have the resistance gene *Sr9e*. The stem rust resistance gene *Sr28*; was found only in the two cultivars, Giza 168 and Sids 14, but *Sr11* was identified in three wheat cultivars *i.e.*, Giza 168, Sids 13 and Gemmeiza 12. A combination or pyramiding of multiple genes for stem rust resistance in one particular wheat cultivar prolonged their life expectancy and gives it the potentiality to be more durable.

**Keywords:** Wheat, Resistance genes, Stem rust, Adult-plant resistance, Genetic analysis.

Stem rust of wheat caused by *Puccinia graminis* Pers. f.sp. *tritici* Eriks. & E. Henn., is the most destructive disease of wheat in Egypt, and worldwide. Successful control of such disease over the last five decades, through the use of host genetic resistance or the release of new resistant cultivars, resulted in a sharp decline in an annual yield loss, and avoidance of severe, epidemics. Grain yield losses due to stem rust infection ranged from 5 to 8% in a slight disease attack. While, it reached up to

50% under severe infection (Anonymous, 2012). In other cases, yield losses reached to 100% in the highly susceptible wheat cultivars during severe epidemic years.

The sudden evolution of new virulent races in a regional pathogenic population can be attributed, in a large extent, to the long-distance dispersal or migration from an external source. The occurrence of an aggressive pathogen race considered to be a great threat to the new released resistant cultivars. The emergency and rapidly spread of race TTKSK, in East Africa, commonly known as Ug99, is of high significance, as most wheat cultivars currently grown in it,s migration path, as well as in Egypt, are highly susceptible to this aggressive race, and the environment is conducive to the disease epidemics (Singh *et al.*, 2009 and Patpour *et al.*, 2014).

Developing adapted resistant cultivars, in a relatively short time, replacing the susceptible cultivars before rust migrates out of it's origin, proved to be an effective strategy to mitigate potential losses (Singh *et al.*, 2006). So, breeding strategy could be implemented to incorporate diverse resistance genes to such race and it's variants into wheat germplasm, perior to the further migration to other wheat growing areas (Christiansen *et al.*, 2002).

Adult plant resistance (APR) has often been considered as a polygenic resistance, but there is also an evidence that it is oligogenic type of resistance (Barcellos et al., 2000). The value of such resistance in protecting wheat cultivars against most of virulent stem rust races could be achieved by combining or pyramiding many genes of resistance in a single variety that conferred high level of generalized resistance against the target pathogen race. In this respect, Brennan (1975) stated that a breeding program should develop and release rust resistant cultivars, conditioning with resistance genes (both race- specific and race-non-specific resistance), exist in these wheat cultivars. The identification of genes conferring adult plant resistance to stem rust would be an initial and a significant step towards a good and an effective control for such disease (Abou-Zeid., 2013, Abou-Zeid *et al.*, 2015 and Abdalla *et al.*, 2015).

The present study was, therefore, aimed to evaluate and characterize more accurately adult plant resistance (APR) to stem rust in seven Egyptian bread wheat cultivars under artificial inoculation and natural infection in the field. In addition to identify some effective genes controlling stem rust disease in the tested wheat cultivars.

## Materials and Methods

*Field Studies:*

The experiments of the current study were carried out under field conditions, at Sids Agricultural Research Station during three successive growing seasons *i.e.*, 2014/15, 2015/16 and 2016/17. Seven Egyptian bread wheat cultivars *i.e.*, Giza 168, Misr 1, Misr 2, Sakha 94, Gemmeiza 12, Sids 13 and Sids 14, were crossed with the selected monogenic lines *i.e.*, *Sr 9e, Sr 11,.Sr 13, Sr 28* and *Sr Pl*. All wheat genotypes of the study were tested for their disease reaction to stem rust, at adult plant stage under field conditions.

The parental wheat cultivars and monogenic lines (*Sr,s*) were grown in plots (12 m2). During 2014/15 growing season each plot contained 10 rows of 4 m long and 30 cm apart. Monogenic lines under study were used as male parents for crosses with each of the tested wheat cultivars to obtain F1 seeds. The F1 seeds were grown in the following season (2015/16) in rows, 4 m long and 30 cm apart, in order to facilitate production of F2 seeds. In 2016/17 growing season, the two parents and F2 plants were also grown in plots, each plot of the parents contained six rows (4m long and 30 cm between rows). While each plot of the F2 plants contained eight rows 4 m long and seeds were planted 20 cm apart, each row therefore contained 25 F2 plants. All plots were surrounded by a spreader area sown with the highly susceptible wheat variety Morocco.

**Table 1. Pedigree and year of release for the seven wheat cultivars used in this study.**

| No. | Cultivars | Pedigree | Year of release |
|---|---|---|---|
| 1 | Sakha-94 | OPATA/RAYON//KAUZ. CMBW90Y3180-OTOPM-3Y-010M-010M-010Y-10M-015Y-0Y-0AP-0S. | 2004 |
| 2 | Giza 168 | MRL/BUC//SERI.CM93046-8M-0Y-0M-2Y-0B-0GZ. | 1999 |
| 3 | Gemmeiza12 | OTUS/3/SARA/THB//VEECMSS97Y00227S-5Y-010M-010Y-010M-2Y-1M-0Y-0GM | 2011 |
| 4 | Misr 1 | OASIS/SKAUZ//4*BCN1312*PASTOR.CMSSOOYO1881T-050M-030Y-030M-030WGY-33M-0Y-0S. | 2011 |
| 5 | Misr 2 | SKAUZ"S"BAV92. CMSS96M03611S-1M-010SY-010M-010SY-8M-0Y-0S. | 2011 |
| 6 | Sids 13 | AMAZ19=KAUZ"S"//TSI/SNB"S". ICW94-0375-4AP-2AP-030AP-0APS-3AP-0APS-050AP-0AP- 0SD. | 2010 |
| 7 | Sids 14 | Bow"s"/vee"s"// Bow"s"/TSI/3Babi Swef 1 SD239-1SD-2SD-4SD-Osd | 2014 |

**Table 2. Near isogenic lines (NIL's), genome location, source and tester line.**

| No. | Sr gene | Genome location | Original source | Tester |
|---|---|---|---|---|
| 1 | ***9e*** | 2BL | *T. turgidum* | Vernstein |
| 2 | ***11*** | 6BL | Isr H-Ra | ISrl1-Ra |
| 3 | ***13*** | 6AL | *T. turgidum (Kaphli emmer)* | W2691 Sr 13 |
| 4 | ***28*** | 2BL | *Marquis* | LC |
| 5 | ***PL*** | | *T. turgidum* | Peliss |

*Inoculation and disease assessment:*

Spreader plants of the highly susceptible wheat variety Morocco were artificially inoculated. They were sprayed with water and dusted with spores powder mixture of the most prevalent and aggressive stem rust races (one volume of fresh urediospores: 20 volumes of talcum powder). Dusting was carried out in the early evening (at sunset), before dew point formation. The inoculation of all spreader plants was carried out at booting stage, according to the method of Tervet and Cassell (1951). Data of stem rust severity (%) were recorded at the adult plant stage of the tested plant, using the modified Cobb,s scale (Peterson et al., 1948). Disease reaction expressed in wheat plants was recorded as five infection types (Roelfs et al., 1992). The infection type; very or highly resistance = (0), resistance = (R), moderately resistance = (MR), moderately susceptible = (MS) and susceptible = (S).

Stem rust severity (%) was recorded for each of the two parents and all wheat plants of F2 crosses, when the susceptible (check) variety; Morocco reached it's maximum and final rust severity (%), according to Das et al., (1993). The F2 plants of each cross were grouped into eight classes, depending on their percentage of disease severity (%) under field conditions. The disease severity classes were: 0-10; >10-20; >20-30; >30-40; >40 50; >50-60; >60-70 and >70-80 (Das et al., 1993). Plants grouped in the first three classes were considered as resistant genotypes, while plants of the other five classes (more than 30%), were considered as the susceptible genotypes (Singh and Huerta-Espino, 1995).

For identification of the adult stem rust resistance genes (Sr,s) in each cross under study, goodness of fit of the observed to the expected ratio of the phenotypic classes concerning the stem rust severity (%), was determined using Chi-squared ($\chi$ 2) analysis, according to Steel and Torrie (1960).

*Assessment of yield components:*

Plots were harvested and threshed, then grain yield per plot (kg) and 1000-kernel weight (g) were determined. Yield loss (%) was estimated as the difference among the protected (healthy) and infected (diseased) wheat plants, using simple equation adopted by Calpouzos *et al.* (1976) as follows:

Loss (%) = 1- yd / yh × 100

Where: Yd = yield of diseased plants.

Yh = yield of healthy plants.

*Statistical analysis:*

The epidemiological parameters and yield traits were subjected to an analysis of variance (ANOVA), which was done for each year separately. Then, a combined analysis of variance was done using the mean data of each year (Table 3). The least significant difference (LSD) at 5% level of significance was used to compare treatment means. All this computation was done as described by Gomez and Gomez 1984. This analysis was performed using SPSS 20 computer software.

## Results

Analysis of variance for the obtained data showed that mean squares (MS) of the tested wheat cultivars, as well as the susceptible (check) variety, Morocco, were highly significant for all the studied disease parameters and yield components, during the two growing seasons of the study. In addition, the interaction between years and these genotypes was highly significant (Table, 3). These results indicated that all the tested genotypes differed in their response to stem rust during the two years of the study.

**Table. 3. Combined analysis of variance for the studied traits during the two growing seasons, *i.e.* 2014/2015 and 2015/2016**

| S.O.V | d.f. | Mean sum of squares for | | | | | |
|---|---|---|---|---|---|---|---|
| | | FRS (%)[a] | AUDPC[b] | 1000-KW[c] (g) | | GY[d] (kg) | |
| | | | | Protected | Infected | Protected | Infected |
| **Replications** | 2 | 47.396 | 3264.583 | 16.8 | 44.37 | 0.201 | 0.096 |
| **Treatments** | 15 | 1837.743** | 469210.24** | 43.605** | 78.363** | 0.321** | 0.625** |
| **Genotypes (G)** | 7 | 3790.997** | 975376.12** | 86.538** | 166.77** | 0.583** | 1.304** |
| **Years (Y)** | 1 | 150.521** | 82917.188** | 37.577** | 1.181 | 0.587** | 0.163** |
| **G x Y** | 7 | 125.521** | 18229.092** | 1.533 | 0.981 | 0.022 | 0.011 |
| **Error** | 30 | 34.618 | 1270.139 | 2.116 | 3.926 | 0.018 | 0.011 |

** Significant at 0.01 probability level, a. (FRS) Final Rust severity, b. (AUDPC) Area under disease progress curve, c. (1000-KW) 1000-kernel weight, d. (GY) Grain yield.

*1. Characterization of adult plant resistance (APR) in the tested wheat cultivars:*

To gain more details on the variation of the expression of adult plant resistance (APR) to stem rust, and to characterize more accurately this type of resistance displayed on the tested wheat cultivars, the two epidemiological parameters i.e. FRS (%) and AUDPC, as a more reliable estimators were recorded for each of these cultivars during the two growing seasons of the study (Table, 4).

*a. Final rust severity (FRS %):*

Due to the slight changes in the environmental conditions from one year to another, stem rust epidemic was found to be more severe in it,s degree during the second growing season 2015/16 than that in the first season; 2014/15. Data in Table (4) reveal that stem rust severity (%) expressed on the tested wheat cultivars in 2015/2016 was higher than that in the previous season. All the tested wheat cultivars showed different percentages of final rust severity (%) in the first season (ranged between 13.33 and 90.00 %). Disease severity (%) was very high for the highly susceptible check variety Morocco (90.00 - 80.00%), as well as the tested wheat cultivar, Misr 2 (55.00 - 75.00 %). Whereas, the lowest percentages of final rust severity (%) were recorded on wheat cultivars showing the high levels of adult plant resistance to stem rust *i.e.*, Giza 168 (25.00 -28.00%), Sids 14 (13.33 -16.67%), Sakha 94 (21.97 - 15.00%) and Gemmeiza 12 (21.67 - 26.67%), in the two growing seasons, respectively (Table 4).

**Table. 4. Mean performance of the two stem rust parameters i.e. FRSa (%) and AUDPCb, and impact of disease incidence on grain yield per plot and 1000 kernel weight (g) of some Egyptian wheat cultivars, under field conditions at Sids Research Station during 2014/2015 and 2015/2016 growing seasons**

| Wheat cultivar | | Disease parameters | | Yield components & Losses (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | FRS (%) [a] | AUDPC [b] | 1000-KW [c] (g) | | Losses % | GY [d] (kg) | | Losses % |
| | | | | Protected | Infected | | Protected | Infected | |
| **2014/2015** | | | | | | | | | |
| **Giza 168** | | 25.00 | 240.00 | 39.27 | 37.83 | 4.33 | 2.45 | 2.20 | 14.04 |
| **Misr 1** | | 41.67 | 530.00 | 42.08 | 38.35 | 11.21 | 2.48 | 2.15 | 18.54 |
| **Misr 2** | | 55.00 | 853.33 | 42.57 | 38.44 | 12.41 | 2.59 | 2.15 | 24.72 |
| **Sids 13** | | 48.33 | 401.67 | 41.2 | 38.1 | 9.32 | 2.37 | 2.09 | 15.73 |
| **Sids 14** | | 13.33 | 180.00 | 44.78 | 43.27 | 4.54 | 2.63 | 2.44 | 10.67 |
| **Sakha 94** | | 21.67 | 236.67 | 39.96 | 38.62 | 4.03 | 2.32 | 2.30 | 1.12 |
| **Gemmeiza12** | | 21.67 | 260.00 | 41.14 | 39.38 | 5.29 | 2.50 | 2.36 | 7.87 |
| **Morocco (Check)** | | 80.00 | 1176.67 | 33.27 | 25.57 | 23.14 | 1.78 | 0.99 | 44.38 |
| **Mean** | | 38.33 | 484.79 | 40.53 | 37.45 | 9.28 | 2.39 | 2.09 | 17.13 |
| **2015/2016** | | | | | | | | | |
| **Giza 168** | | 28.00 | 280.33 | 37.83 | 36.54 | 4.40 | 2.22 | 2.00 | 16.92 |
| **Misr 1** | | 45.00 | 716.67 | 40.57 | 38.93 | 5.59 | 2.35 | 2.8 | 20.77 |
| **Misr 2** | | 75.00 | 906.67 | 40.53 | 35.94 | 15.65 | 2.32 | 1.99 | 25.38 |
| **Sids 13** | | 46.67 | 496.67 | 40.24 | 37.28 | 10.09 | 2.21 | 1.97 | 18.46 |
| **Sids 14** | | 16.67 | 200.00 | 43.58 | 42.21 | 4.67 | 2.43 | 2.29 | 10.77 |
| **Sakha 94** | | 15.00 | 200.00 | 37.63 | 36.61 | 3.48 | 2.22 | 2.18 | 3.08 |
| **Gemmeiza12** | | 26.67 | 256.67 | 40.35 | 39.33 | 3.48 | 2.34 | 2.12 | 16.92 |
| **Morocco (Check)** | | 90.00 | 1476.67 | 29.33 | 22.2 | 24.31 | 1.30 | 0.70 | 46.15 |
| **Mean** | | 42.87 | 566.71 | 38.76 | 36.13 | 8.96 | 2.17 | 2.01 | 19.81 |
| **LSD 0.05** | **(G)[e]** | 6.937 | 42.017 | 1.715 | 2.336 | - | 0.156 | 0.126 | - |
| | **(Y)[f]** | 3.468 | 21.008 | 0.857 | 1.168 | - | 0.078 | 0.063 | - |
| | **(GxY)[h]** | 9.81 | 59.42 | 2.425 | 3.304 | - | 0.221 | 0.179 | - |
| **Coefficient of Variation (CV%)** | | 15.65 | 6.88 | 3.67 | 5.33 | - | 5.81 | 5.25 | - |

a. (FRS) Final rust severity, b. (AUDPC) Area under disease progress curve, c. (1000-KW) 1000-kernel weight, d. (GY) Grain yield, e. (g) Genotypes, f. (Y) Year h. (GxY) Genotypes x Year *b. Area under disease progress curve (AUDPC):*

According to the obtained data in Table 4 and on the basis of AUDPC estimates, the wheat cultivars under study could be substantially classified into two main groups. The first group included wheat cultivars with the lowest AUDPC estimates (less than 300) *i.e.*, Giza 168, Sids 14, Sakha 94 and Gemmeiza 12, and "in the same time" they showed few or little grain yield loss (%). These cultivars were, therefore, designated or characterized as the partially resistant (PR) wheat cultivars to stem rust. In contrast, another group included the highly susceptible or fast-rusting cultivars; *i.e.*, Misr 1, Misr 2 and Sids 13, as well as the check variety; Morocco. Were they showed the highest AUDPC values, ranged from 380.00 to 1176.67, in the first season and from 323.33 to 1476.67, in the other season (Table 4).

*2. Assessment of yield components and losses (%) in grain yield:*

To measure the potentiality or capacity of the tested wheat cultivars to tolerate stem rust, two yield components *i.e.*, 1000 K.W. and GY/plot and their loss (%) due to artificial infection were estimated for these cultivars under field conditions (Table 4).

*a. Thousand kernel weight (1000-KW/ g):*

The 1000 kernel weight (g) was differed between protected (healthy wheat plant) and infected wheat plants of the tested wheat genotypes due to the differences in the level of disease severity (%) of stem rust (Table, 4). In 2014/2015, the highest loss (%) in 1000 kernel weight; ranged from 4.03 to 23.14 %, in the susceptible wheat cvs. Misr 2, Misr 1 and Sids 13 as well as the check variety Morocco. While the lowest losses (%) in 1000 K.W. during this season (not exceeded up to. 8.21%) were recorded in the wheat cvs. Sids 14, Sakha 94, Gemmeiza 12 and Giza 168, which showed high levels of APR, under field conditions, compared to the other genotypes. In the second season (2015/2016), the susceptible cvs. Misr 2, Misr 1, Sids 13 as well as the check variety Morocco showed the highest values of loss (%) in 1000 kernel weight (reached to 24.31%).

*b. Grain yield per plot (kg):*

Grain yield per plot (kg) was significantly differed between protected (healthy) and infected (diseased) wheat plants of the tested genotypes due to the differences in their levels of stem rust severity (%) (Table 4). In 2014/2015, the highest loss % in grain yield/plot, ranged from 1.12 to 44.38 %, in the three susceptible wheat cvs. Misr 2, Misr 1, Sids 13 and the check variety Morocco. While the lowest values of loss (%) in grain yield during this season (ranged from 1.12 to 14.04%) were recorded in the partially resistant wheat cvs. Sids 14, Sakha 94, Gemmeiza 12 and Giza 168. Where these wheat cultivars showed in general high levels of APR, under filed conditions, compared to the other tested genotypes. In the second season the susceptible cvs. Misr 2, Misr 1, Sids 13 and the check variety Morocco were severely affected by stem rust infection under field conditions as they showed the highest values of loss (%) of the grain yield (reached to 46.15%).

*3. Evaluation of stem rust resistance genes (Sr,s) at adult plant stage:*

Data in Table 5 indicate that the three stem rust resistance genes (*Sr,s*) *i.e., Sr13*, *Sr34* and *Sr36* proved to be the most effective genes, as they showed high levels of adult plant resistance during the two years of the study. They were completely resistant as they showed (5 R, 0 and 0 percentages, respectively). While, *SrPl* showed moderate resistance level (10 MR and 5 MR) to stem rust under field conditions during 2014/2015 and 2015/16 growing seasons, respectively. On the other hand, the other tested monogenic lines showed moderately susceptible (MS) to susceptible (S) reaction to stem rust infection ranged from 5 MS to 40 S (Table, 5).

**Table. 5. Response of the nine monogenic lines to stem rust under field conditions, during 2014/15 and 2015/16 growing seasons at Sids Research Station.**

| No. | ***Sr* genes** | **Diseases response/ Growing season** | | **Gene efficacy** |
|---|---|---|---|---|
| | | **2014/15** | **2015/16** | |
| 1 | *Sr 13* | 5 R | 5 R | R* |
| 2 | *Sr 34* | 0 | 0 | R* |
| 3 | *Sr 36* | 0 | 0 | R* |
| 4 | *Sr Pl* | 10 MR | 5 MR | MR** |
| 5 | *Sr 9e* | 5 MS | 10 MS | MS*** |
| 6 | *Sr 28* | 5 MS | 20 MS | MS*** |
| 7 | *Sr 11* | 10 S | 30 S | S**** |
| 8 | *Sr 17* | 30 S | 40 S | S**** |
| 9 | *Sr 9a* | 10 S | 20 S | S**** |

* R: Resistance, ** MR: Moderately resistance, ***MS Moderately susceptible, **** S: Susceptible

*4. Identification of adult plant resistance genes in the tested cultivars:*

In this part of the current study, five stem rust resistance genes (*Sr,s*) were identified in the tested wheat cultivars using a genetic analysis method, and the obtained results are presented in Tables (6 & 7).

*Sr 9e:*

All the resulted F2 plants *i.e.*, 177, 204, 193 and 210 from the crosses between this gene and the wheat cvs. Misr 1, Gemmeiza 12, Sids 13 and Sakha 94, respectively, showed resistance reaction to stem rust infection without any segregations. These results indicated that these wheat cultivars have an adult-plant resistance gene *Sr9e*. Whereas, F2 plants of the crosses between this gene and the other wheat cvs. Giza 168, Misr 2 and Sids 14 segregated to the ratios of 55 R:182 S, 16 R: 204 S and 11 R: 213 S, respectively, indicating that these cultivars do not have the resistance gene *Sr9e* (Table 6).

**Table. 6 Segregations and Chi square analysis of $F_2$ plants of the crosses between nine stem rust monogenic lines (*Sr's*) and seven bread wheat cultivars at adult plant stage under field conditions at Sids Research Station during 2016/17 growing season.**

| Cross | No. of $F_2$ plants | | Expected ratio | $\chi^2$ | *P* value | Grain yield (kg) |
|---|---|---|---|---|---|---|
| | Resistant (R) | Susceptible (S) | | | | |
| ***Sr 13*× Giza 168** | 197 | 0 | No segregation | - | - | 2.33 |
| ***Sr 13*× Misr 1** | 203 | 0 | No segregation | - | - | 2.2 |
| ***Sr 13*× Misr 2** | 199 | 59 | 3:1 | 0.625 | 0.429 | 1.9 |
| ***Sr13*× Gemmeiza 12** | 206 | 0 | No segregation | - | - | 1.92 |
| ***Sr 13*×Sids 13** | 195 | 0 | No segregation | - | - | 2.1 |
| ***Sr 13*× Sids 14** | 167 | 0 | No segregation | - | - | 2.3 |
| ***Sr 13*× Sakha 94** | 189 | 0 | No segregation | - | - | 2.1 |
| ***Sr 11*× Giza168** | 192 | 0 | No segregation | - | - | 2.4 |
| ***Sr 11*× Misr 1** | 155 | 49 | 3:1 | 0.105 | 0.746 | 2.3 |
| ***Sr 11*× Sids 14** | 85 | 89 | 1:1 | 0.092 | 0.762 | 1.89 |
| ***Sr 11* ×Sids 13** | 231 | 0 | No segregation | - | - | 1.91 |
| ***Sr 11*× Gemmeiza 12** | 198 | 0 | No segregation | - | - | 2.02 |
| ***Sr Pl*× Giza 168** | 16 | 197 | 1:15 | 0.579 | 0.447 | 1.8 |
| ***Sr Pl*× Misr 1** | 13 | 181 | 1:15 | 0.067 | 0.795 | 1.87 |
| ***Sr Pl*× Misr 2** | 34 | 172 | 3:13 | 0.682 | 0.409 | 1.93 |
| ***Sr Pl*×Gemmeiza 12** | 197 | 0 | No segregation | - | - | 1.92 |
| ***Sr Pl*× Sids 14** | 202 | 0 | No segregation | - | - | 1.92 |
| ***Sr Pl* × Sids 13** | 71 | 169 | 1:3 | 2.689 | 0.101 | 2.01 |
| ***Sr Pl* ×Sakha 94** | 242 | 0 | No segregation | - | - | 1.97 |
| ***Sr 9e*×Giza 168** | 55 | 182 | 1:3 | 0.406 | 0.524 | 1.72 |
| ***Sr 9e*× Misr 1** | 177 | 0 | No segregation | - | - | 1.73 |
| ***Sr 9e*× Misr 2** | 16 | 204 | 1:15 | 0.393 | 0.531 | 1.82 |
| ***Sr 9e*×Gemmeiza 12** | 204 | 0 | No segregation | - | - | 1.87 |
| ***Sr 9e*×Sids 14** | 11 | 213 | 1:15 | 0.686 | 0.408 | 1.93 |
| ***Sr 9e*× Sids 13** | 193 | 0 | No segregation | - | - | 1.94 |
| ***Sr 9e*×Sakha 94** | 210 | 0 | No segregation | - | - | 1.91 |
| ***Sr 28*× Giza 168** | 222 | 0 | No segregation | - | - | 2.01 |
| ***Sr 28*× Misr 1** | 198 | 15 | 15:1 | 0.228 | 0.633 | 2.03 |
| ***Sr 28*× Misr 2** | 215 | 67 | 3:1 | 0.232 | 0.630 | 2.14 |
| ***Sr 28*× Sids 14** | 123 | 0 | No segregation | - | - | 1.99 |
| ***Sr 28*× Sids 13** | 172 | 11 | 15:1 | 0.018 | 0.894 | 1.98 |
| ***Sr 28* ×Sakha 94** | 227 | 45 | 13:3 | 0.869 | 0.351 | 2.05 |

*P* values higher than 0.05 indicate non-significant of $\chi^2$.

*Sr 11:*

Data of the crosses between the five wheat cvs. Giza 168, Sids 14, Gemmeiza 12, Misr 1 and Sids 13 and the stem rust resistance gene *Sr11*, are presented in Table (6). All F2 plants *i.e.*, 192, 231 and 198 of the crosses between this gene and the three wheat cvs. Giza 168, Sids 13 and Gemmeiza 12 displayed low stem rust severity (%), and showed no segregation, indicating that these cultivars have the resistance gene *Sr11*. Whereas, F2 plants of the crosses between Sr 11 gene and the wheat cvs. Sids 14 segregated to an observed ratio 85 R: 89 S, which fitted the expected ratio 1 R: 1 S indicating that, this wheat cultivar does not have this gene and may carry different minor gene (s) which cause low disease severity (%). In addition, F2 plants of the crosses between this gene and wheat cv. Misr 1 segregated to the ratio of 155 R: 49 S, which fitted the theoretical ratio 3 R: 1 S, indicating that this cultivar does not have this stem rust resistance gene.

*Sr 13:*

Genetic analysis of the F2 crosses between the seven bread wheat cultivars under study *i.e.*, Giza 168, Misr 1, Gemmeiza 12, Misr 2, Sids 13, Sids 14 and Sakha 94 and the stem rust resistance gene Sr 13 was carried out and the obtained data are presented in Table (6). As indicated in this Table, all the resulted F2 plants *i.e.*, 197, 203, 206, 195, 167 and 189 from the crosses between this gene and the above-mentioned wheat cultivars, respectively, showed resistance response to stem rust with no segregations, which indicated the presence of *Sr13* in each of these six wheat cultivars. On the other hand, F2 plants of the cross between this gene and Misr 2 segregated to the observed ratio *i.e.*, 199 R: 59 S. This ratio fitted the expected ratio 3 R: 1 S, indicating that this cultivar does not have the resistance gene; *Sr13*.

*Sr28:*

Data in Table 6 reveall that a total of 222 and 123 F2 plants of the crosses between this gene and the two wheat cultivars; Giza 168 and Sids 14, respectively, displayed low stem rust severity (%), and all showed the resistance response to stem rust without any segregation. These results indicated that this gene is present in these two wheat cultivars. On the other hand, F2 plants of the crosses between *Sr28* gene and Misr 1, Misr 2 and Sakha 94 segregated to 198 R: 15S, 215 R: 67 S and 227 R: 45 S, respectively. These observed ratios fitted the expected ratios 15:1, 15:1 and 9:7, respectively. Based on these results it could be suggested that each of the three wheat cultivars Misr 1, Misr 2 and Sakha 94 do not have stem rust resistance gene; *Sr28*. Meanwhile, F2 plants of a cross between this gene and the wheat cv. Misr 2 segregated to the observed ratio; 215 R: 53 S, which fitted the theoretical ratio of 3 L: 1 H, indicating that this new cultivar does not have *Sr28* gene, and it may carry different minor gene(s) that cause low disease severity (%).

*SrPl:*

The obtained data in the same Table 6 reveal in general that all of the F2 plants resulted from the three crosses between wheat cvs. Gemmeiza 12, Sids 14 and Sakha 94, and stem rust resistance gene *Srl i.e.*, 197, 202 and 242, respectively, had low rust severity (%), also showed no segregation. These results indicated that each of the three cultivars have *SrPl* gene. Whereas, F2 plants of the crosses between this gene and the other wheat cultivars under study *i.e.*, Giza 168, Misr 1, Misr 2 and Sids 13, segregated to 16 R: 197 S, 13 R: 181 S, 34 R: 172 S and 71 R: 169 S, respectively. This result gave evidence to the absence of this gene in these four wheat cultivars.

For all of the wheat genotypes, grain yield (kg) per plot, as a good component to increase wheat production was estimated under field conditions. A wide variability for this character was found between the tested genotypes ranged from 2.33 Kg. in *Sr13* × Giza 168 to 1.72 Kg in *Sr9e* × Giza 168, with an average of 1.99 Kg in 2015/2016 season (Table 6). This result revealed a better opportunity for wheat breeders to select genotypes with high grain yield and have in the same time an adequate level of stem rust resistance, to develop new wheat cultivars resistant to such disease with high yield potentiality.

**Table. 7. Resistance genes for stem rust (*Sr's*) identified in the tested seven Egyptian wheat cultivars at adult -plant stage under filed conditions at Sids Research Station during 2016/17.**

| No. | Wheat cultivar | Stem rust resistance genes (*Sr's*) | | | | | Total* |
|---|---|---|---|---|---|---|---|
| | | *Sr9e* | *Sr11* | *Sr13* | *Sr28* | *SrPl* | |
| 1 | Giza 168 | - | + | + | + | - | 3 |
| 2 | Misr 1 | + | - | + | - | - | 2 |
| 3 | Misr 2 | - | abs | - | - | - | 0 |
| 4 | Sids 13 | + | + | + | - | - | 3 |
| 5 | Sids 14 | - | - | + | + | + | 3 |
| 6 | Sakha 94 | + | abs | + | - | + | 3 |
| 7 | Gemmeiza 12 | + | + | + | - | + | 4 |
| No. of genes & frequency (%) | | 4 (57%) | 3 (42%) | 6 (85%) | 2 (28%) | 3 (42%) | - |

* Total No. of genes detected in a particular cultivar.

Data in Table 7 summarize the obtained results and it can lead to the conclusion that adult plant resistance to stem rust in the partially resistant Egyptian wheat cultivars under study *i.e.*, Giza 168, Sids 14, Sakha 94 and Gemmeiza 12 were found to be effectively controlled by at least three genes for stem rust resistance. These genes are *Sr9e* (Sakha 94 and Gemmeiza 12), *Sr11* (Giza 168 and Gemmeiza 12 ), *Sr13* (Giza 168, Sids 14, Sakha 94 and Gemmeiza 12), *S 28* (Giza 168 and Sids 14) and *SrPl* (Sids 14, Sakha 94 and Gemmeiza 12).

## Discussion

Host-genetic resistance is, still, the most effective control method that widely applied, as a main breeding strategy to successfully face wheat rust diseases, especially stem rust.

As mentioned before in the previous reports, the genetic resistance in wheat plants could be divided into two main types *i.e.*, adult plant resistance (APR) and seedling resistance (Johnson, 1984 and Agenbag *et al.*, 2012.).
Adult plant resistance is a type of genetic resistance that conditioned by several numbers of minor genes with additive effects (polygenic resistance). It is, generally, quantitatively inherited, race-non-specific or general resistance. Thus it remains effective for a long period of time (many years) and hope to be long-lasting and more durable (Boorner *et al.*, 2000).

Meanwhile, seedling resistance, has been qualitatively inherited as a simple inherited trait, also it is defined as a monogenic resistance. Therefore, it is easily overcome or breakdown by the sudden emergency of new virulent races in the rust pathogen population (Johnson, 1981).

A combination or pyramiding of more than one stem rust resistance gene into individual cultivar resulted in a considerable success in reducing and minimizing the rate of evolution of new pathogen races, particularly in the situations where the pathogen does not reproduce sexually, as in the case of *Puccinia graminis* f.sp. *tritici* population in Egypt. Considerable arguments for the durability of cultivar resistnce with pyramided race-specific resistance genes have been previously reported (Kolmer *et al.*, 1991and Mundt, 1991).

The present study demonstrated in general that wheat cvs. Giza 168, Sakha 94, Gemmeiza 12 and Sids 14 showed high levels of APR to stem rust under field conditions in the two growing seasons, under study. This type of resistance was accurately characterized by the lowest percentages of FRS (%) and the least values of AUDPC, compared to the fast-rusting or the highly susceptible wheat cultivars, as well as the check variety Morocco.

A wide application of AUDPC as a more reliable estimator for characterization "more precise" PR that expressed in the tested wheat cultivars, rather than other epidemiological parameters, is mainly due to it’s enclosure of all factors that influence or affect the disease development (Pandey *et al.*, 1989). Estimating yield loss (%) caused by a disease is a prerequisite to develop an effective strategy for disease control, particularly through breeding for disease resistance (Simmonds, 1988). Resistance of any wheat genotypes to stem rust can be described as its capacity to decrease the amount of loss in grain yield, as a result of infection. The grain yield losses were estimated under field conditions in 2014/15 and 2015/16 growing seasons for all wheat cultivars under study, the loss (%) in the thousand kernel weight (1000-KW/ g) and grain yield /plot (Kg), were estimated for the seven wheat cultivars under investigation. The obtained results in this part of the study revealed, in general that the partially resistant cultivars Giza 168, Sids 14, Sakha 94 and Gemmeiza 12 were slightly affected by stem rust infection under field conditions. Therefore, they showed few or little grain yield loss (%) during the study. In contrast the fast-rusting cultivars and the check variety Morocco were severely affected by the disease infection, thus high amount of grain yield loss was estimated as a result of stem rust infection during the two years of the study; these results are in agreement with those reported by Ashmmawy *et al.* (2013).

To identify the gene (s) governing adult plant resistance to stem rust in the tested wheat cultivars; genetic analysis method was conducted. This experiment included crossing of the tested wheat cultivars with the five monogenic lines *i.e.*, *Sr9e*, *Sr11*, *Sr13*, *Sr28* and *SrPl*. The F2 plants were evaluated at adult plant stage under field conditions, and goodness of fit of the observed ratio to the expected ratio of the phenotypic classes were determined by Chi-square analysis according to Steel and Torrie (1960).

Stem rust resistance gene 9e that proved to be an effective gene against the aggressive race Ug99 was transferred from goat grass (*Aegilops speltoides*) to wheat cultivar Marquis (Kerber and Dyck, 1990). This gene also, showed high efficacy against almost all stem rust races prevalent, under field conditions in Egypt during the two-growing seasons of the study. In the current study, F2 plants of the crosses between this gene and the three wheat cvs. Gemmeiza 12, Sids 13 and Sakha 94, had low stem rust severity (%) and showed no segregation, indicating that these cultivars have the resistance gene *Sr9e*. Whereas, this gene is not detected in the wheat cvs. Giza 168, Misr 1, Misr 2 and Sids 14. Also, no segregation in F2 plants for the crosses between the three tested wheat cultivars *i.e.*, Giza 168, Sids 13 and Gemmeiza 12 and *Sr11*, which means the presence of this gene in these three cultivars. While, Misr 1 and Sids 14 did not have the resistance gene *Sr11*. In addition, hybridization process did not succeed between the two cvs. Misr 2 and Sakha 94 and *Sr11*. The third stem rust resistance gene *Sr13* is widely distributed with high frequency (85%) in all the tested wheat cultivars except Misr 2. In addition, this gene showed high efficacy against stem rust, as it displayed and exhibited high levels of adult plant resistance to stem rust infection under field conditions during the two years of the study. Therefore, it is considered as an important gene in Egypt and should be put into consideration in the national breeding program. *Sr13* gene was early translocated to hexaploid wheat in the 1920s from tetraploid emmer wheat cultivar ‘Yaroslav’. This gene confers only moderate levels of resistance when present alone (Hare and McIntosh, 1979 and Singh *et al.*, 2010). In the present study, six wheat cultivars *i.e.*, Giza 168, Misr 1, Gemmeiza 12, Sakha 94, Sids 13 and Sids 14 proved to have this gene. While, only one cultivar, Misr 2 don’t have the stem rust resistance gene *Sr13*.

On the other hand, stem rust resistance gene *Sr28* which has been translocated from *Triticum timopheevii* and was mapped on chromosome 2BS (Dyck, 1992), is closely linked with other stem rust resistance gene Sr 36 (Wu et al., 2008). Results of this study revealed the presence of Sr 28 in only two wheat cultivars *i.e.*, Giza 168 and Sids 14. Meanwhile, the other five cultivars under study *i.e.*, Misr 1, Misr 2 Sids 13, Sakha 94 and Gemmeiza 12 do not have the stem rust resistance gene *Sr28*. In addition, gene *SrPl* proved to be present in some US wheat cultivars and translocated from "Triumph" to these cultivars (Jin and Singh 2006). However, crosses between stem rust resistance gene *SrPl* and the wheat cvs; Gemmeiza 12, Sids 14 and Sakha 94, had low rust severity (%) and showed no segregation in the F2 plants. This result indicated that, the above three cultivars have the adult-plant resistance gene *SrPl*. Whereas, the crosses between this gene and the wheat cvs. Giza 168, Misr 1, Misr 2 and Sids 13 revealed that these cultivars do not have this gene. Similar results were previously obtained under the Egyptian conditions during the studies of Hassan (2006); Youssef *et al.* (2012); Abou-Zeid (2013) and Abou-Zeid *et al.* (2015).

In conclusion, adult plant resistance (APR) to stem rust in the Egyptian wheat cultivars under study are controlled by three or more of the tested genes, some of them are *Sr13*, *Sr11*, *Sr9e*, *Sr28* and *SrPl*. These genes are part of the Egyptian genetic pool, since it was expected to be found in the tested wheat cultivars. However, these five stem rust resistance genes are conditioning effective field resistance or adult plant resistance to stem rust, and should be incorporated into a particular wheat cultivar. to diverse and strength, stem rust resistance in this wheat cultivar.

The enhancement and/or increment the level of adult plant resistance (APR) to stem rust in the tested wheat cultivars depending to a large extent, on the number of genes involved. However, combining or pyramiding of more than one gene of stem rust resistance into individual cultivar often led to a considerable success in decreasing or minimizing the rate of new evolution of pathogen races within it's population, particularly in the situations where the pathogen does not reproduce sexually, as in the case of *Puccinia graminis* f.sp. *tritici* population in Egypt Considerable arguments for durability of cultivars with pyramided race-specific resistance genes have been already reported (Kolmer *et al.*, 1991and Mundt, 1991).

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# التحليل الوراثي وتعريف بعض جينات المقاومة لمرض صدأ الساق فى طور النبات البالغ فى بعض أصناف من قمح الخبز المصرية

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أجري هذا البحث لدراسة وتشخيص مقاومة النباتات البالغة (APR) لمرض صدأ الساق في سبعة أصناف من قمح الخبز المصرية، وهم جيزة ١٦٨، مصر ١، مصر ٢، سخا ٩٤، جميزة ١٢، سدس ١٣، سدس ١٤ وخمس جينات من جينات المقاومة لذلك المرض، وهي *(SrPl ، Sr28 ، Sr13 ، Sr11 ، Sr 9e)* خلال مواسم الزراعة ٢٠١٤-٢٠١٥ و ٢٠١٥-٢٠١٦ و ٢٠١٦-٢٠١٧ في محطة البحوث الزراعية بسدس .تم قياس وتشخيص المقاومة لمرض صدأ الساق في السبعة أصناف من القمح تحت الدراسة باستخدام اثنين من مقاييس المرض الوبائية وهما شدة الإصابة النهائية (FRS٪) والمساحة تحت منحنى تقدم المرض (AUDPC) وقد أوضحت النتائج المتحصل عليها من تلك الدراسة انخفاض قيم تلك المقاييس علي الأصناف التي تتمتع بالمقاومة الجزئية للمرض وهم (جيزة ١٦٨ ، سخا ٩٤ ، جميزة ١٢ و سدس ١٤) مقارنةً بالاصناف عالية القابلية للاصابة (الحساسة أو سريعة الصدأ) وهي (مصر ١ ، مصر ٢ و سدس ١٣) وأيضاً الصنف موركو(المستخدم كمقارنة). ومن ناحية أخري فقد تأثرت الأصناف التي تتمتع بالمقاومة الجزئية تأثراً طفيفاً بعدوى صدأ الساق مما أدي إلى تقليل الفقد في محصول الحبوب الناتج وذلك بمقارنتها بالاصناف عالية القابلية للاصابة (سريعة الاصابة بالصدأ) والتي قد تأثرت تاثراً شديداً بالاصابة المرضية مما أدى الي ظهور خسائر عالية في محصول الحبوب الناتج من تلك الأصناف تم زراعة نباتات الجيل ألاول (F1) الناتجة عن إجراء التهجين بين السبعة أصناف تحت الدراسة وكلا من الجينات الخمسة المسئولة عن المقاومة لمرض صدأ الساق خلال موس الزراعة ٢٠١٤/٢٠١٥ وذلك لانتاج حبوب الجيل الثاني (F2) وقد تم اختبار النباتات الجيل الثاني في طور البلوغ لخليط من سلالات الفطر المسبب للمرض تحت ظروف الحقل في موسم ٢٠١٦\٢٠١٧ وقد أوضحت الانعزلات الوراثية في نباتات هذا الجيل (F2) تعريف وجود جين المقاومة *Sr13* في جميع الاصناف محل الدراسة باستثناء مصر٢ بينما تم تحديد جين المقاومة *SrPl* في ثلاثة أصناف قمح وهي سخا ٩٤، جميزة ١٢ وسدس ١٤، وقد اتضح من الدراسة أيضاً أن أصناف القمح الثلاثة جيزة ١٦٨ وسدس ١٣ وجميزة ١٢يحتوي كلاً منها علي جين المقاومة *Sr 9e* كذلك فإن جين المقاومة *Sr 28* لم يثبت وجوده إلا في صنفين فقط من الأصناف المختبرة وهما جيزة ١٦٨ وسدس ١٤. ومن ناحية أخري فقد تم تعريف جين المقاومة *Sr11* في ثلاثة أصناف قمح وهم جيزة ١٦٨؛ سدس ١٣ وجميزة ١٢؛ ويتضح من تلك الدراسة أن تجميع (Pyramiding) العديد من الجينات المسئولة عن مقاومة مرض صدأ الساق في صنف محدد من أصناف القمح يؤدي الي إطالة العمر المتوقع من تلك المقاومة بذلك الصنف ويكسبه القدرة على المقاومة المستدامة "طويلة الأمـــد" لمرض صدأ الساق.