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Pyramiding Stripe Rust Resistant Genes *Yr5*, *Yr10* and *Yr15* in Sids 12 and Gemmeiza 11 Wheat Derived Lines

Hagras, A.A.[1], Kh.E. Ragab[1], S.A.M. Abdelkhalik[1]*, A.A. Shahin[2], Samar M.A. Esmail[2], W.A. Yosof[2], M.D. Sehsah[2], A.Y. Salah[3], N. M. Fouad[3], S.A. Tawkaz[3] and A. Hamwieh[3]

[1] *Wheat Research Department, Field Crops Research Institute (FCRI), Agricultural Research Center (ARC), Egypt*
[2] *Wheat Disease Research Department, Plant Pathology Research Institute, Agricultural Research Center, Egypt*
[3] *International Center for Agricultural Research in the Dry Areas (ICARDA), Egypt*

A TOTAL of 173 beard wheat lines with resistance to stripe rust (Yr) were created by crossing the highly susceptible cultivars Sids 12 and Gemmeiza 11 with the three monogenic lines carrying *Yr5*, *Yr10* and *Yr15* genes from 2016 through 2020. These lines were then evaluated at Sakha Agricultural Research Station in Egypt from 2021 to 2023. Stripe rust resistance at seedling and adult plant stages, molecular marker detection for the three Yr genes as well as agronomic characteristics for these lines were studied. The results showed that 126 lines were resistant at both seedling and adult plant stages. Out of them, 87 lines (69%) showed positive markers for one gene (*Yr5*, *Yr10* or *Yr15*), while 39 (31%) lines had positive markers for two genes combination of the three genes (*Yr5+Yr10*, *Yr5+Yr15* or *Yr10+Yr15*). Yield evaluation indicated that the grain yield improvement was 3% for Gemmeiza 11 and 25% for Sids 12 lines. Lines have only one Yr gene showed greater grain yield compared to lines having a combination of two Yr genes. Fifteen lines showed higher grain yield compared to Giza 171 check cultivar. These lines can be used as a novel source of *Yr5*, *Yr10* and *Yr15* for wheat breeding aiming to enhance stripe rust resistance. Additionally, they can be introduced to the national wheat yield trials for potential release as new cultivars.

Keywords: Wheat, Pyramiding Genes, Stripe Rust, MAS.

Introduction

As an imperative food crop, bread wheat (Triticum aestivum L.) feeds ~40% of the global population (Zhao et al. 2023). With the growing population, annual progress in yield improvement will hardly fulfil wheat production requirements by 2050 (Rasheed and Xia 2019). In addition, many challenges threaten the increase in wheat yield; therefore, modern cultivars need to improve biotic resistance to various diseases, including rust diseases (Xiao et al. 2022). The diseases of wheat, mainly the fungal diseases are crucial yield constraints in nearly all wheat-developing environments (McIntosh 1997). Among the three rust diseases, stripe or yellow rust of wheat as a result of Puccinia striiformis f. sp. tritici is a devastating foliar disease and is considered of immense importance in almost all the wheat growing parts of the world (Khan et al., 2012a; Singh et al., 2014). Stripe rust can cause yield losses ranging from 10% to 70%, with extreme conditions causing up to 100% (Bolat and Altay, 2007). Stripe rust continues to pose a threat to wheat cultivation worldwide (Sareen et al., 2012). In Egypt, the highest grain yield losses were recorded with wheat genotype Gemmeiza 11 (64.20%),

followed by genotype Misr 1 (62.38%), as well as Misr 2 (57.66%) and Sids 12 (50.89%) genotypes in the North Delta region, Egypt (Shahin et al., 2020). Stripe rust pathogen is serious due to mutation, rapid generation turnover, race development, and widespread spread, causing significant yield losses in major wheat producing countries (Wellings, 2011; Hu et al., 2020). Moreover, the use of fungicides is considered unfriendly to the environment. Therefore, breeding for resistance is the most effective and efficient management strategy because it does not increase the operating costs of the farmer and is environmentally safe (Yang and Liu 2004). Conventional breeding has played a critical role in wheat improvement in the last few decades. However, it was quite limited to manipulating more alleles of genes for further improvement (Rasheed and Xia 2019; Anderson 2007).

The identification and knowledge of the stripe rust resistance genes in commonly used parental germplasm and released cultivars is very important for utilizing them to control the rust to its full potential. Therefore, a long-term and economic strategy could be better to select for resistance by deploying rust resistance genes

efficiently in space and time (Ragab et al., 2020; Hagras et al., 2024). Various programs aimed at developing disease-resistant cultivars require diverse, well-characterized and efficient resistance genes. More than 70 major genes for resistance to stripe rust have been identified and assigned (Zhou et al., 2014; McIntosh et al., 2022). Genes expressed at the adult plant stage are of particular significance because cultivars with such genes show that resistance remains effective for longer periods (Rajaram, et al. 1988; Khan et al. 2012b). Selection of cultivars for interest genes based on molecular markers is referred to as marker-assisted selection (MAS). As a requisite breeding tool, MAS could make it faster and easier to select target traits in breeding for breeders (Kuchel et al., 2008).

To date, many genes or gene complexes conferring long-term linked or unlinked rust resistance have been identified and indicated. Molecular markers for these resistance genes have been intensively used in wheat breeding programs in Egypt and worldwide. Genes *Yr5*, *Yr10*, and *Yr15* are still effective against the most virulent pathotypes and are used to determine their presence in wheat cultivars (Ragab et al., 2020). Molecular tagging of the *Yr5* gene (STS7/STS8) (Dyck 1987); *Yr10* gene (Xpsp3000) (McIntosh 1992; Wang et al. 2002) and *Yr15* gene (Xbarc8) (Gerechter et al 1989) has facilitated the detection of these genes in isolated populations and germplasm collections worldwide (Murphy et al. 2009).

Pyramiding entails stacking multiple genes leading to the simultaneous expression of more than one gene in a variety to develop durable resistance expression (Malav and Chandrawat 2016). In comparison to traditional selection in plant breeding, MAS offers a number of advantages, including being more efficient with time, money, and goals (Song et al. 2023). MAS allows breeders to pyramid multiple disease genes in one cultivar, improving resistance durability. It enhances selection efficiency by allowing assays at early generations. (William et al., 2007; Miedaner and Korzun 2012). MAS enhances crop breeding efficiency by pyramiding multi-allelic traits, allowing selection for traits dependent on a single plant, pyramiding a few QTL/genes at a time, efficiently selecting recessive genes, selecting phenotypes in restricted field conditions, and decreasing replications while increasing selection intensity (Liu et al., 2007; Paux et al., 2012). The main objective of this investigation is to identify superior and adapted high yielding wheat germplasm carrying *Yr5*, *Yr10* and *Yr15* stripe rust resistance genes individually or in combinations to be evaluated in the national yield trials for release as new bread wheat cultivars.

Materials and Methods

This investigation was conducted on the Research Farm of Sakha Agricultural Research Station, Kafrelsheikh, Egypt from 2016 through 2023 wheat

seasons. The geographical position of the farm lies between 31° 5' N latitude and 30° 56' E longitude and 7 m above sea level, in the North Delta as a hot spot for stripe rust disease. In 2016/2017 wheat season, the two bread wheat cultivars Sids 12, Gemmeiza 11 and the three monogenic lines carrying the stripe rust resistance genes *Yr5*, *Yr10* and *Yr15* (Table 1) were used to produce simple crosses between the two cultivars and each of the three stripe rust monogenic lines. In 2017/2018 wheat season, double crosses combining two stripe rust resistance genes were made from F_1 crosses. In 2018/2019 wheat season, the F_2 simple and double crosses (500 plants per cross) in addition to the two cultivars and the three monogenic lines was evaluated in the field for stripe rust. Ten resistant plants were selected based on phenotypic from each double cross (these plants are expected to carry one or two resistant genes). The selected plants were planted in summer season (off-season, 2019, following bulk method) in order to speed up generation. In 2019/2020, F_3 simple and double were planted for stripe rust evaluation and generation advancement following pedigree method. In 2020/2021 wheat season, out of 3000 plants, only 236 lines were selected based on yellow rust resistance and yield data.

Agronomic performance evaluation

In 2021/2022 wheat season, the F_5 wheat lines were tested in the field in two separate experiments using an augmented design (each line planted in two rows, 3.5 m long and 30 cm apart where each plot area is 2.1 m2). The first experiment included 110 lines derived from Gemmeiza 11, and the second experiment included 126 lines derived from Sids 12, while Sakha 95, Giza 171, and Misr 3 were used as check cultivars. The selected lines based on grain yield and rust data, were re-evaluated in the next season.

In 2022/2023 wheat season, the F_6 wheat lines were evaluated in the field in large plots using alpha lattice design in two replicates; two trials for Gemmeiza 11 derived lines (containing 42 lines each in addition to Gemmeiza 11, Giza 171 and Misr 3 as check cultivars) and two trials for Sids 12 derived lines (containing 47 and 42 lines in addition to Sids 12, Giza 171 and Misr 3 as check cultivars). Each genotype was planted in six rows, 3.5 m long and 20 cm apart (plot area is 4.2m2). All the recommended agricultural practices for wheat cultivation in north delta was applied for the experiments at the proper time in both seasons. Plots of the two cultivars "Gemmeiza 11 and Sids 12" were chemically protected against rust. During all evaluation steps, plant materials were surrounded by wheat rust spreader containing a mixture of highly susceptible lines to ensure uniformity of yellow rust infection. The inoculation of spreader row plants was carried out at wheat booting stage according to the method

of Tervet & Cassel (1951). The collected data were days to 50% heading and physiological maturity, plant height, number of spikes per square meter, number of kernels per spike, 1000 kernels weight, grain yield per plot, harvest index and final yellow rust field response.

Statistical analysis has been conducted by R package version 0.1.6 (Aravind et al. 2023) and "GENSTAT" microcomputer program via analysis of variance using unbalanced designs, VSN International (2011). The means of genotypes were assessed using Duncan's multiple range test (Duncan 1955).

Table 1. Name, pedigree and source of plant materials used in this study.

Name	Pedigree	Origin
Gemmeiza 11	BOW"S"/KVZ"S"//7C/SER182/3 /GIZA168/SAKHA 61	Egypt
Sids 12	BUC//7C/ALD/5/MAYA74/ON//1160.147/3/BB/GLL/4/CHAT"S"/6/MAYA/VUL//CMH74A.630/4*SX	Egypt
Yr5 monogenic line	*Yr5*/6*Avocet S	CIMMYT [†]
Yr10 monogenic line	*Yr10*/6*Avocet S	CIMMYT
Yr15 monogenic line	*Yr15*/6*Avocet S	CIMMYT

[†]CIMMYT; Centro Internacional de Mejoramiento de Maiz y Trigo (International Maize and Wheat Improvement Center).

Stripe rust evaluation at seedling stage (greenhouse)

All plant materials were grown in plastic pots (10 cm in diameter) in the greenhouse at Sakha Agriculture Research Station. Each pot contained four entries clockwise in each corner. The method of inoculation was carried out as described by (Stakman et al., 1962). The inoculated plants were incubated in a dark dew chamber overnight at 10ºC and 95% relative humidity then moved to the benches in the greenhouse and maintained at 12ºC-15ºC and 95-100% relative humidity. Light intensity was adjusted at 7600 lux in a photoperiod of 16 hours light and 8 hours dark (Stubbs, 1988). After approximately two weeks from inoculation, infection types (IT's) on the plants (0 - 9) were scored as described by (McNeal et al., 1971). Plants with IT's of 0, 0;, 1, 2, 3, 4 and 5 were considered as resistant response, while IT's of 6, 7, 8 and 9 were considered as susceptible response.

Stripe rust evaluation at adult plant stage (field response)

Adult plant resistance was evaluated on the same set of materials in field experiments during 2022/2023 growing season. A mixture of the most virulent Pst pathotypes isolates was used to inoculate the plants in the field experiments. The inoculation was carried out at wheat booting stage according to the method of Tervet and Cassel 1951. Disease severity was assessed using the modified Cobb's Scale (Peterson et al., 1948) when the flag leaf reaction of the susceptible control rust severity reached 100S. Infection response was scored as resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible (S), as described by Roelfs et al., (1992).

Make assisted selection using *Yr5, Yr10* and *Yr15* markers

Molecular marker study was conducted at ICARDA laboratory, Giza, Egypt. For DNA extraction, three

fresh young leaves were collected and air-dried from each genotype in the field experiments of the 2022/2023 season. About 1 cm leaf sample for each of the three leaves were cut put into 1.5 ml Eppendorf tube. DNA extraction was performed using the CTAB method according to Doyle and Doyle (1990). Marker assisted selection (MAS) was applied on the samples using simple sequence repeats (SSRs) primers (Table 2). PCR amplifications were performed in a total volume of 25 μl of the reaction mixture comprising, 2.5 mM of MgCl2, 10 mM of dNTPs, 0.2 U of GoTaq® DNA Polymerase (GoTaq_Flexi, Promega, Madison, Wisconsin, USA). The PCR amplifications were performed in a thermal cycler (GeneAmp PCR system 9700, Applied Biosystems). The molecular markers used for MAS against stripe rust are *Yr5, Yr10, Yr15*. The PCR program for the *Yr10* (Xpsp3000) was conducted as follows: one cycle of 2 minutes at 95°C, 35 cycles of 45 seconds at 95°C, 45 seconds at 55°C and 72°C for 30 seconds, followed by a final extension cycle at 72°C for 10 minutes. The PCR program for the *Yr15* (xbarc8) was conducted as follows: one cycle of 2 minutes at 95°C, 35 cycles of 30 seconds at 95°C, 30 seconds at 57°C and 72°C for 30 seconds, followed by a final extension cycle at 72°C for 10 minutes. The PCR program for the *Yr5* was conducted using a touchdown program as follows: 10 cycles, -0.5 °C per cycle starting from 67°C and the remaining 25 cycles at 62°C.one cycle of 2 minutes at 95°C, 35 cycles of 30 seconds at 95°C, 30 seconds at 45°C and 72°C for 30 seconds, followed by a final extension cycle at 72°C for 7 minutes. The PCR product of the *Yr5* marker is CAPS (cleaved amplified polymorphic sequence), was subsequently digested with the Sau3AI enzyme (New England Biolabs, Cat no.: R0169S). The PCR products were separated on a 2.5% agarose gel (1X TAE running buffer) and stained with redsafe (intron, Cat no. 21141, Korea).

Table 2. SSR primer names, sequences and required annealing temperatures (Ta°C).

No.	Primer name	Sequence	Ta°C
1	Yr-10-F (Xpsp3000)	GCAGACCTGTGTCATTGGTC	55
2	Yr-10-R (Xpsp3000)	GATATAGTGGCAGCAGGATAC	55
3	Yr-15-gwm11-F	GCGGGAATCATGCATAGGAAAACAGAA	57
4	Yr-15-gwm11-R	GCGGGGGGCGAAACATACACATAAAAACA	57
5	*Yr5*_insertion_F	CTC ACG CAT TTG ACC ATA TAC AAC T	62
6	*Yr5*_insertion_R	TAT TGC ATA ACA TGG CCT CCA GT	62

Results

Preliminary evaluation in season 2021/2022

Analysis of variance for days to 50% heading, plant height, and grain yield and its components is presented in Tables 3. Regarding Gemmeiza 11 derived lines, the differences among treatments (ignoring blocks) and among treatment tests were significant for number of days to 50% heading, plant height, number of spikes per square meter however, the differences were insignificant for number of kernels per spike, 1000 kernels weight and grain yield (Table 3). For Sids 12 derived lines, the differences among treatments (ignoring blocks) and among treatment tests were significant for all studied traits except for grain yield in the treatment test (Table 3).

Frequency distribution of Gemmeiza 11 and Sids 12 derived lines comparing with the three check cultivars for the six studied traits is illustrated in Figures 1 and 2. Out of the 110 wheat lines derived from Gemmeiza 11, 78 lines were earlier heading than Giza 171 (the earliest check cultivar) where their number of days to 50% heading ranged from 100 – 86 days with maximum improvement percentage of 13% (Fig. 1). The same trend was obtained for Sids 12 derived lines where 86 lines were earlier heading than Giza 171 where their number of days to 50% heading ranged from 100 – 89 days with maximum improvement percentage of 11%. For plant height, 77 and 110 lines ranged from 121-103 cm and from 122-87 cm were shorter than Sakha 95 (the highest check cultivar) with maximum improvement percentage of 16% and 29% were obtained from Gemmeiza 11 and Sids 12 derived lines, respectively (Fig. 1). For number of spikes per square meter, 57 and 78 lines recorded higher number than the check cultivar Sakha 95 with maximum improvement percentage of 96% and 91% for Gemmeiza 11 and Sids 12 derived lines, respectively (Fig. 1). For number of kernels per spike, 39 and 79 lines ranged from 69-110 kernels and from 70-119 kernels were higher than Giza 171 cultivar with maximum improvement percentage of 59% and 70% were obtained from Gemmeiza 11 and Sids 12 derived lines, respectively (Fig. 2). Thirty-five and 34 lines ranged from 48-64 and from 48-59 1000 kernels weight higher than Misr 3 cultivar with

maximum improvement percentage of 36% and 23% were obtained from Gemmeiza 11 and Sids 12 derived lines, respectively (Fig. 2). For grain yield, 18 and 16 lines ranged from 1.660 to 1.920 kg/plot and from 1.660-1.870 kg/plot were higher than Sakha 95 cultivar with maximum improvement percentage of 16% and 13% were obtained from Gemmeiza 11 and Sids 12 derived lines, respectively (Fig. 2).

Evaluation in season 2022/2023

Grain yield and agronomic traits evaluation for Gemmeiza 11 derived lines

Analysis of variance according to alpha lattice design for the studied traits of Gemmeiza 11 derived lines in the first and second trials is presented in Table 4. It was noticed that mean square values for reps.block in some traits (number of spikes per square meter and days to 50% maturity in the first trial and spikes per square meter, 1000 kernels weight, kernels per spike, grain yield per plot and harvest index in the second trial) were lower than the mean squares values for residuals. In these cases, analysis of variance was applied following the randomized complete block design procedure. The analysis indicated significant differences among genotypes for all traits in both trials except for number of spikes per square meter in the second trial.

Gemmeiza 11 derived lines 1ˢᵗ trial (G1)

Means of the studied traits for Gemmeiza 11 derived lines, season 2022/2023 is presented in supplemented Table S1. For grain yield, 11 wheat lines recorded significantly higher grain yield per plot than the check (Giza 171). Out of the 11 lines, 3 lines were insignificantly higher and 8 were insignificantly lower than the highest check cultivar Misr 3. The highest grain yield improvement percentage over Gemmeiza 11 parent was 3%.

For biological yield, 18 wheat lines recorded significantly higher biological yield than the check (Giza 171). Out of the 18 lines, the line # 23 was insignificantly higher than the highest check cultivar Misr 3 where the improvement percentage reached 11% over Gemmeiza 11 parent.

**Table 3. Mean squares of the studied traits for Gemmeiza 11 and Sids 12 derived lines, season 2021/2022.**

Source of variance	d.f	DH†	PH	SM	KS	1000KW	GY
Gemmeiza 11							
Treatment (ignoring Blocks)	112	25.960 **	52.270 **	8861.15 **	127.800 ns	36.140 ns	0.030 ns
Treatment: Check	2	4.360 *	238.960 **	12997.91 *	82.950 ns	46.580 ns	0.320 **
Treatment: Test	109	25.760 **	48.840 **	8744.68 **	129.740 ns	34.150 ns	0.020 ns
Treatment: Test vs. Check	1	91.450 **	52.500 **	13283.51 ns	6.510 ns	231.92 *	0.100 *
Block (eliminating Treatments)	9	1.330 ns	5.110 ns	4974.95 ns	107.110 ns	19.570 ns	0.030 ns
Residuals	18	0.930	4.600	3240.19	75.720	29.270	0.020
Sids 12							
Treatment (ignoring Blocks)	128	31.570 **	62.200 **	21561.73 **	348.110 **	49.64 **	0.050 *
Treatment: Check	2	4.930 *	204.060 **	9383.85 ns	3.600 ns	2.440 ns	0.290 **
Treatment: Test	125	31.740 **	53.190 **	20829.79 **	343.460 **	49.12 **	0.040 ns
Treatment: Test vs. Check	1	64.250 **	904.220 **	137410.3 **	1618.79 **	209.03 **	0.230 **
Block (eliminating Treatments)	10	1.240 ns	3.580 ns	3551.80 ns	75.170 ns	2.930 ns	0.030 ns
Residuals	20	1.000	6.140	3137.41	51.590	7.740	0.020

† DH; days to 50% heading, PH; plant height, SM; number of spikes per square meter, KS; number of kernels per spike, 1000KW; 1000 kernels weight and GY; grain yield. ns P > 0.05; * P <= 0.05; ** P <= 0.01

Table 4. Mean squares of the studied traits for Gemmeiza 11 derived lines, season 2022/2023.

S.O.V	d.f (lattice)	d.f (RCBD)	DH†	DM	PH	SM	KS	BY	1000KW	GY	HI
First trial (G1)											
Reps	1	1	7.144	16.044	0.044	30372	27.89	0.8047	70.1*	0.0401	0.691
Reps. Blocks	8	-	56.727**	-	46.267**	-	89.91*	1.6951*	60.85**	0.2697*	21.088**
Genotypes	44	44	81.738**	21.69**	67.674**	13050*	110.84**	1.2828*	72.36**	0.405**	16.397**
Residual	36	44	1.929	2.363	7.447	7904	38.2	0.6625	17.2	0.1062	3.24
Total	89	89	46.874	-	40.629	-	78.65	1.0636	48.98	0.268	3.24
Second trial (G2)											
Reps	1	1	18.678**	3.211	1.11	7593	64.74	0.4694	59.88	0.1408	40.603**
Reps.Blocks	8	-	27.806**	13.461**	25.42	-	-	0.7762	-	-	-
Genotypes	44	44	57.183**	19.923**	86.81**	8708	134.67**	1.3055**	71.2**	0.2831**	12.925**
Residual	36	44	1.881	2.039	13.49	4683	53.7	0.5962	20.39	0.0873	3.961
Total	89	89	31.74	11.92	50.67	-	-	0.9616	-	-	-

†DH; days to 50% heading, DM; days to 50% maturity, PH; plant height, SM; number of spikes per square meter, KS; number of kernels per spike, 1000KW; 1000 kernels weight and GY; grain yield per plot. ns P > 0.05; * P <= 0.05; ** P <= 0.01.

Gemmeiza 11 derived lines   **Sids 12 derived lines**
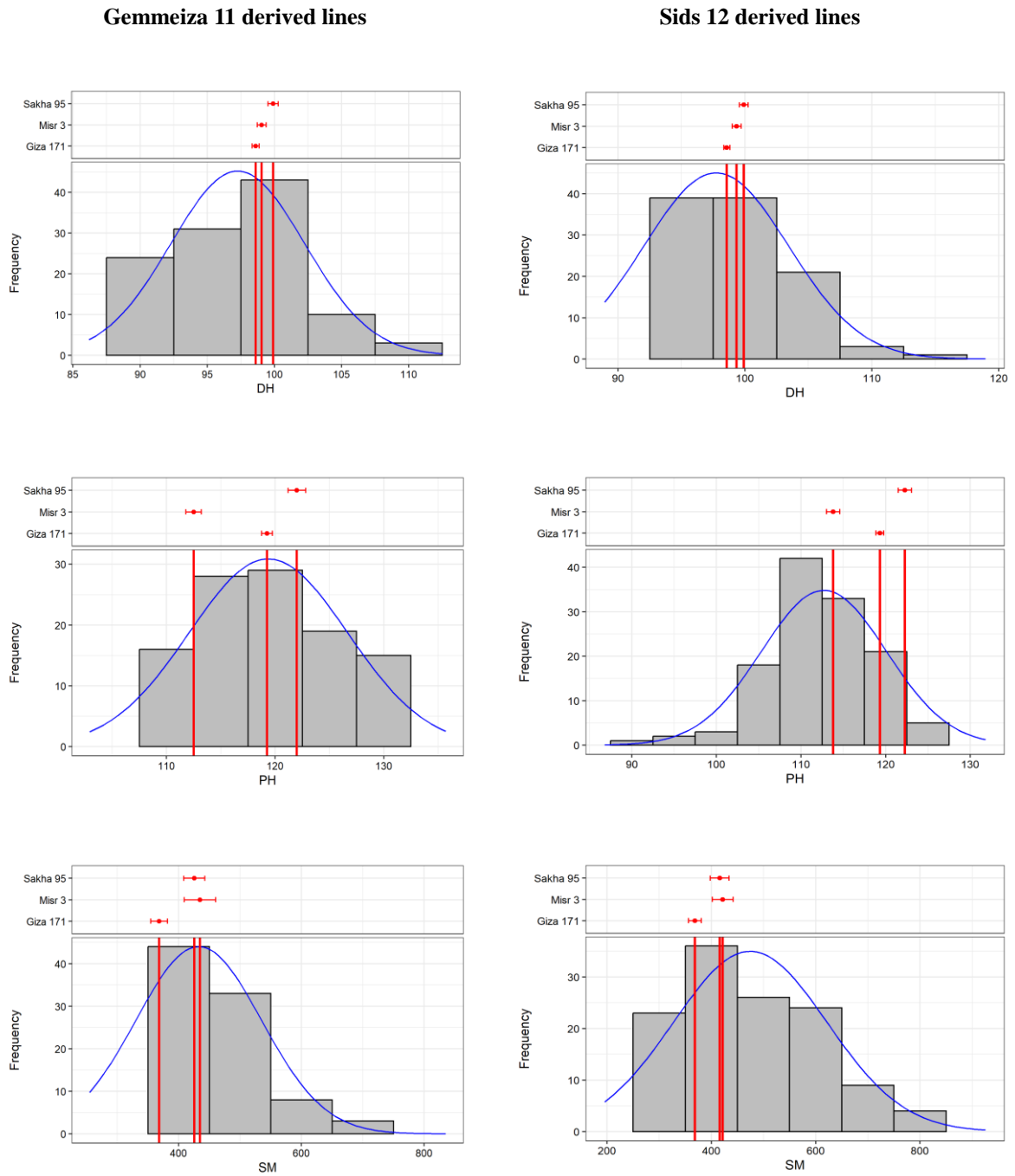
Fig. 1. Frequency distribution for number of wheat lines derived from both Gemmeiza 11 (left) and Sids 12 (right) for number of days to 50% heading (DH), plant height (PH) and number of spikes per square meter (SM) comparing to the three check cultivars Sakha 95, Misr 3 and Giza 171 during 2021/2022 wheat season.

Gemmeiza 11 derived lines   **Sids 12 derived lines**
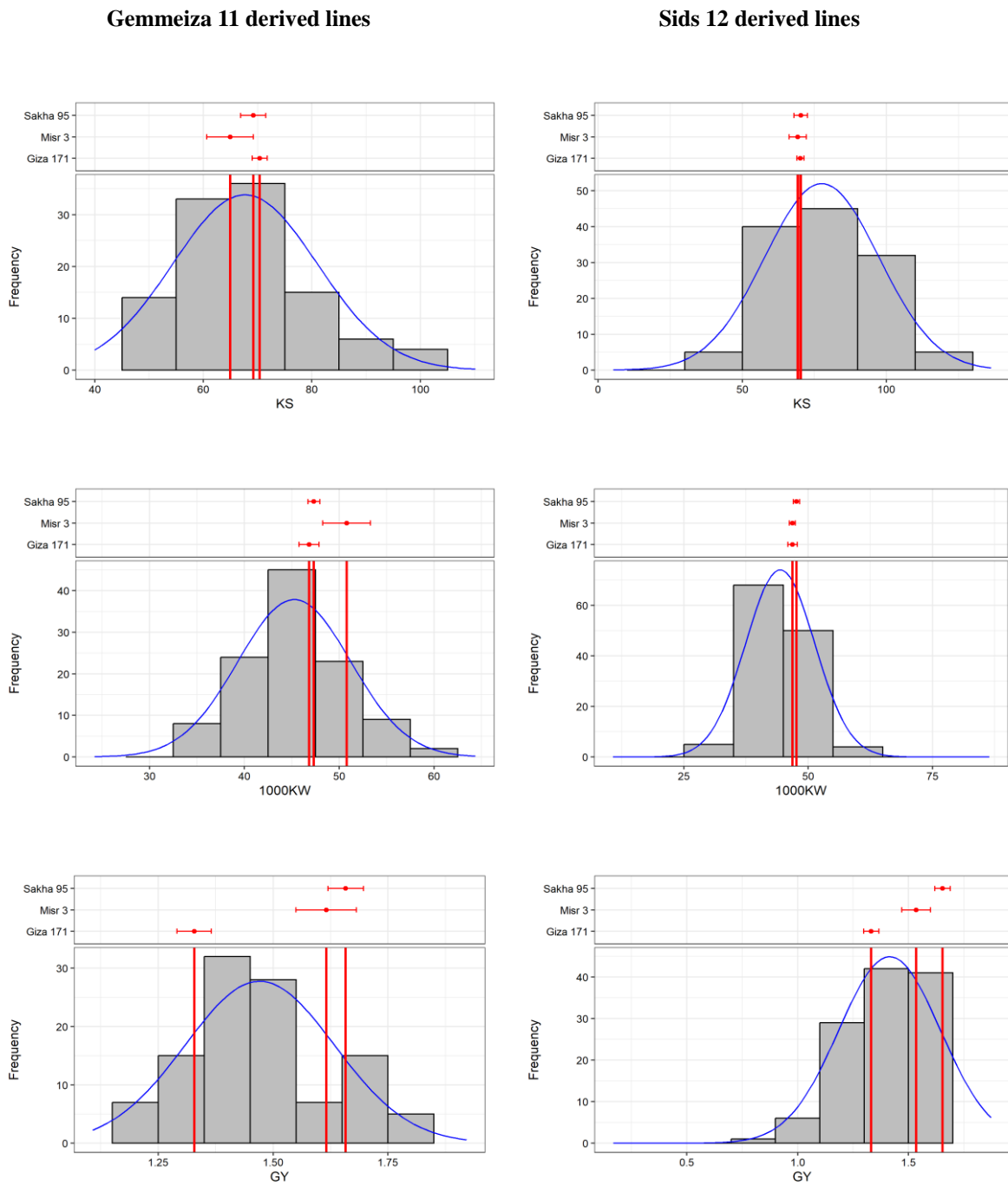
Fig. 2. Frequency distribution for number of wheat lines derived from both Gemmeiza 11 (left) and Sids 12 (right) for umber of kernels per spike (KS), 1000 kernels weight (1000KW) and grain yield (GY) comparing to the three check cultivars Sakha 95, Misr 3 and Giza 171 during 2021/2022 wheat season.

For harvest index, 13 wheat lines recorded significantly higher harvest index than the check (Misr 3). Out of the 13 lines, 7 lines were significantly higher than the highest check cultivar Giza 171 where the improvement percentage reached 3% over Gemmeiza 11 parent.

For number of spikes per square meter, 41 wheat lines recorded significantly higher number than the check (Giza 171). Out of the 41 lines, 7 lines were insignificantly higher than the highest check cultivar Misr 3 where the improvement percentage reached 90% over Gemmeiza 11 parent.

For number of kernels per spike, 20 wheat lines recorded significantly higher number of kernels than the check (Misr 3). Out of the 20 lines, 7 lines were insignificantly higher than the highest check cultivar Giza 171 where the improvement percentage reached 34% over Gemmeiza 11 parent.

For 1000 kernels weight, 21 wheat lines recorded significantly higher weight than the check (Misr 3). Out of the 21 lines, 6 lines were significantly higher than the highest check cultivar Giza 171.

For plant height, 24 wheat lines were significantly shorter than the check (Giza 171). Out of the 24 lines, 3 lines were significantly shorter than the shortest check cultivar Misr 3 where the improvement percentage reached 4% over Gemmeiza 11 parent.

For number of days to 50% heading, 22 wheat lines were significantly earlier than the check (Misr 3). Out of the 22 lines, 12 lines were significantly earlier than the earliest check cultivar Giza 171 where the improvement percentage of earliness reached 3.7% over Gemmeiza 11 parent.

For number of days to 50% maturity, 34 wheat lines were significantly earlier than the check (Misr 3). Out of the 34 lines, 17 lines were insignificantly earlier than the earliest check cultivar Giza 171 where the improvement percentage of earliness reached 3.1% over Gemmeiza 11 parent.

Gemmeiza 11 derived lines 2nd trial (G2)

Means of the studied traits for Gemmeiza 11 derived lines (second trial), season 2022/2023 are presented in supplemented Table S2. For grain yield, 23 wheat lines recorded high grain yield and did not differ significantly with the check (Giza 171). Out of the 23 lines, 14 lines were insignificantly higher and 9 lines were insignificantly lower than the highest check cultivar Misr 3. The highest grain yield improvement percentage over Gemmeiza 11 parent was 2.1%.

For biological yield, 11 wheat lines were significantly higher than the check (Giza 171). Out of the 11 lines, 6 lines were insignificantly higher than the highest check cultivar Misr 3 where the improvement percentage reached 10.2% over Gemmeiza 11 parent.

For harvest index, 30 wheat lines were significantly higher than the check (Misr 3). Out of the 30 lines, 20 lines were insignificantly higher than the highest check cultivar Giza 171 where the improvement percentage reached 4% over Gemmeiza 11 parent.

For number of spikes per square meter, 25 wheat lines were significantly higher than the check (Giza

171). Out of the 25 lines, 7 lines were insignificantly higher than the highest check cultivar Misr 3 where the improvement percentage reached 69.6% over Gemmeiza 11 parent.

For number of kernels per spike, 24 wheat lines were significantly higher than the check (Misr 3). Out of the 24 lines, 21 lines were insignificantly higher than the highest check cultivar Giza 171 where the improvement percentage reached 47.1% over Gemmeiza 11 parent.

For 1000 kernels weight, 23 wheat lines recorded significantly higher weight than the check (Misr 3). Out of the 23 lines, 21 lines were significantly higher than the highest check cultivar Giza 171 where the improvement percentage reached 3.4% over Gemmeiza 11 parent.

For plant height, 9 wheat lines were significantly shorter than the check (Giza 171). Out of the 9 lines, 4 lines were insignificantly shorter than the shortest check cultivar Misr 3 where the improvement percentage reached 2.1% over Gemmeiza 11 parent.

For number of days to 50% heading, 27 wheat lines were significantly earlier than the check (Misr 3). Out of the 27 lines, 17 lines were significantly earlier than the earliest check cultivar Giza 171 where the improvement percentage of earliness reached 4.3% over Gemmeiza 11 parent.

For number of days to 50% maturity, 30 wheat lines were significantly earlier than the check (Misr 3). Out of the 30 lines, 20 lines were significantly earlier than the earliest check cultivar Giza 171 where the improvement percentage of earliness reached 4.2% over Gemmeiza 11 parent.

Grain yield and agronomic traits evaluation for Sids 12 derived lines

Analysis of variance according to alpha lattice design for the studied traits of Sids 12 derived lines in the first and second trials is presented in Table 5. It was noticed that reps.block mean square values in some traits (for biological yield, grain yield per plot, plant height and number of spikes per square meter in the first trial and number of spikes per square meter, 1000 kernels weight, number of kernels per spike and harvest index in the second trial) were lower than reps mean squares. In these cases, analysis of variance was applied following randomized complete block design procedure. The analysis indicated significant differences among genotypes for all traits in both trials except for 1000 kernels weight and plant height in the second trial.

Table 5. Mean squares of the studied traits for Sids 12 derived lines, season 2022/2023.

S.O.V	d.f (latice)	d.f (RCBD)	DH†	DM	PH	SM	KS	BY	1000KW	GY	HI
First trial (S1)											
Reps	1	1	2.178	0.1	33.61	11185	40	3.5601	1.23	0.1555	2.755
Reps.Blocks	8	-	64.26**	14.91**	-	-	276.5**	-	96.71**	-	15.29**
Genotypes	44	44	103.26**	26.32**	39.1**	15671*	200.4**	2.067**	54.52**	0.356**	23.11**
Residual	36	44	1.552	3.311	10.32	9031	70.99	0.7944	13.78	0.0955	4.61
Total	89	89	57.479	15.691	-	-	153.11	-	41.23	-	14.697
Second trial (S2)											
Reps	1	1	7.21	25**	72.25	14240	812.82	2.756	46.755	0.0643	45.326
Reps.Blocks	8	-	68.53**	33.31**	143**	30755**	-	2.476*	-	0.6947**	-
Genotypes	49	49	124.72**	32.89**	37.51	19430**	216.88**	2.689**	39.8	0.5911**	43.624**
Residual	41	49	12.24	3.4	49.63	6602	65.95	1.042	10.51	0.1553	3.951
Total	99	99	73.03	20.63	51.46	14980	-	1.991	-	0.4137	-

† DH; days to 50% heading, DM; days to 50% maturity, PH; plant height, SM; number of spikes per square meter, KS; number of kernels per spike, 1000KW; 1000 kernels weight and GY; grain yield per plot. [ns] P > 0.05; * P <= 0.05; ** P <= 0.01

Sids 12 derived lines 1st trial (S1)

Means of the studied traits for Sids 12 derived lines (first trial), season 2022/2023 is presented in supplemented Table S3. For grain yield, 22 wheat lines recorded high grain yield and did not differ significantly with the check (Giza 171). Out of the 22 lines, 9 lines were insignificantly lower than the highest check cultivar Misr 3. The highest grain yield improvement percentage over Sids 12 parent was 24.9%.

For biological yield, 27 wheat lines were significantly higher than the check (Giza 171). Out of the 27 lines, the line #22 was insignificantly higher than the highest check cultivar Misr 3 where For number of kernels per spike, 29 wheat lines were significantly higher number than the check (Giza 171). Out of the 29 lines, 17 lines were significantly higher than the highest check cultivar Misr 3 where the improvement percentage reached 5% over Sids 12 parent.

For 1000 kernels weight, 6 wheat lines recorded significantly higher weight than the check Giza 171. These lines were insignificantly higher than the highest check cultivar Misr 3 where the improvement percentage reached 11.9% over Sids 12 parent.

For plant height, 19 wheat lines were significantly shorter than the check (Giza 171). Out of the 19 lines, 9 lines were insignificantly shorter than the shortest check cultivar Misr 3 where the improvement percentage reached 2.3% over Sids 12 parent.

For number of days to 50% heading, 28 wheat lines were significantly earlier than the check (Misr 3). Out of the 28 lines, 19 lines were significantly earlier than the earliest check cultivar Giza 171 where the improvement percentage of earliness reached 11% over Sids 12 parent.

For number of days to 50% maturity, 20 wheat lines were significantly earlier than both checks (Misr 3 and Giza 171). The improvement percentage of earliness reached 3.8% over Sids 12 parent.

the improvement percentage reached 28.6% over Sids 12 parent.

For harvest index, 15 wheat lines recorded high harvest index and did not differ significantly with the check (Misr 3). Out of the 15 lines, 3 lines were insignificantly higher than the highest check cultivar Giza 171.

For number of spikes per square meter, 41 wheat lines were significantly higher number than the check (Giza 171). Out of the 41 lines, 35 lines were significantly higher than the highest check cultivar Misr 3 where the improvement percentage reached 293% over Sids 12 parent.

Sids 12 derived lines 2nd trial (S2)

Means of the studied traits for Sids 12 derived lines (Second trial), season 2022/2023 are presented in supplemented Table S4. For grain yield, 21 wheat lines were insignificantly higher than Giza 171 and insignificantly lower than the highest check cultivar Misr 3. The improvement percentage over Sids 12 parent was 22.3%.

For biological yield, 23 wheat lines did not differ significantly with both checks (Giza 171 and Misr 3) where the improvement Percentage reached 60.3% over Sids 12 parent for line#30.

For harvest index, 22 wheat lines were significantly higher than the check (Misr 3) and insignificantly higher than Giza 171.

For number of spikes per square meter, 11 wheat lines were significantly higher than the check (Giza 171). Out of the 11 lines, 7 lines were insignificantly higher than the highest check cultivar Misr 3 where the improvement percentage reached 322% over Sids 12 parent.

For number of kernels per spike, 38 wheat lines were significantly higher than the check (Misr 3). Out of the 38 lines, 18 lines were significantly higher than the highest check cultivar Giza 171.

For 1000 kernels weight, 7 wheat lines recorded insignificantly higher weight than the check (Misr

3). Out of the 7 lines, 2 lines were insignificantly higher than the highest check cultivar Giza 171 where the improvement percentage reached 2.3% over Sids 12 parent.

For plant height, 33 wheat lines were significantly shorter than the check (Giza 171). Out of the 33 lines, 5 lines were significantly shorter than the shortest check cultivar Misr 3 where the improvement percentage reached 2.3% over Sids 12 parent.

For number of days to 50% heading, 41 wheat lines were significantly earlier than the check (Misr 3). Out of the 41 lines, 19 lines were significantly earlier than the earliest check cultivar Giza 171 where the improvement percentage for earliness reached 12.8% over Sids 12 parent.

For number of days to 50% maturity, 31 wheat lines were significantly earlier than both checks (Misr 3 and Giza 171). The improvement percentage for earliness reached 3.4% over Sids 12 parent of line #34.

Yr5, *Yr10* and *Yr15* molecular marker detection

Summary of detecting molecular markers for *Yr5*, *Yr10* and *Yr15* genes and their combinations in wheat lines derived from Gemmeiza 11 and Sids 12 is presented in Figure 3 and Tables 6 and 7, respectively. *Yr5* monogenic line (positive control) produced 1281bp band while no products were obtained with both Sids 12 and Gemmeiza 11 susceptible cultivars. In some cases, 507bp PCR product was obtained as alternate *Yr5* allele (Xiaoqin Zhang) (Fig. 3d). Out of 110 Sids 12 and Gemmeiza 11 derived lines, 50 lines (45%) showed 1281bp PCR product and only 4 lines (4%) showed 507bp PCR product. *Yr10* monogenic line parent showed a single band at 220 bp, confirming its positive status for the *Yr10* gene. Meanwhile, both Sids12 and Gemmeiza 11 susceptible parents displaying bands ranging between 200-300 bp, affirming their negative status for the *Yr10* gene (Fig. 3e). Out of 216 Sids 12 and Gemmeiza 11 derived lines, 75 lines (35%) showed positive marker and 35 lines (16%) showed positive hetero 2 allele and 5 lines (2%) showed positive 3 allele. *Yr15* monogenic line parent showed a single band at 220bp, while Sids12 and Gemmeiza 11 parent samples displaying bands at 200 bp and 260 bp, respectively, indicating their negative status for the targeted genes Fig. 3f). Out of 195 derived lines, 72 lines (37%) showed 220bp bands and 39 lines (20%) showed both 220bp and 260bp bands indicated heterozygous status.

Table 6. PCR product, number of genotypes, percentage and description of the tested wheat lines for *Yr5*, *Yr10* (*Xpsp3000*) and *Yr15* (*Xbrac8*) markers.

Markers	PCR product	No. wheat lines	%	Description
Yr5	1281 bp	50	45	Positive
	507 bp	4	4	Positive
	No amplification	56	51	Negative
	Total	110	-	-
Yr10	200 bp	75	35	Present
	230 bp	36	17	Absent
	260 bp	44	20	Absent
	300 bp	7	3	Absent
	(200 + 260) or (230+ 300)	35	16	Present (Hetero 2 allele)
	200 + 230 +260	5	2	Present (Hetero 3 allele)
	Missing	14	6	-
	Total	216	-	-
Yr15	220 bp	72	37	Present
	260 bp	64	33	Absent
	220 + 260	39	20	Present (Hetero)
	Missing	20	10	-
	Total	195	-	-

Table 7. Summary of PCR markers detected for *Yr5*, *Yr10* and *Yr15* and their combinations in Gemmeiza 11 and Sids 12 wheat derived lines.

Source	No. of tested wheat lines	No. of wheat lines with positive markers							
		Yr5	*Yr10*	*Yr15*	*Yr5+Yr10*	*Yr5+Yr15*	*Yr10+Yr15*	Total	%
Gemmeiza 11 derived lines	84	22	2	9	15	4	13	65	77
Sids 12 derived lines	89	10	13	31	2	2	3	60	67
Total	173	32	15	40	17	6	16	126	73
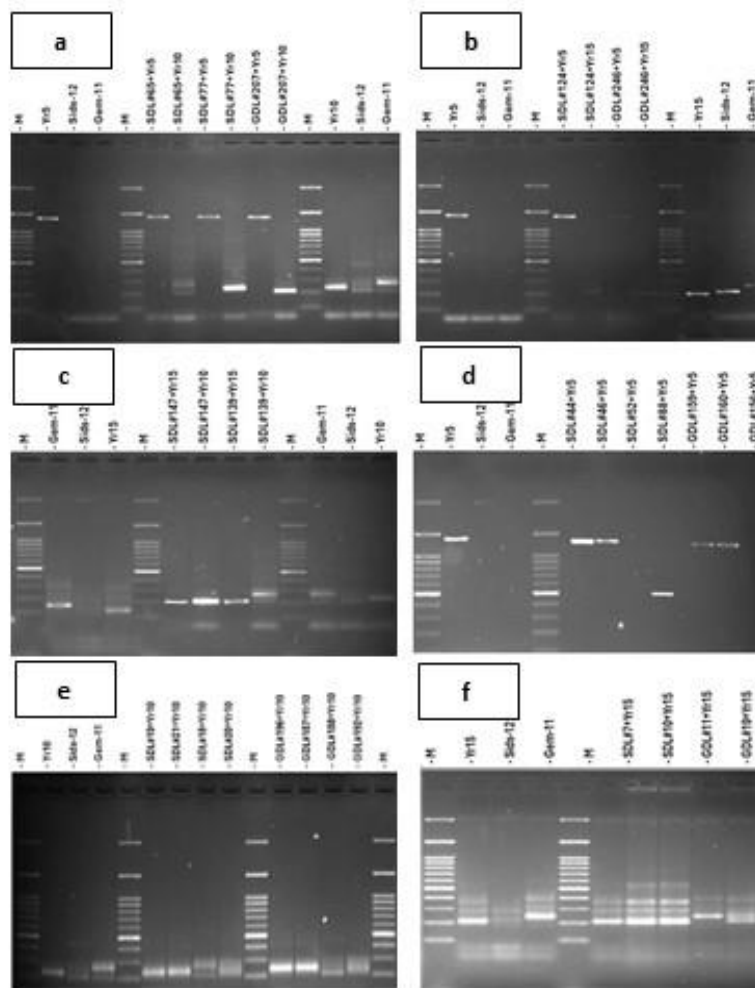
Fig. 3. Representative images of PCR product for Sids 12 and Gemmeiza 11 wheat cultivars and their derived line with *Yr5*, *Yr10* and *Yr15* SSR primers: M refers to the DNA ladder 100bp. Sids-12 and Gem-11 are negative control; *Yr5*, *Yr10* and *Yr15* are positive control; SDL and GDL are Sids and Gemmeiza wheat derived lines. a. derived lines showing *Yr5+Yr10* combination, b. derived lines showing *Yr5+Yr15* combination, c. derived lines showing *Yr15+Yr10* combination, d, e & f derived lines showing *Yr5, Yr10 and Yr15* alone, respectively.

Seedling and adult plant stripe rust evaluation

Wheat stripe rust trap nursery is a routine work for wheat pathology research department aiming to tracking the dynamics of yellow rust pathogen population all over the country as well as its pathogenicity on wheat yellow rust resistance genes. Seedling and adult plant stage reaction for wheat yellow rust trap nursery from 2021 to 2023 at Sakha station as hot spot for yellow rust in north Egypt is presented in Table 8. Efficiency of Yr genes based on the seedling and adult plant stage reaction can be grouped in to five groups: 1st group including three genes (*Yr5*, *Yr8* and *Yr15*) which showed resistance in both seedling and adult plant sages; 2nd group including three genes (*Yr17*, *Yr26* and *Yr54*) which showed resistance at seedling only; 3rd group including six genes (*Yr37*, *Yr4BL*, *Yr51*, *Yr57*, *YrKK* and *YrAld*) which showed resistance at adult plant

stage only; 4th group including three genes (*Yr10* and *YrSp*) which showed moderate resistance at both seedling and adult plant stages; 5th group including nine genes (YrA, Yr6, Yr7, Yr9, Yr18, Yr24, Yr27, Yr35 and YrCV) which showed susceptibility at both seedling and adult plant stages. Based on this result it could be concluded that the importance of the three genes *Yr5*, *Yr8* and *Yr15* in wheat breeding program in Egypt for improving yellow rust resistance.

Results of DNA markers for wheat lines derived from Gemmeiza 11 and Sids 12 cultivars, confirmed the presence of *Yr5*, *Yr10* and *Yr15* positive markers in 32, 15 and 41 wheat lines, respectively. Yellow rust reaction of these lines in both seedling and adult plant stages is presented in Table 9 and 10. Ninety-one percent of wheat lines carrying *Yr5* marker showed complete resistance in both seedling and

adult plant stages for yellow rust. In the same direction, 73% and 97.5% of wheat lines carrying *Yr10* and *Yr15* marker showed complete resistance in both seedling and adult plant stages for yellow rust, respectively. These results confirmed the findings in Table 8 indicating the effectiveness of both *Yr5* and *Yr15* at both seedling and adult plant stages in Egypt.

Table 8. Seedling and adult plant stage reaction for wheat yellow rust trap nursery from 2021 to 2023 at Sakha station.

No.	Genotype/ *Yr* gene [a]	IT [b] at seedling (2023)		IT [c] at adult plant stage		
		Bulk	262E31	2021	2022	2023
1	Morocco	9	9	100S	100S	100S
2	Avocet S – *YrA*	5	9	30S	30S	80S
3	Avocet A + *YrA*	6	7	50S	50S	70S
4	*Yr1/6*AOC*	9	9	20S	20S	70S
5	SIETE CERROS T66	8	9	20S	20S	60S
6	TATARA	0;	0;	TrS	TrS	0
7	*Yr5/6* Avocet S*	0	1	0	0	0
8	*Yr6/6* Avocet S*	7	7	60S	60S	70S
9	*Yr7/6* Avocet S*	8	8	70S	70S	80S
10	*Yr8/6* Avocet S*	0;	0;	0	0	0
11	*Yr9/6* Avocet S*	6	7	60S	60S	80S
12	*Yr10/6* Avocet S*	9	9	0	0	10MS
13	*Yr15/6* Avocet S*	0;	0;	0	0	0
14	*Yr17/ 6* Avocet S*	0;	3	30MSS	30MSS	50MSS
15	*Yr18/6* Avocet S*	7	8	60MS	60MS	30MS
16	*Yr24/6* Avocet S*	7	6	30S	30S	30MSS
17	*Yr26/6* Avocet S*	0;	0;	30MS	30MS	60MRMS
18	*Yr27/6* Avocet S*	9	0;	10S	10S	20MRMS
19	*YrSp/6* Avocet S*	9	0;	0	0	5MS
20	PAVON F 76	9	8	20S	20S	30S
21	SERI M82	9	6	30S	30S	60S
22	OPATA M 85	9	9	10MR	10MR	10MR
23	SUPER KAUZ	7	8	0	0	0
24	*YrCv/6* Avocet S (Yr32)*	6	6	30S	30S	60S
25	PBW343	4	6	10S	10S	30S
26	AOC-YR*3/3/ALTAR 84/AE.SQ//OPATA	0;	0;	30S	30S	30S
27	AOC-YR*3//LALBMONO 1*4/PVN	9	7	40S	40S	60S
28	AOC-YR*3 / PASTOR	7	7	70S	70S	100S
29	POLLMER	6	7	30S	30S	30S
30	PASTOR	9	8	40S	40S	60S
31	REBECA F2000	3	4	30S	30S	50S
32	FRANCOLIN #1	8	7	40S	40S	80S
33	AOC-YR/QUAIU/#3	9	9	30S	30S	80S
34	OPATA/PASTOR CO5607 A020	9	9	0	0	0
35	OPATA/PASTOR CO5607 A047	7	7	0	0	0
36	AOC-YR/QUAIU # 3	7	6	30S	30S	80S
37	M10 (MUTATED C-306) / AOC-YR	6	5	10S	10S	80S
38	CHUAN NONG 19	6	7	10R	10R	20MR
39	IRAGI	9	9	10MR	10MR	10MR
40	KOELZ W 11192:AE	8	8	30MS	30MS	60MSS
41	PBW343/KKU	9	7	0	0	5S
42	AOC-YR*3 //LALBMONO1*4/PVN	7	8	30S	30S	80S
43	*Yr33*	4	3	0	0	10S
44	*Yr34*	4	7	20MR	20MR	20MR
45	*Yr35* 98M71	7	8	30S	30S	70S
46	*Yr37*	9	7	0	0	0
47	*Yr4BL*	6	9	20MR	20MR	20MR
48	*Yr51*	3	5	TrMR	TrMR	5R
49	*Yr54*	3	3	50S	50S	80S
50	*Yr57*	8	9	5MR	5MR	5R
51	*YrKK*	9	3	0	0	0
52	*YrAId*	7	4	5MR	5MR	0

[a] Resistance gene based on the studies of Chen, (2005).; [b] Infection type at seedling stage based on the 0–9 scale as described by McNeal et. al., (1971);[c] ITs based on Roelfs et al. (1992)., 0=Immune. R = resistant (necrosis with few uredinia); MR = moderately resistant (necrosis with small to moderate number of uredinia); MS = moderately susceptible (moderate number of uredinia with chlorotic areas); and S = susceptible (large number of uredinia, no necrosis but chlorosis may be evident).

Table 9. Gemmeiza 11 and Sids 12 derived lines showing positive PCR marker for *Yr5*, *Yr10* and *Yr15* and their adult plant and seedling response against yellow rust during 2022/2023 season.

PCR identified marker	No of tested lines	Yellow rust response		
		Adult [a]	Seedling (bulk) [b]	Seedling (race)
Yr5	17	0	0;	0;
	6	5 R	0;	0;
	4	0	1	0;
	1	0	3	1
	1	0	0;	1
	1	0	0;	9
	1	0/10MS	3	9
	1	0/5R	6	0;
Total	32	-	-	-
Yr10	10	0	0;	0
	1	0	0;	1
	1	0	6	0;
	1	0	7	0
	1	0	0;	9
	1	0/TR	6	0;
Total	15	-	-	-
Yr15	25	0	0;	0;
	7	0/TR	0	0
	1	0	3	3
	1	0	3	3
	1	0	0;	3
	1	0	2	4
	1	0	1	0;
	1	0	1	0;
	1	0	3	0;
	1	0/5R	3	0;
	1	0/100S	9	0
Total	41	-	-	-
Gemmeiza 11		100S	8	9
Sids 12		40S	3	0;

[a] Infection type based on the 0–9 scale as described by McNeal et. al., (1971); [b] infection type based on Roelfs et. al.; (1992). 0= Immune; R = resistant; MR = moderately resistant; MS= moderately susceptible; and S= susceptible.

Table 10. Gemmeiza 11 and Sids 12 derived lines showing positive PCR marker for *Yr5*, *Yr10* and *Yr15* combinations and their adult plant and seedling response against yellow rust during 2022/2023 season.

PCR identified marker	No of tested lines	Yellow rust response		
		Adult	Seedling (bulk) [b]	Seedling (race)
Yr5+Yr10	10	0	0;	0
	1	0	0;	1
	1	0	3	0;
	1	0	0;	1
	1	0	1	0;
	1	0	1	0;
	1	0/100 S	3	0;
	1	T R	4	0;
Total	17	-	-	-
Yr5+Yr15	1	0	0;	0;
	1	0	1	3
	1	0	1	0;
	1	0	2	0;
	1	0	0;	3
Total	5	-	-	-
Yr10+Yr15	7	0	0;	0;
	2	TR	0;	0;
	3	0	2	0
	1	0	1	0
	1	0	1	3
	1	0	0;	1
	1	TR	1	5
Total	16	-	-	-
Gemmeiza 11		100S	8	9
Sids 12		40S	3	0;

[a] Infection type based on the 0–9 scale as described by McNeal et. al., (1971); [b] infection type based on Roelfs et. al.; (1992). 0= Immune; R = resistant; MR = moderately resistant; MS= moderately susceptible; and S= susceptible.

Nine lines derived from Sids 12 showed resistance to stripe rust at both seedling and adult plant stages as well as superiority in grain yield over Giza 171 check with increase percentage ranged from 0.6 to 5.7% (Table 11) and can be promoted to the national wheat yield trials. Those are line S2# 35 (carry *Yr5*); Line S1# 20 (carry *Yr10*); Lines S1# 7, S2# 3, 34, 38, 44 & 50 (carry *Yr15*); S1# 14 (carry combination of *Yr5* and *Yr15*). Meanwhile, Sids 12 derived lines carrying *Yr5+Yr10* and *Yr10+Yr15* combinations did not show superiority in grain yield over Giza 171 check. For Gemmeiza 11 derived lines, eighteen lines showed resistance to stripe rust at both seedling and adult plant stages as well as superiority in grain yield over Giza 171 check with increase percentage ranged from 0.4 to 14.6% (Table 12) and can be promoted to the national wheat yield trials. Five lines carrying *Yr5* (Line G1# 14, 28, 36 & G2# 3, 15); one line carrying *Yr10* (G1# 42); five lines carrying *Yr15* (G1# 11, 19 & G2# 11, 21, 28); five lines carrying *Yr10+Yr15* combination (G1# 45 & G2# 19, 37, 42, 45); two line carrying *Yr5+Yr15* combination (G2# 25, 39). Meanwhile, Gemmeiza 11 derived lines carrying *Yr5+Yr10* combination did not show superiority in grain yield over Giza 171 check.

Generally, the results in Tables 11 & 12 indicated that derived lines from both cultivars with single Yr gene recorded superiority in grain yield over lines with two Yr genes combination. Also, Gemmia 11 derived lines were higher in grain yield than those derived from Sids 12.

Discussion

In Egypt, stripe rust attacked many commercial bread wheat cultivars causing severe infection in North Delta Area such as Sakha 93, Gemmiza 9, Sids 12 and Gemmiza 11 cultivars. The two bread wheat cultivars Sids12 and Gemmeiza11 were the most popular cultivars in Egypt, especially for small farms. The reason for farmers preference for those cultivars is due to its good bread making quality (Mahrous et al., 2009, Sadek et al., 2013, Hagras, 2014 and Ragab, & Mohamed, 2014). Area cultivated with both cultivars reached 25% of total wheat area in the 2015/2016 season (Economic Affairs Section, Ministry of Agriculture and Land Reclamation, Egypt, 2016). Unfortunately, stripe rust resistance of both cultivars was breakdown in season 2016/2017 and great yield loss occurred. The best way to control yellow rust and reduce crop loses is to pyramid the effective resistant genes into new cultivars (Singh et al., 2005). The wheat breeding program developed new rust resistant wheat cultivars depending only on phenotypic evaluation for crossing blocks, segregating populations and promising lines in rust hot spots. So, the newly released cultivars may carry the same rust resistance gene(s) causing resistance breakdown of more than

one cultivar at the same time. One important method to increase the durability of rust resistance would be through producing wheat cultivars carrying different effective resistance gene(s) (Ragab et al, 2020). The pyramiding of efficient stripe rust resistance genes in the new wheat promising lines may be facilitated using marker-assisted selection (MAS) techniques.

Virulence to *Yr5* and *Yr15* genes rarely occur in wheat producing areas of the world (Chen, 2005 and Sharma et al., 2013). In Egypt, the three strip rust resistant genes *Yr5, Yr10* and *Yr15* are effective against the dominating *Pst* races where, the two genes *Yr5* and *Yr15* show complete resistance at both seedling and adult plant stages (Shahin 2017 and Ragab et al., 2020). Meanwhile, it should be mentioned herein that *Yr5* was attacked by race 494E158 once, 2008; (Shahin and Abu El-Naga, 2011). During the period of the current study, the effectiveness of these genes is confirmed. Therefore, pyramiding combinations of these genes in one wheat background is expected to enhance resistance durability for stripe rust in Egypt.

Tracking Yr targeted genes in selected plants through segregating generation based on phenotyping (0 type field response, the same of Yr genes doners) led to obtaining lines harboring the Yr targeted genes based on genotyping with 73% success. This was an advantage of using Yr gene doners showing 0 type field response and highly susceptible recurrent parent. The remaining number of lines (27%) showing 0 type field response did not show the target band. This is might be because the polymorphism of a marker could not be distinguished in some wheat germplasms, such as Owm45F3R3 for *Yr51* (Randhawa et al. 2014). Haider et al. (2023) used 13 Yr gene-tagged markers for the detection of known Yr genes *(Yr5, Yr10, Yr15, Yr24/Yr26)* in 45 stripe rust resistance accessions and they reported that the targeted band for *Yr5* was not detected with marker *Xgwm120* while it was detected with marker *Xwmc175* for the same accession.

Breeders prefer the use of major stages resistance (ASR) genes in wheat breeding. However, this strategy seems to be not so successful because stripe rust frequently occurs on a large scale when the underlying gene is circumvented by the emergence of new virulent races (McIntosh et al. 2018). In China, wheat breeders introduced the three APR genes *Yr18*, *Yr28*, and *Yr36* into elite wheat lines through gene-based marker selection and indicated that pyramiding of these genes provides long-term resistance to stripe rust (Fang et al., 2023). In this study, *Yr* monogenic lines were used as a source for R genes through pre breeding to transfer and pyramid some effective stripe rust resistance genes into agronomically acceptable wheat lines. A total of 173 strip rust resistant lines were derived from Sids

12 and Gemmeiza 11. Of these lines, 32 lines showed marker for *Yr5*; 15 lines showed marker for *Yr10* and 40 lines showed marker for *Yr15*. Fortunately, a total of 38 lines showed markers for two gene combination where 17 lines showed markers for both *Yr5* and *Yr10*; five lines showed markers for both *Yr5* and *Yr15*; and 16 lines showed markers for both *Yr10* and *Yr15*. In this study, phenotypic selection followed by MAS allowed to pyramid *Yr5*, *Yr10* and *Yr15* gene combination and enhance selection efficiency at early generations.

Improvement percentage in yield components reached about 300% for number of spikes per square meter which may be due to the higher tillering ability if the Yr genes doners. Despite this, the improvement percentage for grain yield reached only about 25% that may be due to the directed selection toward stripe rust resistance in segregating generation regardless grain yield in addition to the undesirable genetic background of the Yr gene doners. This was confirmed by the grain yield superiority of lines with single over lines with two Yr genes combination. Accordingly, low percentage (30%) of selected lines with two gene combination compared with one gene (70%) was obtained. Also,

Gemmia 11 derived lines were higher in grain yield than those derived from Sids 12 this may be due to the cultivar`s genetic background. In accordance with our result, Mandea et al. (2019) reported that most correlations among the wheat yield components were negative, illustrating the difficulty of combining high values of more than one component. The strongest negative correlation was found between the number of spikes per unit area and the number of grains per spike, but most correlations between number of grains/spike and 1000 kernels weight were negative.

Conclusion

In this study 126 elite bread wheat lines were developed from the stripe rust highly susceptible cultivars Gemmeiza 11 and Sids 12. Phenotypic and genotypic testing indicated that these lines are resistance to the local pathotypes of stripe rust races in both seedling and adult plant stag test and carry markers for *Yr5*, *Yr10* or *Yr15* genes individually or in combinations. The developed resistant genotypes can be released after testing in multi-environment trials and also used as parental lines for crosses with potential and adapted wheat cultivars to develop stripe rust durable resistant varieties.

Table 11: Summary of the selected lines derived from Sids 12 based on Yr gene detection, adult and seedling yellow rust resistance as well as superiority in grain yield over Giza 171 check and harvest index percentage.

Line	Identified Yr gene	Yellow rust reaction		Grain yield (kg/plot)	Change % over Giza 171 mean	Harvest index %
		Adult plant [a]	Seedling [b]			
S2#35	5	0	0;	4.505	4.4	34.9
S2#19	5	TR	0;	4.275	-0.9	33.2
S2#18	5	0	0	4.113	-4.7	34.7
S1#26	5	0	0;	3.920	-9.2	30.9
S2#4	5	0	0;	3.885	-10.0	33.7
S1#20	10	0	0;	4.360	1.0	32.1
S2#24	10	0	0;	3.835	-11.1	32.1
S1#40	10	0	0;	3.535	-18.1	28.9
S2#30	10	0	0	3.313	-23.2	25.8
S2#34	15	0	0;	4.560	5.7	37.4
S2#50	15	0	0;	4.555	5.6	35.1
S2#38	15	0	0;	4.520	4.8	32.9
S1#7	15	0	0	4.425	2.5	34.3
S2#44	15	0	0;	4.357	1.0	35.0
S2#3	15	0	0	4.343	0.6	35.9
S2#11	15	0	0;	4.313	0.0	36.1
S2#12	15	0	0;	4.280	-0.8	35.0
S2#21	15	0	0;	4.178	-3.2	36.7
S2#13	15	0	0;	4.070	-5.7	36.3
S1#10	10+15	0	1	3.342	-22.5	29.5
S2#43	10+15	0	0;	2.648	-38.6	23.4
S2#7	10+15	0	0;	2.618	-39.3	20.7
S2#25	5+10	TR	4	3.280	-24.0	28.7
S2#16	5+10	0	3	3.253	-24.6	26.0
S1#14	5+15	0	0;	4.347	0.7	35.3
Giza 171	-	0	2	4.315	0.0	33.4

[a] Infection type based on the 0–9 scale as described by McNeal et. al., (1971); [b] infection type based on Roelfs et. al.; (1992). 0= Immune; R = resistant; MR = moderately resistant; MS= moderately susceptible; and S= susceptible.

Table 12. Summary of the selected lines derived from Gemmeiza 11 based on Yr gene detection, adult and seedling yellow rust resistance as well as superiority in grain yield over Giza 171 check and harvest index percentage.

| Line | Identified Yr gene | Yellow rust reaction | | Grain yield (kg/plot) | Change % over Giza 171 mean | Harvest index % |
		Adult plant [a]	Seedling [b]			
G1#28	5	0	0	4.810	12.4	40.3
G2#3	5	0	3	4.643	8.5	38.2
G2#15	5	0	1	4.483	4.7	37.8
G1#36	5	0	0	4.375	2.2	37.7
G1#14	5	0	0	4.297	0.4	32.9
G1#40	5	0	0;	4.275	-0.1	34.6
G1#39	5	0	0;	4.267	-0.3	35.4
G1#43	5	0	0;	4.205	-1.8	33.8
G2#29	5	0	1	4.168	-2.6	36.9
G1#6	5	0	0;	4.125	-3.6	36.6
G1#27	5	0	0;	4.122	-3.7	34.4
G1#23	5	0	0;	4.010	-6.3	33.7
G2#27	5	0	1	3.998	-6.6	31.5
G1#42	10	0	0;	4.435	3.6	37.9
G2#16	10	0	0;	4.250	-0.7	37.5
G1#11	15	0	1	4.907	14.6	39.2
G2#21	15	0	0;	4.878	13.9	37.0
G1#19	15	TR	0;	4.817	12.5	36.8
G2#28	15	0	3	4.795	12.0	38.1
G2#11	15	0	9	4.318	0.9	37.4
G2#17	15	0	3	4.268	-0.3	36.0
G2#31	15	0	2	4.085	-4.6	39.4
G2#19	10+15	0	0;	4.770	11.4	38.8
G2#45	10+15	0	2	4.425	3.4	38.8
G2#37	10+15	0	0;	4.358	1.8	38.9
G2#42	10+15	0	0;	4.343	1.4	40.6
G1#45	10+15	0	2	4.310	0.7	38.3
G1#17	10+15	TR	0;	4.227	-1.3	34.6
G1#18	10+15	TR	0;	4.215	-1.5	35.6
G1#38	10+15	0	0;	4.207	-1.7	36.2
G1#10	10+15	0	2	4.127	-3.6	36.4
G2#32	10+15	0	0;	4.098	-4.3	35.5
G2#7	10+15	0	1	3.960	-7.5	34.3
G2#18	10+15	TR	1	3.948	-7.8	37.6
G2#38	10+15	0	0;	3.933	-8.1	33.5
G2#34	5+10	0	0;	4.255	-0.6	36.7
G2#30	5+10	0	0;	4.250	-0.7	32.4
G1#29	5+10	0	0;	4.207	-1.7	36.3
G2#14	5+10	0	0;	4.195	-2.0	37.8
G2#6	5+10	0	1	4.088	-4.5	33.9
G1#30	5+10	0	0	4.027	-5.9	33.9
G1#34	5+10	0	0;	4.025	-6.0	36.5
G2#39	5+15	0	2	4.705	9.9	36.9
G2#25	5+15	0	1	4.463	4.3	38.6
G2#20	5+15	0	0;	4.240	-1.0	34.9
G2#36	5+15	0	1	3.955	-7.6	35.3
Giza 171	-	0	2	4.281	0.0	36.3

[a] Infection type based on the 0–9 scale as described by McNeal et. al., (1971); [b] infection type based on Roelfs et. al.; (1992). 0= Immune; R = resistant; MR = moderately resistant; MS= moderately susceptible; and S= susceptible.

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تجميع جينات مقاومة الصدأ الأصفر *Yr5* و *Yr10* و *Yr15* في السلالات الناتجة من صنفي قمح الخبز سدس 12 وجميزة 11

عادل عبدالعزيز هجرس[1]، وخالد الدمرداش رجب[1]، وسيدهم عبدالخالق[1]، وعاطف عبدالفتاح شاهين[2]، وسمر محمد عادل إسماعيل[2]، وواصف عبدالصمد يوسف[2]، ومحمد درغام صحصاح[2]، والاء يوسف صلاح[3]، ونورهان محمود فؤاد[3]، وسوسن توكز[3]، وعلاء الدين حموية[3]

[1] قسم بحوث القمح- معهد بحوث المحاصيل الحقلية – مركز البحوث الزراعية - مصر
[2] قسم بحوث أمراض القمح- معهد بحوث أمراض النباتات – مركز البحوث الزراعية - مصر
[3] المركز الدولي للبحوث الزراعية في المناطق الجافة - مصر

تم إنتاج وتقييم مائة وثلاثة وسبعون سلالة من قمح الخبز المقاوم للصدأ الأصفر، الناتجة من التهجين بين صنفي القمح سدس 12 وجميزة 11 القابلة للإصابة، والسلالات الثلاثة أحادية الجين التي تحمل جينات *Yr5* و*Yr10* و*Yr15* في محطة البحوث الزراعية بسخا، خلال الفترة من عام 2016م وحتى 2023م. تمت دراسة مقاومة الصدأ الأصفر في مرحلتي البادرة والنبات البالغ، والكشف عن الواسمات الجزيئية للجينات الثلاثة وكذلك تقييم الصفات المحصولية لهذه السلالات. أظهرت النتائج أن 126 سلالة كانت مقاومة في مرحلتي البادرات والنباتات البالغة. من بين هذه السلالات، أظهرت 87 سلالة وجود واسمات لجين واحد (*Yr5*، *Yr10* أو *Yr15*) وأظهر 39 سلالة وجود واسمات لتوليفة من ازواج الجينات (*Yr5+Yr10*، *Yr5+Yr15* أو *Yr10+Yr15*). أوضح التقييم المحصولى إلى أن أعلى نسبة تحسين في محصول الحبوب بلغت 3% و25% للسلالات الناتجة من هجن جميزة 11 وسدس 12 على التوالي. سجلت السلالات الحاملة لواسمات جين واحد تفوقا في محصول الحبوب على السلالات الحاملة لواسمات زوج من الجينات. تفوقت خمسة عشر سلالة في محصول الحبوب مقارنة بالصنف جيزة 171، وهذه السلالات هى S2#35، G1#28، G2#3 والتى تحمل جين *Yr5*؛ السلالات S1#20، G1#42 والتى تحمل جين *Yr10*؛ والسلالات S2#34 و G1#11, G1#19, S2#50 و G1#21, G1#28 والتى تحمل جين *Yr15* ؛ السلالات G2#19, G2#45 والتى تحمل الجينين*Yr10+Yr15* معا ؛ والسلالات G2#25, G2#39 والتى تحمل الجينين *Yr5+Yr15* معا. يمكن استخدام هذه السلالات كمصدر جديد للجينات *Yr5* و*Yr10* و*Yr15* لتربية القمح لمقاومة الصدأ الأصفر و ترشيحها إلى التجارب القومية لتقييم المحصول لتسجيلها كأصناف جديدة.

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Supplementary Table S1: Means of the studied traits for Gemmeiza 11 derived lines (first trial; G1), season 2022/2023.

Genotype	DH†		DM		PH		BY		GY		HI%		SM		KS		1000KW	
G1#1	79	a	146	bcdef	113	h	11.3	efgh	4.125	cdefgh	36.6	abcdef	533	abcdefg	42.1	klmn	55.9	abcd
G1#2	90	mnop	151	ijk	120	efg	11.9	cdef	4.027	defgh	33.9	fghijklm	522	abcdefg	50	cdefghijklmn	44.3	hijklmn
G1#3	90.5	mnop	149	efghij	118	fgh	12	abcdef	4.122	cdefgh	34.4	efghijklm	502	abcdefg	43.8	hijklmn	48.8	cdefghijkl
G1#4	81.5	abcde	146	bcdefg	135	a	11	efgh	3.957	defgh	36	bcdefgh	457	bcdefg	43.2	jklmn	53.9	bcdef
G1#5	86	ghijk	148	defghij	120	efg	11.6	cdefg	4.207	bcdefg	36.3	bcdefgh	505	abcdefg	43.5	ijklmn	51.4	cdefghi
G1#6	79.5	ab	140	a	130	abc	11	efgh	4.025	defgh	36.5	bcdefg	383	defg	41.2	lmn	61.2	ab
G1#7	88	jklm	148	defghij	125	cde	11	efgh	3.857	defghi	35.2	defghijk	512	abcdefg	45.9	ghijklmn	46.4	efghijklm
G1#8	90.5	mnop	149	efghij	133	ab	13.1	abcd	4.297	abcdef	32.9	ghijklm	405	defg	56.9	abcdefg	50.9	cdefghij
G1#9	102.5	t	155	lm	120	efg	12.6	abcde	3.525	hi	28.1	no	375	efg	55.4	abcdefghij	38.8	mno
G1#10	85.5	fghij	148	defghij	120	efg	11.9	bcdef	4.01	defgh	33.7	fghijklm	512	abcdefg	51.8	bcdefghijklm	54.5	abcde
G1#11	92.51	pq	151	jk	128	bcd	12.4	abcde	3.917	defghi	31.7	jklmn	457	bcdefg	53	abcdefghijkl	57.2	abc
G1#12	90	mnop	148	defghij	123	def	12	abcdef	3.737	efghi	31.2	mn	553	abcdefg	39.8	mn	55.8	abcd
G1#13	91.51	op	150	hijk	115	gh	12.5	abcde	4.205	bcdefg	33.8	fghijklm	442	bcdefg	56.7	abcdefg	44.8	hijklmn
G1#14	86.03	ghijk	149	efghij	130	abc	12.4	abcde	4.275	abcdef	34.6	efghijklm	463	bcdefg	46.2	fghijklmn	44.4	hijklmn
G1#15	91.02	nop	149	fghijk	120	efg	12.6	abcde	3.947	defghi	31.5	lmn	573	abcdef	62.2	abc	45.3	ghijklmn
G1#16	90.5	mnop	148	defghij	123	def	12.1	abcdef	4.267	abcdef	35.4	defghi	597	abcd	54.8	abcdefghij	45.9	fghijklm
G1#17	80.5	abc	146	bcdefg	115	gh	11.6	cdefg	4.375	abcde	37.7	abcde	537	abcdefg	39.3	n	48.9	cdefghijkl
G1#18	99.01	s	153	kl	130	abc	11.4	defgh	3.59	ghi	31.5	lmn	350	g	64	ab	47.6	defghijkl
G1#19	100	st	150	ghijk	128	bcd	12.5	abcde	3.945	defghi	31.6	klmn	408	defg	46.8	fghijklmn	50.5	cdefghij
G1#20	81.5	abcde	147	bcdefgh	123	def	10	gh	3.287	ij	32.9	ghijklm	413	defg	53.6	abcdefghijkl	45.3	hijklmn
G1#21	83.5	defg	148	defghij	113	h	12	abcdef	4.81	ab	40.3	a	455	bcdefg	46.5	fghijklmn	48.6	defghijkl
G1#22	82	bcde	144	bc	118	fgh	11.1	efgh	3.957	defgh	35.8	bcdefghi	473	bcdefg	46.5	fghijklmn	54.5	abcde
G1#23	88.51	klmn	148	defghij	128	bcd	13.6	a	4.47	abcd	33.1	fghijklm	650	ab	49.9	cdefghijklmn	43.1	ijklmn
G1#24	88.5	klmn	146	bcdef	125	cde	10.6	fgh	3.647	fghi	34.4	efghijklm	518	abcdefg	55	abcdefghij	45.7	fghijklm
G1#25	82	bcde	147	bcdefgh	115	gh	11.5	cdefgh	3.945	defghi	34.7	defghijklm	583	abcde	53.5	abcdefghijkl	45.2	hijklmn
G1#26	98	rs	148	defghij	115	gh	12.4	abcde	4.067	defgh	32.8	hijklm	470	bcdefg	60.1	abcde	45.9	fghijklm
G1#27	86.5	hijk	146	bcdefg	118	fgh	11.8	cdef	3.937	defghi	33.5	fghijklm	442	bcdefg	54.3	abcdefghijk	47.1	efghijklm
G1#28	86.5	hijk	145	bcde	128	bcd	12	abcdef	3.855	defghi	32.2	ijklm	470	bcdefg	52.3	bcdefghijklm	41.4	klmn
G1#29	85.53	fghij	148	defghij	120	efg	11.5	cdefg	4.067	defgh	35.3	defghij	635	abc	57.1	abcdefg	41.8	klmn
G1#30	89.52	lmno	148	defghij	118	fgh	11.3	efgh	3.542	hi	31.5	lmn	542	abcdefg	48.2	efghijklmn	32.6	o
G1#31	82.01	bcde	148	defghij	130	abc	12.4	abcde	4.352	abcde	35.1	defghijkl	425	cdefg	52	bcdefghijklm	53.7	bcdefg
G1#32	83	cdef	146	bcdef	120	efg	11.7	cdef	4.435	abcd	37.9	abcde	487	bcdefg	53.1	abcdefghijkl	49.8	cdefghijk
G1#33	85.01	fghi	147	bcdefgh	128	bcd	12.3	abcdef	4.227	bcdefg	34.6	efghijklm	582	abcdef	65.3	a	50.5	cdefghij
G1#34	84.02	efgh	148	defghij	114	h	11	efgh	3.737	efghi	34	fghijklm	588	abcde	58.5	abcdef	42.7	jklmn
G1#35	85.03	fghi	143	ab	133	ab	11.9	cdef	4.215	bcdefg	35.6	cdefghi	428	cdefg	48.8	defghijklmn	45.3	hijklmn
G1#36	82	bcde	147	cdefghi	128	bcd	13.1	abc	4.817	ab	36.8	abcdef	532	abcdefg	63.6	ab	51.3	cdefghi
G1#37	95	qr	146	bcdefg	128	bcd	11.4	efgh	4.127	cdefgh	36.4	bcdefgh	445	bcdefg	49.3	defghijklmn	52.2	cdefgh
G1#38	80.5	abc	143	ab	128	bcd	11.3	efgh	4.31	abcde	38.3	abcd	498	bcdefg	60.7	abcd	48.1	defghijkl
G1#39	88	jklm	147	bcdefgh	120	efg	12.5	abcde	4.907	a	39.2	ab	567	abcdefg	56	abcdefghi	40.8	lmno
G1#40	101	st	158	m	123	def	11.3	efgh	2.75	j	24	p	720	a	43.7	hijklmn	32.6	o
G1#41	110.5	u	158	m	133	ab	9.8	h	2.633	j	27.1	op	495	bcdefg	25.3	o	37.3	no
G1#42	81.01	abcd	147	bcdefgh	118	fgh	11.6	cdefg	4.207	bcdefg	36.2	bcdefgh	457	bcdefg	56.2	abcdefgh	47.8	defghijkl
Gemmeiza 11 (protected)	82	bcde	145	bcd	118	fgh	12.3	abcdef	4.78	abc	39.1	abc	378	defg	48.7	defghijklmn	62.6	a
Misr 3	87	ijkl	150	ghijk	115	gh	13.6	ab	4.81	ab	35.5	cdefghi	580	abcdef	51.7	bcdefghijklmn	46.6	efghijklm
Giza 171	83.5	defg	147	bcdefgh	125	cde	11.9	cdef	4.242	bcdefg	35.8	bcdefghi	362	fg	57.2	abcdefg	53.8	bcdef
%cv	1.58		1		2.23		6.89		8.03		5.24		18		12.1		8.67	
se	1.389		1.54		2.73		0.81		0.326		1.8		88.9		6.18		4.15	

† DH; days to 50% heading, DM; days to 50% maturity, PH; plant height (cm), By; biological yield per plot (kg), GY; grain yield per plot (kg), HI% harvest index percentage, SM; number of spikes per square meter, KS; number of kernels per spike, 1000KW; 1000 kernels weight**.**

Supplementary Table S2: Means of the studied traits for Gemmeiza 11 derived lines (second trial; G2), **season 2022/2023.**

Genotype	DH†	DM	PH	BY	GY	HI%	SM	KS	1000KW
G2#1	83 cdefg	146 ghijk	125 def	12 abcdefgh	4.1 bcdefghij	34 efghijkl	417 ef	40 hij	46 fghij
G2#2	90 mn	151 n	118 gh	13 abc	4.3 abcdefghi	32 jkl	525 abcdef	51 bcdefghij	45 fghij
G2#3	91 n	149 lmn	120 fgh	13 abcd	4 cdefghijk	32 kl	642 a	37 j	42 hij
G2#4	80 ab	141 abc	123 efg	11 efghij	3.9 efghijk	35 bcdefghijk	475 bcdef	40 hij	53 bcdefgh
G2#5	87 ijkl	148 jklm	115 h	11 defghij	4.2 abcdefghij	37 abcdefghij	587 abcd	47 efghij	53 bcdefgh
G2#6	81 abc	144 cdefg	125 def	12 cdefghij	4.3 abcdefghi	37 abcdefghij	408 ef	48 defghij	59 abcde
G2#7	82 bcde	142 bcde	125 def	11 defghij	4.3 abcdefghi	38 abcdefghi	388 f	57 abcdefghi	53 bcdefgh
G2#8	81 abcd	146 ghijk	120 fgh	11 efghij	4.2 abcdefghij	38 abcdefghi	458 bcdef	49 bcdefghij	65 a
G2#9	88 jklm	144 defgh	118 gh	12 cdefghij	3.6 hijk	32 kl	433 def	46 fghij	58 abcde
G2#10	79 a	143 bcdef	123 efg	11 defghij	3.8 efghijk	34 fghijkl	383 f	44 ghij	61 abcd
G2#11	84 efghi	148 jklm	128 cde	14 a	4.9 a	36 abcdefghijk	602 abc	63 abcdef	48 efghij
G2#12	89 lmn	148 jklm	118 gh	11 defghij	3.8 efghijk	33 hijkl	475 bcdef	64 abcdef	38 j
G2#13	83 cdefg	144 cdefg	130 bcd	10 j	3.5 jk	35 bcdefghijk	432 def	51 bcdefghij	55 abcdef
G2#14	97 o	149 lmn	118 gh	12 defghij	3.8 efghijk	33 ijkl	427 def	53 bcdefghij	41 ij
G2#15	90 mn	150 mn	115 h	11 ghij	3.7 fghijk	34 cdefghijk	437 cdef	60 abcdefg	49 efghij
G2#16	89 klmn	147 ijkl	115 h	13 abcd	4.7 abc	37 abcdefghij	615 ab	55 abcdefghij	49 efghij
G2#17	90 mn	147 ijkl	133 bc	11 defghij	4 defghijk	35 bcdefghijk	383 f	56 abcdefghi	52 bcdefgh
G2#18	88 jklm	147 hijkl	128 cde	12 abcdefgh	4.2 abcdefghij	35 bcdefghijk	495 abcdef	57 abcdefgh	55 abcdef
G2#19	82 bcde	144 defgh	135 ab	12 cdefghij	4.5 abcdef	39 abcdef	455 bcdef	64 abcdef	52 bcdefghi
G2#20	86 ghij	144 cdefg	123 efg	10 ij	4.1 bcdefghij	39 ab	483 abcdef	63 abcdef	45 fghij
G2#21	84 defgh	147 ijkl	135 ab	13 abcdef	4.8 ab	38 abcdefgh	560 abcde	65 abcde	50 defghi
G2#22	80 ab	141 abc	120 fgh	12 cdefghij	4.5 abcdef	39 abcdef	422 def	52 bcdefghij	56 abcdef
G2#23	87 ijkl	147 ijkl	135 ab	13 ab	4.9 a	37 abcdefghij	425 def	54 abcdefghij	49 efghij
G2#24	90 mn	148 klmn	140 a	14 a	4.4 abcdefg	33 jkl	478 bcdef	67 ab	46 fghij
G2#25	85 ghij	144 defgh	125 def	12 bcdefghi	4.5 abcde	38 abcdefghi	393 ef	53 bcdefghij	49 efghij
G2#26	87 ijkl	147 hijkl	120 fgh	11 ghij	3.7 ghijk	34 fghijkl	380 f	52 bcdefghij	43 ghij
G2#27	109 p	155 o	135 ab	11 defghij	3.3 k	29 l	398 ef	51 bcdefghij	42 hij
G2#28	86 ghij	145 fghij	128 cde	12 abcdefgh	4.6 abcd	38 abcdefgh	472 bcdef	66 abc	49 efghij
G2#29	82 bcdef	141 abc	133 bc	11 defghij	4.4 abcdefgh	39 abcd	375 f	59 abcdefg	55 abcdef
G2#30	86 hijk	144 defgh	125 def	12 cdefghij	4.1 bcdefghij	36 bcdefghijk	413 ef	64 abcdef	55 abcdef
G2#31	84 efghi	141 abc	120 fgh	11 fghij	4.2 abcdefghij	38 abcdefg	438 cdef	62 abcdef	53 bcdefg
G2#32	80 ab	142 bcd	135 ab	11 hij	4.3 abcdefgh	41 a	370 f	51 bcdefghij	53 bcdefgh
G2#33	90 mn	148 klmn	123 efg	12 bcdefghi	4.3 abcdefghi	36 abcdefghijk	398 ef	61 abcdefg	50 efghi
G2#34	83 cdefg	146 ghijk	125 def	12 cdefghij	4 defghijk	34 defghijk	447 cdef	62 abcdef	49 efghij
G2#35	80 ab	139 a	118 gh	11 ij	4 defghijk	38 abcdefghi	408 ef	39 ij	55 abcdef
G2#36	85 fghi	147 hijkl	130 bcd	12 abcdefg	4.8 ab	39 abcde	420 def	66 abcd	50 defghi
G2#37	80 ab	142 bcd	120 fgh	11 ghij	4.1 bcdefghij	38 abcdefghi	467 bcdef	58 abcdefgh	62 abc
G2#38	89 klmn	144 defgh	120 fgh	11 defghij	4.4 abcdef	39 abcde	418 def	55 abcdefghij	52 bcdefghi
G2#39	90 mn	148 klmn	128 cde	12 defghij	4.3 abcdefghi	37 abcdefghi	412 ef	57 abcdefgh	41 ij
G2#40	81 abcd	142 bcd	120 fgh	11 defghij	4.4 abcdefg	39 abc	415 ef	72 a	55 abcdef
G2#41	80 ab	141 ab	115 h	10 ij	3.6 ijk	35 bcdefghijk	412 ef	62 abcdef	51 cdefghi
G2#42	97 o	151 n	130 bcd	12 bcdefghi	3.9 defghijk	34 ghijkl	412 ef	62 abcdef	46 fghij
Gemmeiza 11 (protected)	82 bcdef	145 efghi	118 gh	12 abcdefgh	4.8 ab	39 abcd	378 f	49 cdefghij	63 ab
Misr 3	84 efghi	146 ghijk	120 fgh	12 bcdefghi	4.3 abcdefghi	37 abcdefghij	412 ef	56 abcdefghi	50 defghi
Giza 171	88 jklm	148 Jklm	115 h	13 abcde	4.3 Abcdefghi	34 Defghijk	487 Abcdef	51 bcdefghij	50 efghi
%cv	1.6	0.98	2.96	6.63	7.1	5.5	15.3	13.2	8.9
se	1.37	1.43	3.67	0.77	0.3	1.99	68.4	7.33	4.52

† DH; days to 50% heading, DM; days to 50% maturity, PH; plant height (cm), By; biological yield per plot (kg), GY; grain yield per plot (kg), HI% harvest index percentage, SM; number of spikes per square meter, KS; number of kernels per spike, 1000KW; 1000 kernels weight**.**

Supplementary Table S3: Means of the studied traits for Sids 12 derived lines (first trial; S1), season 2022/2023.

Genotype	DH†		DM		PH		BY		GY		HI%		SM		KS		1000KW	
S1#1	96	o	151	lmno	125	ab	13.9	abcd	4.02	bcdefghij	29.0	ghijklmn	463	cd	54.7	cdefghijk	40.6	jklmno
S1#2	81	de	147	fghijk	125	ab	13.4	abcde	3.98	bcdefghij	29.7	efghijklmn	542	abcd	54.6	cdefghijk	40.8	ijklmno
S1#3	84	efg	147	fghijk	110	ef	12.5	bcde	3.75	defghijk	30.2	defghijklmn	568	abcd	49.9	defghijkl	43.4	fghijklmn
S1#4	99	p	152	no	115	cdef	13.5	abcde	3.67	efghijkl	27.4	lmnopq	480	bcd	57.8	bcdefghi	41.4	hijklmno
S1#5	82	def	145	defgh	115	cdef	14.1	ab	4.68	ab	33.3	bcdefg	505	abcd	50.7	defghijkl	45.6	cdefghijkl
S1#6	86	ghi	148	ghijklm	108	f	13.6	abcde	3.68	defghijkl	27.1	mnopq	593	abcd	50.6	defghijkl	41.8	hijklmn
S1#7	95	o	148	fghijkl	118	bcde	13.0	abcde	3.97	bcdefghij	30.5	defghijklm	540	abcd	47.0	efghijkl	41.8	hijklmn
S1#8	81	d	143	abcde	113	def	12.3	bcde	4.35	abcdef	35.3	b	492	abcd	56.4	cdefghij	53.0	abc
S1#9	90	lm	150	klmn	108	f	12.8	abcde	3.55	ghijkl	27.8	jklmnop	617	abcd	41.9	hijkl	38.9	lmno
S1#10	86	ghij	151	klmno	118	bcde	12.4	bcde	3.52	ghijkl	28.4	ijklmno	517	abcd	43.9	ghijkl	41.2	hijklmno
S1#11	81	de	148	fghijkl	120	bcd	12.8	abcde	3.92	bcdefghij	30.9	cdefghijklm	555	abcd	58.8	bcdefgh	47.9	bcdefghij
S1#12	82	def	150	jklmn	118	bcde	12.6	bcde	3.99	bcdefghij	31.8	bcdefghijk	465	bcd	61.2	bcdef	48.3	bcdefghi
S1#13	77	bc	146	efghij	120	bcd	13.4	abcde	4.09	bcdefghi	30.7	cdefghijklm	617	abcd	54.6	cdefghijk	51.3	abcde
S1#14	81	de	148	ghijklm	115	cdef	12.3	bcde	3.54	ghijkl	28.9	hijklmn	542	abcd	34.7	l	28.5	p
S1#15	89	klm	151	klmno	120	abcd	13.0	abcde	3.55	ghijkl	27.4	klmnopq	618	abcd	51.7	defghijkl	41.0	ijklmno
S1#16	86	ghij	147	fghijk	115	cdef	13.0	abcde	3.81	defghij	29.4	fghijklmn	555	abcd	41.6	ijkl	44.3	defghijklm
S1#17	95	o	157	p	115	cdef	13.0	abcde	3.01	kl	23.2	q	472	bcd	55.0	cdefghijk	34.2	op
S1#18	86	ghi	146	efghij	113	def	13.6	abcde	4.36	abcde	32.1	bcdefghij	592	abcd	39.5	jkl	47.4	bcdefghijk
S1#19	101	p	154	op	118	bcde	13.3	abcde	3.99	bcdefghij	30.1	defghijklmn	570	abcd	62.1	bcdef	39.0	lmno
S1#20	100	p	154	op	118	bcde	12.6	bcde	3.27	jkl	25.9	nopq	582	abcd	73.8	b	39.9	klmno
S1#21	82	def	145	defgh	113	def	11.8	cde	3.94	bcdefghij	33.3	bcdefg	642	abcd	51.1	defghijkl	38.5	lmno
S1#22	86	ghi	144	bcdef	120	abcd	14.9	a	4.61	abc	31.1	bcdefghijklm	575	abcd	56.1	cdefghijk	38.0	mno
S1#23	88	ijkl	149	ijklmn	113	def	12.7	abcde	4.16	bcdefgh	32.9	bcdefgh	535	abcd	52.3	defghijk	40.0	klmno
S1#24	80	d	141	ab	115	cdef	12.7	abcde	3.88	cdefghij	30.6	defghijklm	683	abc	47.2	efghijkl	41.3	hijklmno
S1#25	80	d	143	abcde	113	def	12.2	bcde	4.27	abcdefg	35.0	bc	507	abcd	45.2	fghijkl	50.5	abcdef
S1#26	93	no	151	klmno	123	abc	14.1	ab	4.24	abcdefgh	30.2	defghijklmn	602	abcd	71.5	bc	39.6	lmno
S1#27	80	d	145	cdefg	118	bcde	12.2	bcde	3.99	bcdefghij	32.8	bcdefgh	525	abcd	55.3	cdefghijk	44.2	efghijklm
S1#28	84	fgh	146	efghi	115	cdef	13.2	abcde	3.93	bcdefghij	29.8	efghijklmn	600	abcd	62.1	bcdef	37.1	mno
S1#29	95	o	151	lmno	120	abcd	12.3	bcde	2.96	l	23.9	pq	558	abcd	48.6	efghijkl	38.9	lmno
S1#30	77	b	140	a	128	a	12.8	abcde	3.85	defghij	30.0	defghijklmn	553	abcd	53.1	defghijk	51.4	abcde
S1#31	89	jklm	146	efghij	125	ab	13.1	abcde	3.88	cdefghij	29.8	efghijklmn	457	cd	66.8	bcd	36.6	no
S1#32	82	def	142	abcd	113	def	14.2	ab	4.44	abcd	31.3	bcdefghijklm	722	a	39.1	kl	38.9	lmno
S1#33	73	a	143	abcde	118	bcde	11.7	de	3.51	ghijkl	30.1	defghijklmn	595	abcd	50.4	defghijkl	54.2	ab
S1#34	80	d	145	cdefg	118	bcde	12.9	abcde	4.43	abcde	34.3	bcd	557	abcd	53.5	defghijk	44.6	defghijklm
S1#35	80	cd	143	abcde	120	abcd	11.6	e	3.75	defghijk	32.5	bcdefghi	518	abcd	49.9	defghijkl	48.7	bcdefgh
S1#36	84	fgh	146	efghi	118	bcde	12.5	bcde	4.17	bcdefgh	33.5	bcdef	507	abcd	50.8	defghijkl	47.6	bcdefghij
S1#37	73	a	141	abc	118	bcde	11.4	e	3.34	ijkl	29.5	fghijklmn	415	d	48.2	efghijkl	57.9	a
S1#38	87	hijk	146	efghij	118	bcde	13.2	abcde	4.37	abcde	33.3	bcdefg	450	cd	53.9	defghijk	42.1	ghijklmn
S1#39	111	q	152	mno	115	cdef	14.0	abc	3.36	ijkl	24.1	opq	632	abcd	49.6	efghijkl	40.0	klmno
S1#40	84	efg	146	efghi	123	abc	13.2	abcde	4.16	bcdefgh	31.6	bcdefghijkl	570	abcd	60.0	bcdefg	42.9	ghijklmn
S1#41	87	hijk	150	klmn	115	cdef	12.4	bcde	3.48	hijkl	28.2	ijklmnop	570	abcd	62.4	bcde	44.2	efghijklm
S1#42	91	mn	149	hijklmn	115	cdef	13.2	abcde	3.59	fghijkl	27.4	lmnopq	700	ab	43.3	ghijkl	37.3	mno
Sids 12 (protected)	82	def	146	efghi	110	ef	8.6	f	3.73	defghijk	43.7	a	183	e	102.1	a	51.8	abcd
Misr 3	88	ijkl	148	fghijkl	115	cdef	14.8	a	4.94	a	33.4	bcdefg	483	bcd	50.2	defghijkl	50.5	abcdef
Giza 171	84	fgh	148	fghijkl	118	bcde	12.4	bcde	4.18	bcdefgh	34.0	bcde	407	d	49.1	efghijkl	49.5	bcdefg
%cv	1.45		1.24		2.8		6.9		7.9		7.03		17.5		15.72		8.54	
se	1.246		1.82		3.212		0.891		0.309		2.147		95		8.425		3.712	

† DH; days to 50% heading, DM; days to 50% maturity, PH; plant height (cm), By; biological yield per plot (kg), GY; grain yield per plot (kg), HI% harvest index percentage, SM; number of spikes per square meter, KS; number of kernels per spike, 1000KW; 1000 kernels weight.

Supplementary Table S4: Means of the studied traits for Sids 12 derived lines (second trial; S2), season 2022/2023.

Genotype	DH†		DM		PH		BY		GY		HI%		SM		KS		1000KW	
S2#1	97	qrst	147	fghi	115	Cdef	11.8	bcdefg	2.64	q	22.5	opq	775	a	42.1	hi	46.4	abcdefghijk
S2#2	89	jklmnop	151	jkl	115	Cdef	13.4	abcd	3.78	bcdefghijklmno	28.3	ijklm	567	bcdefghijklmno	60.4	bcdefghi	42.1	defghijklmno
S2#3	97	rst	143	abcde	118	bcdef	12.0	bcdefg	3.84	bcdefghijklmn	32.1	cdefghijk	508	efghijklmno	57.3	bcdefghi	42.2	defghijklmno
S2#4	93	nopqr	150	ijk	115	Cdef	11.4	defg	3.28	ijklmnopq	28.9	ghijklm	722	ab	62.9	bcdefg	39.8	hijklmno
S2#5	90	lmnopqr	141	ab	110	ef	11.2	efg	3.14	mnopq	28.1	ijklm	472	ijklmno	69.0	bcd	45.6	abcdefghijkl
S2#6	85	efghijkl	152	klm	115	cdef	12.9	abcdef	3.64	fghijklmno	28.4	hijklm	675	abcd	48.2	efghi	38.5	klmno
S2#7	95	opqrs	152	klmn	118	bcdef	13.1	abcde	3.55	ghijklmnop	27.3	klmn	455	jklmno	48.4	efghi	36.7	no
S2#8	92	mnopqr	154	lmn	115	cdef	12.8	abcdefg	3.49	hijklmnop	27.0	lmno	418	mno	75.5	b	40.6	ghijklmno
S2#9	103	stu	156	n	120	abcdef	13.7	ab	3.00	opq	21.9	pq	598	bcdefghijk	43.3	ghi	41.5	fghijklmno
S2#10	94	nopqr	150	ijk	120	abcdef	12.6	abcdefg	3.25	klmnopq	26.0	mnop	583	bcdefghijkl	45.1	fghi	39.4	ijklmno
S2#11	80	bcdefg	144	abcdefg	108	f	12.9	abcdef	4.51	abcd	34.9	bcde	525	defghijklmno	53.6	defghi	50.1	abcd
S2#12	75	abc	146	defgh	120	abcdef	12.9	abcdefg	3.31	ijklmnopq	25.8	mnop	477	hijklmno	52.0	defghi	49.1	abcdef
S2#13	72	a	143	abcde	118	bcdef	11.7	bcdefg	3.08	nopq	26.1	mnop	617	abcdefghij	48.2	efghi	53.3	a
S2#14	73	ab	142	abc	118	bcdef	10.8	gh	2.76	pq	24.9	mnopq	552	cdefghijklmno	61.9	bcdefgh	47.7	abcdefgh
S2#15	72	a	142	abcd	110	ef	11.6	cdefg	3.28	ijklmnopq	28.7	hijklm	600	bcdefghijk	48.9	defghi	47.0	abcdefghi
S2#16	81	bcdefg	145	bcdefg	115	cdef	11.1	efg	3.77	bcdefghijklmno	34.0	bcdef	508	efghijklmno	51.4	defghi	42.7	defghijklmn
S2#17	80	bcdef	142	abcd	113	def	9.0	hi	3.27	jklmnopq	35.8	bcde	418	mno	61.7	bcdefgh	44.8	cdefghijklm
S2#18	77	abcd	142	abcd	128	abc	12.3	abcdefg	3.63	fghijklmno	29.6	fghijklm	662	abcde	57.4	bcdefghi	48.3	abcdefg
S2#19	81	bcdefg	142	abcd	123	abcde	11.9	bcdefg	4.11	abcdefgh	34.7	bcde	532	defghijklmno	59.2	bcdefgh	49.5	abcde
S2#20	85	fghijklm	143	abcdef	118	bcdef	12.9	abcdef	4.28	abcdefgh	33.2	bcdefgh	525	defghijklmno	49.2	defghi	41.6	efghijklmno
S2#21	102	tu	156	n	120	abcdef	13.7	ab	3.62	fghijklmno	26.4	mnop	593	bcdefghijk	44.5	ghi	34.4	o
S2#22	88	hijklmnop	149	hijk	133	a	12.0	bcdefg	3.78	bcdefghijklmno	31.5	defghijkl	723	ab	55.8	bcdefghi	39.9	hijklmno
S2#23	82	cdefghijk	145	bcdefg	115	cdef	12.7	abcdefg	3.67	efghijklmno	29.0	ghijklm	508	efghijklmno	40.3	i	41.3	fghijklmno
S2#24	89	ijklmnop	143	abcdef	118	bcdef	13.7	ab	3.98	abcdefghijk	29.1	ghijklm	640	abcdefgh	74.9	bc	46.4	abcdefghijk
S2#25	87	fghijklmn	144	abcdefg	115	cdef	12.7	abcdefg	3.96	abcdefghijkl	31.3	efghijkl	517	defghijklmno	54.0	defghi	42.5	defghijklmn
S2#26	84	defghijkl	141	a	110	ef	11.2	efg	4.07	abcdefghi	36.3	bcd	547	cdefghijklmno	46.4	fghi	46.9	abcdefghi
S2#27	81	cdefgh	144	abcdefg	118	bcdef	12.9	abcdefg	4.52	abc	35.2	bcde	450	klmno	60.3	bcdefghi	43.5	defghijklmn
S2#28	85	efghijkl	143	abcde	113	def	13.1	abcde	4.56	ab	35.1	bcde	547	cdefghijklmno	64.9	bcdef	46.1	abcdefghijk
S2#29	80	bcdef	141	ab	110	ef	12.0	bcdefg	4.31	abcdefg	36.1	bcde	550	cdefghijklmno	49.8	defghi	46.7	abcdefghij
S2#30	88	ghijklmno	146	efghi	118	bcdef	13.8	ab	4.52	abc	32.9	bcdefghi	657	abcdef	46.6	fghi	42.4	defghijklmn
S2#31	81	cdefgh	147	ghij	113	def	12.0	bcdefg	3.83	bcdefghijklmn	32.1	cdefghijk	647	abcdefg	54.5	defghi	53.0	ab
S2#32	80	bcdef	142	abcd	115	cdef	12.2	bcdefg	4.56	ab	37.4	b	525	defghijklmno	58.0	bcdefghi	49.6	abcde
S2#33	77	abcde	144	abcdefg	115	cdef	11.2	efg	3.79	bcdefghijklmno	34.0	bcdef	525	defghijklmno	49.8	defghi	44.9	cdefghijklm
S2#34	94	nopqr	151	klm	115	cdef	11.4	defg	3.17	lmnopq	27.8	jklmn	700	abc	55.1	cdefghi	43.4	defghijklmn
S2#35	81	cdefghi	143	abcde	115	cdef	12.5	abcdefg	4.36	abcdef	35.0	bcde	578	bcdefghijklmn	68.0	bcde	45.3	bcdefghijkl
S2#36	77	abcd	143	abcde	115	cdef	10.9	fgh	3.71	defghijklmno	34.1	bcdef	410	o	49.0	defghi	49.3	abcdef
S2#37	87	ghijklmno	143	abcdef	120	abcdef	12.3	bcdefg	4.28	abcdefgh	35.0	bcde	625	abcdefghi	52.5	defghi	45.9	abcdefghijk
S2#38	81	bcdefg	144	abcdefg	120	abcdef	11.5	cdefg	4.18	abcdefgh	36.7	bc	592	bcdefghij	54.9	defghi	48.5	abcdefg
S2#39	81	bcdefg	145	cdefgh	118	bcdef	11.6	cdefg	3.89	abcdefghijklm	33.7	bcdefg	500	efghijklmno	51.9	defghi	47.0	abcdefghi
S2#40	80	bcdef	143	abcdef	120	abcdef	12.0	bcdefg	4.12	abcdefgh	34.3	bcdef	492	ghijklmno	46.6	fghi	38.7	jklmno
S2#41	88	ghijklmno	147	fghi	118	bcdef	12.5	abcdefg	4.07	abcdefghi	32.9	bcdefghi	495	fghijklmno	59.0	bcdefgh	45.4	abcdefghijkl
S2#42	80	bcdef	145	bcdefg	115	cdef	12.6	abcdefg	4.56	ab	36.3	bcd	425	lmno	61.4	bcdefgh	44.4	cdefghijklmn
S2#43	78	abcde	141	a	120	abcdef	12.1	bcdefg	4.34	abcdefg	35.9	bcde	438	klmno	52.1	defghi	47.7	abcdefgh
S2#44	90	klmnopq	147	ghij	118	bcdef	12.6	abcdefg	4.06	abcdefghij	32.3	cdefghij	582	bcdefghijklm	49.4	defghi	37.0	mno
S2#45	77	abcd	144	abcdefg	130	ab	12.4	abcdefg	4.27	abcdefgh	34.6	bcde	417	no	54.6	defghi	46.0	abcdefghij
S2#46	105	u	155	mn	120	abcdef	11.4	defg	2.65	q	23.4	nopq	407	o	47.0	fghi	37.7	lmno
S2#47	106	u	156	n	120	abcdef	12.7	abcdefg	2.62	q	20.7	q	475	ijklmno	53.8	defghi	42.1	defghijklmno
Sids 12 (protected)	82	cdefghij	146	defgh	110	ef	8.6	i	3.73	cdefghijklmno	43.7	a	183	p	102.1	a	51.8	abc
Misr 3	95	pqrst	147	fghi	113	def	14.4	a	4.66	a	32.5	cdefghij	653	abcdefg	45.4	fghi	48.7	abcdef
Giza 171	82	cdefghij	147	ghij	125	abcd	13.6	abc	4.45	abcde	32.9	bcdefghi	585	bcdefghijkl	49.3	defghi	52.4	abc
%cv	4.1		1.27		6.02		8.38		10.38		6.3		14.95		14.8		7.3	
Se	3.499		1.844		7.045		1.021		0.3941		1.99		81.25		8.121		3.242	

† DH; days to 50% heading, DM; days to 50% maturity, PH; plant height (cm), By; biological yield per plot (kg), GY; grain yield per plot (kg), HI% harvest index percentage, SM; number of spikes per square meter, KS; number of kernels per spike, 1000KW; 1000 kernels weight.