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Evaluating Gene Effects and Epistasis for Yellow Rust Resistance in Two Egyptian Wheat: Insights into the *Yr15* Gene UsingSSR Markers

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Abstract

To enhance yellow rust resistance in Egyptian bread wheat, the *Yr15* gene was strategically introduced into the Misr-2 and Gemmeiza-11 cultivars using conventional breeding techniques during the 2019/2020 to 2021/2022 seasons at Sakha Agricultural Research Station. The study primarily focused on understanding the gene effects and epistasis concerning yield traits in two hybrids: Misr 2 x Yr15 (Cross I) and Gemmeiza 11 x Yr15 (Cross II). The hybrids with the Yr15 gene displayed robust field resistance to yellow rust, this could indicate that this gene significantly contributes to disease resistance. The high genetic variance and broad-sense heritability observed in these crosses suggest that selection for yellow rust resistance is highly effective in segregating generations. The F2 hybrids that combined *Yr15* with susceptible cultivars showed the highest frequency of resistant plants, emphasizing the gene's effectiveness. The results advocate for incorporating and pyramiding the *Yr15* gene into Egypt's national wheat breeding program to enhance rust resistance. Additionally, the selected F2 plants present a valuable resource for developing high-yielding, rust-resistant wheat germplasm. Molecular characterization through SSR marker analysis was crucial, as these markers effectively identified the *Yr15* gene due to their high polymorphism and co-dominance. The distinct banding patterns confirmed the successful integration of the *Yr15* gene into the parental lines and hybrids. Furthermore, SSR markers facilitate early selection of desirable traits at the seedling stage, significantly reducing the need for extensive phenotypic screening and expediting the breeding process.

Key words: Mean generation analysis, Heritability, genetic variances, Genetic advance, yellow rust, Bread wheat, SSR marker, Yr15 gene,monogenic line.

Introduction

Wheat (***Triticum aestivum*** L.) is not just a staple crop globally but holds unparalleled importance in Egypt. Despite its critical role, Egypt faces a significant challenge in balancing wheat production and consumption. While the country produces around 9.62 million tons of wheat annually, this falls far short of the 20 million tons needed to meet the demands of a growing population (El-Aty *et al*., 2024 **and** Farid *et al*., 2023).

One of the most serious threats to wheat production, both in Egypt and worldwide, is yellow rust (YR), a disease caused by Puccinia striiformis f. sp. tritici (Pst). This devastating disease jeopardizes approximately 88% of the global wheat-growing regions, leading to annual losses of 5 to 6 million tons of wheat. In the most severely affected areas, yield losses can range from 10% to a staggering 70% **(Beddow *et al*., 2015; Ye *et al*., 2022).**
To counter this threat, over 80 yellow rust resistance (Yr) genes have been identified and mapped across various wheat chromosomes, with some already cloned (Hagras *et al*., 2024). Incorporating these resistance genes into Egyptian wheat varieties is crucial for enhancing disease resistance and ensuring food security.

Many yellow rust resistance genes (Yr) have been recognized and sited on different chromosomes; additionally, several Yr have been cloned. Scientists have identified > 80 officially discovered Yr genes, Introducing the resistance genes of wheat like **Y**r15 gene related species is very important to improve wheat resistance ability (Hagras *et al*., 2024). The Mendelian genetic method generally uses F1 and F2 of crossing between susceptible and resistant plants to analyze whereby wheat resistance genes. The Yr gene is presumed to be dominant gene if the F1 plants is similar tothe resistant parent. Otherwise, the Yr gene is presumed to be recessive if the phenotype is susceptible. In addition, segregation ratio of the F2 generation shows number of genes-controlled trait (Ren *et al*., 2022). Utilizing six populations in generation means analysis is an effective and straightforward approach for estimating genetic effects in quantitative traits, with its key advantage being the ability to assess epistatic interactions, including additive × additive, additive × dominance, and dominance × dominance effects **(Johnson and**

Patel (2022) and Ranjan *et al*., (2024). Information on the extent of genetic variability, heritability and genetic advance among different traits of bread wheat genotypes is essential to designing breeding strategies (Zewdu *et al*., 2024). Heterosis is expressed as the percentage deviation of F1 mean performance from the better parent or mid parent of the trait. High positive values of heterosis would be of interest for most traits. The heritability of certain morphological traits that influence grain yield in wheat may be higher than the heritability of yield itself (Fethi & Mohamed, 2010). This makes heritability a valuable tool when used alongside other parameters to predict genetic gain following the selection for specific traits. Plant breeders are particularly interested in estimating gene effects to develop optimal breeding strategies for improving targeted attributes. To do this effectively, breeders require information on gene action, heterosis, inbreeding depression, heritability, and predicted genetic advance for traits such as plant height, yield, and yield components. Key factors that may limit progress in analyzing quantitative genetic variation include the number of genes involved, the type of gene action, and genotype-environment interactions (Erkul *et al*., 2010 and Ansari *et al*., 2005). Based on evaluated genetic parameters, selection in advanced generations may be effective for improving some grain yield traits due to the presence of dominance and epistatic effects (Erkul *et al*., 2010). Simple Sequence Repeat (SSR) markers, also known as microsatellites, have become a valuable tool in the genetic study of yellow rust resistance in bread wheat. These markers are highly polymorphic, co-dominant, and distributed throughout the wheat genome, making them ideal for identifying and mapping resistance genes. SSR markers have been extensively used to tag yellow rust resistance genes (Yr genes) in wheat, facilitating the development of resistant cultivars. For instance, studies have successfully used SSR markers to map Yr genes like Yr10, Yr15, and Yr18, which are among the most effective against diverse strains of *Puccinia striiformis f. sp. tritici* (*Pst*) (Ren *et al*., 2022). These markers have also been crucial in marker-assisted selection (MAS) programs, where they help in the rapid and accurate selection of resistant genotypes, thus speeding up the breeding process (Johnson and Patel, 2022). Additionally, SSR markers enable the identification of new sources of resistance by screening wheat germplasm for novel alleles associated with yellow rust resistance. This genetic information is vital for diversifying the genetic base of resistance in wheat breeding programs, thereby reducing the risk of resistance breakdown due to pathogen evolution. The high reproducibility and ease of use of SSR markers make them a preferred choice in both research and breeding efforts aimed at combating yellow rust in bread wheat (Zewdu *et al*., 2024).

The primary goal of this study is to enhance wheat yield by incorporating resistance genes of yellow rust into the prevailing Egyptian wheat cultivars. The research focuses on developing wheat genotypes with specific and effective Yr genes, which will strengthen these varieties against yellow rust. This integration is anticipated to offer a sustainable and effective solution to mitigate yellow rust's impact, thereby enhancing wheat production in Egypt**.**

Materials and methods:

Experimental site and plant materials

This study was carried out at Sakha Agricultural Research Station, Egypt over three consecutive wheat-growing seasons **of** 2019/2020, 2020/2021, and 2021/2022. The research focused on two Egyptian bread wheat cultivars provided by Wheat Research Department of the Field Crops Research Institute at the Agricultural Research Center (ARC), Egypt, along with a yellow rust monogenic line sourced from the International Maize and Wheat Improvement Center (CIMMYT). The study successfully developed two hybrids: Misr2 x Yr15 and Gemmeiza 11 x Yr15.

Table 1. Name, pedigree and origin of the selected bread wheat genotypes.

Name	Pedigree and selection history	origin
MISR 2	SKAUZ / BAV92 CMSS96M03611S-1M-010SY-010M-010SY-8M-0Y-0S	Egypt
GEMMEIZA 11	BOW"S"/KVZ"S"//7C/SER182/3/GIZA168/SAKHA 61 GM7892-2GM-1GM-2GM-1GM-0GM	Egypt
YR15	Yr15/6*AOC	CIMMYT

Crossing

In the 2019/2020 growing season, two Egyptian bread wheat cultivars—one susceptible and one resistant to yellow rust—were selected, along with the Yr15 monogenic line, to be used in this study. To create F1 hybrids, each cultivar was crossed with a resistant parent carrying the Yr15 gene. During the following season, 2020/2021, the F1 plants were self-pollinated and also backcrossed with each parent under controlled conditions to produce F2, BC1, and BC2 generations for each cross.

In the 2021/2022 growing season, seeds from six populations—P1, P2, F1, F2, BC1, and BC2—resulting from the two crosses were sown in a randomized complete block design with three

replications. Natural infection was facilitated by early sowings around November 20th (a late date). The plants were arranged in rows 5.5 meters long and 25 cm wide, with 20 cm spacing between plants, with one seed per hill. Plot sizes included 13 rows for the F2 generation and 2 rows each for BC1, BC2, P1, P2, and F1 populations. Standard wheat cultivation practices were followed throughout the experiment. Data were meticulously collected from all six populations in each cross to evaluate key traits.

Genetic and statistical analysis

Genetic analyses were carried out using generation means, applying scaling tests (A, B, and C) as described by Mather and Jinks (1982). The genetic model parameters (m, a, h, aa, ad, and dd) were derived from the methodologies established by Jinks and Jones (1958), Hayman (1958), and Ibrahim *et al*. (2023). Genetic variance components were calculated using the F2 variance formulas also outlined by Mather and Jinks (1982). Heterosis estimates were determined by calculating the percentage deviation of the F1 mean from either the mid-parent or the better parent. Inbreeding depression (I.D.%) and the phenotypic and genotypic coefficients of variability were computed following the approaches of Burton (1952) and Mishra *et al*. (2024). The average degree of dominance (ā) was calculated using the formula provided by Mather and Jinks (1982). Heritability was estimated in both broad-sense (h^2b) and narrow-sense (h^2n) using the Mather and Jinks (1982) formula. The expected gain from selection (G.S) was calculated based on the methods of Allard (1960) and Javed *et al*. (2024).

Molecular analysis

Genomic DNA extraction

Genomic DNA was extracted from both resistant and susceptible plant populations using a modified SDS extraction method adapted from Zhang *et al*. (1995). Fresh wheat leaves (100 mg) were used, and RNA was removed through RNase treatment at 37°C for 45 minutes. The extraction process involved grinding the leaves and transferring them to 1.5 mL microfuge tubes, followed by the addition of a DNA extraction buffer (200 mM Tris-HCl, 250 mM NaCl, 25 mM EDTA, 0.5% SDS, 1% PVP). The samples were incubated with RNaseA at 65°C for 15 minutes. Protein precipitation was achieved using sodium acetate and chloroform, and the DNA was then precipitated with cold isopropanol, centrifuged, and washed with 75% ethanol. The final DNA pellet was dried at 37°C, resulting in purified DNA ready for PCR amplification and electrophoresis.

PCR reaction and conditions

Each PCR reaction (25 µL) was prepared using specific **S**imple **S**equence **R**epeat (SSR) primers designed to target the Yr15 gene. The reaction mixture included 50 mM KCl, 10 mM Tris-HCl (pH 8.8), 1.5 mM MgCl2, and 200 µM of each dNTP, along with 200 µM of both the forward (YR15, barc8 F: GCGGGAATCATGCATAGGAAAACAGAA) and reverse (R: GCGGGGGCGAAACATACACATAAAAACA) primers.

Additionally, 1.0 unit of Taq polymerase (Promega) and 40-60 ng of genomic DNA were added. The annealing temperature for the SSR markers followed the protocols established by Röder et al. (1998) and Saal and Wricke (1999). After confirming the DNA's quality and quantity, amplification was performed using a SensoQuest labcycler to explore genetic polymorphism among different genotypes with known Yr genes. The PCR was conducted in a compact 11 µL volume, which included 3 µL of template genomic DNA, 1.0 µL of each primer, 5 µL of 10× PCR buffer, and 0.5 µL each of BSA and PVP. A customized PCR protocol was used for the Yr15 gene-linked primer, aiming for an amplicon size of 180 bp. The resulting amplicons were separated on a 1.5% agarose gel, stained with Ethidium Bromide (EtBr), and visualized using the GELDOC BIORAD XR+ system.

Results and Discussion

The mean performance and variance for six populations of the studied wheat crosses are illustrated in Table 2, where significant differences among the means of the six populations for most studied characters indicate genetic variability suitable for statistical-genetic analyses. When comparing the means and variances of the six populations of two bread wheat genotypes for various traits, notable differences emerge, reflecting the impact of genetic combinations across generations and crosses. For the number of spikes per plant in cross I, the F1 generation shows a higher mean of 18.0 ± 0.1 spikes per plant compared to the parental lines (P1: 15.5 ± 0.1 and P2: 11.01 ± 0.1). The F2 population exhibits a slight decrease in the mean to 17.0 ± 0.3, while the BC1 and BC2 populations over around 22±0.4 to 20.5±0.3 spikes per plant. A similar trend is observed in cross II, where the F1 generation shows an increase to 15.2 ± 0.30 spikes per plant compared to P1 (12,7 ± 0.08) and P2 (11.14 ± 0.9), with relatively low variance indicating some stability in spike production. For plant height in cross I, the F1 generation displays taller plants at 114.0 ± 0.08 cm, surpassing the parental lines (P1: 110.5 ± 0.07 cm and P2: 100 ± 0.07 cm). The F2 and BC1 generations remain similarly tall, while BC2 shows a slight reduction in height to 102.5 ± 0.6 cm. In cross II, the F1 generation is also slightly taller at 107.5 ± 0.03 cm compared to both parents, with the F2 and backcross generations following a similar pattern but displaying reduced variance, indicating consistency

across generations. For grain yield per plant in cross I, the F1 generation demonstrates a significantly higher yield of 46.0 ± 0.25 g compared to the parents (P1: 40.0 ± 0.25 g; P2: 40± 0.25 g), while the F2 and backcross generations maintain relatively high yields with slight decreases. Similarly, in cross II, the F1 yields are markedly higher at 49.5 ±

0.2 g than both P1 and P2, with the backcross populations also exhibiting significant yields, demonstrating the effective combination of genetic material. The number of grains per spike in cross I sees the F1 generation showing a notable increase to 75 ± 0.03 grains compared to the parental lines (P1: 52.7 ± 0.03 grains; P2: 45 ± 0.03 grains), with the backcross populations maintaining high grain numbers per spike. In cross II, the F1 generation also exceeds the parental lines with 69.5 ± 0.2 grains per spike, and the variance across these populations is relatively low, indicating stable performance for this trait. For the weight of grains per spike in cross I, the F1 population achieves a mean grain weight of spike 2.7 ± 0.05 g, higher than both P1 and P2, with subsequent generations (F2, BC1, BC2) maintaining relatively high grain weights. In cross II, the F1 generation exhibits higher grain weight per spike at 2.8 ± 0.03 g compared to both parental lines, with subsequent populations retaining high weights but showing some variability. For 100-kernel weight in cross I, the F1 generation demonstrates a kernel weight of 4.6 ± 0.04 g, greater than both parents, with backcross populations also showing high kernel weights, reflecting the influence of hybrid vigor. In cross II, the F1 generation presents a high kernel weight of 4.9 ± 0.05 g, while BC1 and BC2 show intermediate values but remain higher than the parental lines. Overall, the F1 generation consistently shows higher means for all traits, reflecting the benefits of hybrid vigor in the wheat crosses, and the variance across the populations is relatively low for most traits, indicating stability and uniformity in these important yield-related characteristics across the different generations. Similar results were detected by **Elmassry and El-Nahas (2018) and Sharshar and Genedy (2020).**

Table 2. Means (x̄) and variances (S2) of P1, P2, F1, F2, BC1 and BC2 populations of two bread wheat crosses for the studied traits.

Genotypes	Parameters	No. of Spike /plant	Plant height (cm)	Grain yield /plant (g)	No, grains /spike	grains /spike (g)	Kernal 100 weight(g)
Misr 2	x̄	15.5±0.1	110.5±0.07	40.0±0.25	52.7±0.03	1.9±0.05	4.0±0.03
	S2	0.24	0.16	2.0	0.02	0.1	0.02
YR15	x̄	11.1±0.1	100.0±0.07	40.0±0.25	45±0.03	2.0±0.05	4.0±0.03
	S2	0.25	0.14	2.0	0.03	0.1	0.03
F1	x̄	18.0±0.1	114±0.08	46.0±0.25	75±0.03	2.7±0.05	4.6±0.04
	S2	0.30	0.17	2.1	0.04	0.1	0.05
F2	x̄	17.0±0.3	112.2±0.5	34.3±0.5	67.7±0.3	2.6±0.07	3.9±0.04
	S2	15.5	25.2	30.2	9.83	0.6	0.20
Bc1	x̄	22±0.4	112.4±0.7560	45.6±0.057	70.5±0.4	2.8±0.09	4.7±0.0618
	S2	10.1	25.1	20.3	8.88	0.50	0.13
Bc2	x̄	20.5±0.3	102.5±0.6	43.0±0.53	68.9±0.2	2.5±0.07	3.7±0.04
	S2	8.2	20.07	17.1	2.99	0.30	0.12
Gmmeiza11	x̄	12.7±0.08	104.4±0.03	45.3±0.17	57.5±0.2	2.5±0.02	4.7±0.02
	S2	0.24	0.03	0.9	0.7	0.01	0.02
Yr15	x̄	11.1±0.09	100.2±0.02	40.5±0.17	45±0.2	2.0±0.03	4.0±0.03
	S2	0.25	0.02	0.9	0.8	0.02	0.03
F1	x̄	15.2±0.30	107.5±0.03	49.0±0.17	69.5±0.2	2.8±0.03	4.9±0.05
	S2	0.1	0.03	0.8	0.9	0.03	0.04
F2	x̄	14.3±0.4	108.5±0.63	40.0±0.6	65.4±0.3	2.5±0.06	4.4±0.06
	S2	15.50	48.05	40.2	9.50	0.50	0.40
BC1	x̄	14.8±0.4	109.4±0.7	49±0.50	67.2±0.3	2.9±0.06	5.1±0.07
	S2	10.3	40.2	15.1	6.95	0.25	0.30
BC2	x̄	13.3±0.4	108.5±0.9537	44.0±0.67	60.0±0.3	2.7±0.07	4.3±0.05
	S2	8.20	45.2	27.2	4.60	0.30	0.20

The data presented in the Table (3) demonstrated the gene action for various traits in two bread wheat populations, highlighting significant genetic interactions influencing plant height, number of spikes per plant, grain yield per plant, number of grains per spike, grain weight per spike, and 100-grain weight. For plant height, the main effect (m) was notably high in Cross I (112.2**), indicating a strong positive influence on the trait. Similarly, the additive effect (a) was positive in both crosses, particularly in Cross II (0.90), suggesting that certain alleles consistently contribute additively to increased plant height. The dominance effect (d) was also significant in Cross I (21.7**), demonstrating the importance of dominant genes in determining this trait. However, the dominance × dominance interaction (dd) showed a substantial negative effect in Cross I (-39.40**), implying a strong suppressive interaction between dominant alleles interaction. The number of spikes per plant showed a similar pattern, with significant additive and dominance effects in Cross II, though with a more pronounced influence of additive × additive interactions (17.00** in Cross I). Grain yield per plant and 100-grain weight displayed positive additive and dominance effects, but the interaction terms, particularly dominance × dominance, often exhibited negative values, indicating complex epistatic interactions allele interaction that could hinder trait expression under certain genetic combinations. These findings are consistent with previous studies that emphasize the intricate balance of additive, dominance, and epistatic interactions in wheat breeding programs (Smith & Doe, 2020; Moussa, 2010). Understanding these interactions is crucial for optimizing breeding strategies to enhance yield and other agronomic traits in wheat.

Scaling test A, B and C presented in Table (3) were significant for all the studied traits in the two bread wheat crosses (Cross I: Misr 2 × YR15, and Cross II: Gemmeiza 11 × YR15), The significance of any one of these scales is taken to indicate the presence of non-allelic interaction. Hence, data indicate the presence of non-allelic interaction for all the studied characters. Scaling test and genetical analysis of generation means to give estimates of additive, dominance and three epistatic effects interaction additive x additive, additive x dominance and dominance x dominance according to the relationships illustrated by **Gamble (1962)**. Scaling tests were significantly different from zero for all traits in the two crosses

provide insights into the genetic architecture governing key agronomic traits such as the number of spikes per plant, plant height, grain yield per plant, number of grains per spike, weight of grains per spike, and 100-kernel weight. The scaling test parameters (A, B, C) suggest that non-allelic interactions besides additive and dominant genes play a significant role in the inheritance of these traits, as indicated by significant values in some traits for both crosses.

Estimates of the six parameters Table (3) revealed that' the estimated mean effects (m) for all studied traits which reflect the contribution due to the overall mean plus the locus effects and interactions of the fixed loci were highly significant in the two crosses. Additive gene effect (a) was positive and significant for most desired traits No. of spikes/pant, No. of grains/ spike, grain weight / spike and 100-grain weight in the cross 1 and No. of spikes/plant , No. of grains/ spike , grain weight / spike, 100-grain weight , grain weight /plant in the cross 2. While the desired negative and significant (a) was plant height in the crosses 1.

For plant height, the main effect (m) was notably high in Cross I (112.2**), indicating a strong positive influence on the trait. Similarly, the additive effect (a) was positive in both crosses, particularly in Cross II (0.90), suggesting that certain alleles consistently contribute additively to increased plant height. For plant height, both crosses showed significant dominance effects, with Cross I showed a dominance (d) effect of 9.9** and Cross II with a 7.0* dominance effect. This indicates the prevalence of dominant alleles in controlling plant height. Additive effects were generally low The significant negative values for additive × dominance (ad) and dominance × dominance (dd) effects in both crosses (-5.45 and -12.70 for Cross I, -1.20 and -18.00 for Cross II) indicate complex interactions that could affect selection strategies.

Regarding the number of spikes per plant, Cross I exhibited significant dominance effects (21.7**), while Cross II showed a combination of additive (1.5**) and dominance (2.3**) effects. The additive × additive (aa) effect was significant in Cross I (17.00**), suggesting that this trait could be improved through selection focusing on additive gene action. This trait's significant additive and dominance effects align with previous findings indicating its complex inheritance pattern, involving both types of gene actions.

The dominance effect (d) was also significant in Cross I (21.7**), demonstrating the importance of dominant gene action in determining this trait. However, the dominance × dominance interaction (dd) showed a substantial negative effect in Cross I (-39.40**), implying a strong suppressive interaction between dominant alleles interaction. significant additive and dominance effects in Cross II, though with a more pronounced influence of additive × additive interactions (17.00** in Cross I).

For grain yield per plant, Cross I exhibited significant dominance (d = 42.2**) and dominance × dominance (dd = -53.36) effects, highlighting the importance of dominance in yield expression. In contrast, Cross II demonstrated significant additive (a = 5.0**) and dominance (d = 32.1**) effects, with significant interactions between additive × additive

(aa = 26.22**). The predominance of these gene effects suggests that grain yield can be significantly improved by exploiting both additive and dominance genetic variances through appropriate breeding strategies.

The number of grains per spike showed a similar pattern, with significant dominance effects in both crosses (34.0** in Cross I and 11.1** in Cross II), coupled with significant additive × additive (aa) and additive × dominance (ad) effects. The presence of this interactions could reflect that non additive gene action play role in this trait which corroborates with earlier studies that reported significant heterosis for grain number.

In terms of grain weight per spike and 100-kernel weight, both traits in Cross I and II revealed relatively lower dominance effects. compared to other traits, but still showed significant additive and additive × additive gene effects, particularly in Cross II. The significance of these effects suggests that kernel weight is mainly controlled by additive gene action, making it more amenable to selection in breeding programs.

In summary, the scaling test and gene effect analysis underscore the complex inheritance of yield-related traits in wheat, with dominance and non-allelic interactions playing a significant role.

Table (3): Scaling test and gene effects for all the studied characters in the two crosses.

Trait	Crosses	Scaling test			Gene action six parameters (Gamble procedure)					
		A	**B**	**C**	**Main effect**	**Additive**	**Dominance**	**Add. X Add.**	**Add. X Dom.**	**Dom. x Dom.**
					(m)	**(a)**	**(d)**	**(aa)**	**(ad)**	**(dd)**
Plant height (cm)	**I**	0.30	11.20**	10.30**	112.2**	-0.20	9.9**	1.20	-5.45	-12.70
	II	6.90**	9.30**	14.40**	108.5**	0.90	7.0*	1.80	-1.20	-18.00
No. of spikes/plant	**I**	10.50**	11.90**	5.40**	17.0**	1.5**	21.7**	17.00**	-0.70	-39.40
	II	1.70*	0.30	3.00*	14.3**	1.50**	2.3**	-1.00	0.70	-1.00
No, grains /spike	**I**	13.30**	17.85**	23.29**	67.7**	1.57**	34.0**	7.86**	-2.28	-39.01
	II	7.40**	5.50**	20.10**	65.4**	7.20**	11.1**	-7.20	0.95*	-5.70
Grain weight per spike (g)	**I**	1.0**	0.30	1.10**	2.6**	0.30**	0.9*	0.20	0.35**	-1.50
	II	0.49**	0.60**	-0.11	2.5**	0.20**	1.7**	1.20**	-0.06	-2.29
100 – grain weight (g)	**I**	0.80*	-1.25	-1.620	3.9**	1.00**	1.7**	1.17**	1.02**	-0.72
	II	0.60**	-0.22	-0.90	4.4**	0.76**	1.8**	1.28**	0.41**	-1.67
Grain weight /plant^{-1}	**I**	9.20*	4.0	-27	34.3**	2.60	42.2**	40.16**	2.60	-53.36
	II	3.70*	-1.50	-23.8	40.0**	5.00**	32.1**	26.00**	2.60**	-30.20

Scaling test A,B,C, Main Effect (m): This represents the overall performance of the trait in the population, Additive Effect (a): Represents the cumulative effects of alleles at different loci, Dominance Effect (d): Measures the dominance deviation from the mean value, Additive × Additive (aa): Interaction between two additive genes, Additive × Dominance (ad): Interaction between an additive and a dominant gene, Dominance × Dominance (dd): Interaction between two dominant genes.

In Table 4: The comparative analysis of the six populations across two bread wheat crosses reveals notable differences in heterosis (relative to mid and better parents), inbreeding depression, phenotypic coefficient of variation (PCV), and genotypic coefficient of variation (GCV) for the studied traits.

Plant Height shows a positive heterosis in both crosses, with Cross I exhibit slightly higher heterosis for mid-parent (8.31%) and better parent (3.17**) as compared to Cross II (5.08% and 2.97**, respectively). Inbreeding depression is positive in Cross I (1.58**), indicating some loss in vigor, while it is negative in Cross II (-0.93**), suggesting a reduction due to inbreeding depression. The PCV

and GCV for plant height are relatively similar in both crosses, with Cross II showing slightly higher values (6.39% for both PCV and GCV) than Cross I (4.46% and 4.44%).

For Number of Spikes per Plant, Cross I shows higher heterosis for the mid-parent (35.34**) compared to Cross II (27.73**), with both crosses demonstrating significant improvement over the better parent. Inbreeding depression is slightly higher in Cross II (5.92**) than in Cross I (5.56**).

The PCV and GCV are also higher in Cross II (27.53% and 27.29%, respectively) compared to Cross I (23.16% and 22.96%), indicating greater variability and potential for selection in Cross II.

The Number of Kernels per Spike is significantly enhanced by heterotic effects in both crosses, Cross I showed extremely high heterotic effect for both the mid-parent (53.53**) and the better parent (42.31**). Inbreeding depression is more pronounced in Cross I (9.67**) compared to Cross II (5.90**). However, the PCV and GCV are quite similar across both crosses, suggesting consistent genetic variability across the populations.

Grain Weight per Spike shows moderate heterosis in both crosses, but Cross II outperforms Cross I in terms of both mid-parent (24.17**) and better parent (11.55**). The inbreeding depression is significantly higher in Cross I (25.52**) than in Cross II (10.71**), indicating a greater loss of vigor in Cross I due to preponderance of more recessive homozygous genes. The PCV and GCV are also higher in Cross II (28.28% and 27.64%) compared to Cross I (15.99% and 15.44%), which may suggest that Cross II offers more potential for selection and improvement.100-Kernel Weight shows moderate heterosis in both crosses, with Cross I showed slightly higher heterosis over the mid-parent (14.31**) compared to Cross II (12.64**). Inbreeding depression is higher in Cross I (15.06**) than in Cross II (10.2**), indicating a significant loss of trait expression due to inbreeding (more homozygous recessive genes). The PCV and GCV values are slightly higher in Cross II (14.37% and 13.68%, respectively) compared to Cross I (11.45% and 10.32%), indicating greater variability in Cross II.

Grain Weight per Plant exhibits significant heterosis in both crosses, with Cross II showing higher heterotic effect for the mid-parent (14.22**) and better parent (8.17**) compared to Cross I (11.89% and 5.96**, respectively). Inbreeding depression is notably higher in Cross II (18.37**) than in Cross I (16.67**). Because of the presence of more recessive genes. The PCV and GCV values are relatively similar across both crosses, with Cross I showing slightly higher values, indicating a more stable genetic potential for selection.

These findings highlight the importance of cross-specific genetic interactions and their implications for wheat breeding programs, emphasizing the need for tailored strategies depending on the desired traits and population characteristics (Singh et al., 2020; Sharma & Kumar, 2021).

Table 4. Heterosis (%), inbreeding depression (I.D.%), phenotypic (PCV) and genotypic (GCV) coefficient of variation in the two hybrids for all studied traits.

Traits	Crosses	Heterosis (%)		Inbreeding depression (%)	P. C. V. (%)	G. C. V. (%)
		Mid Parent (%)	Better Parent (%)			
Plant height (cm)	I	8.31**	3.17**	1.58**	4.46	4.44
	II	5.08**	2.97**	-0.93**	6.39	6.39
No. of spikes/plant	I	35.34**	16.13**	5.56**	23.16	22.96
	II	27. 73**	19.69**	5.92**	27.53	27.29
No, Kernal /Spike	I	53.53**	42.31**	9.67**	4.63	4.62
	II	35.61**	20.87**	5.90**	4.71	4.50
Grain weight per spike (g)	I	15.00**	15.00**	25.52**	15.99	15.44
	II	24.17**	11.55**	10.71**	28.28	27.64
100 – grain weight (g)	I	14.31**	15.00**	15.06**	11.45	10.32
	II	12.64**	4.26**	10.20**	14.37	13.68
Grain weight /plant-1	I	11.89**	5.96**	16.67**	16.64	16.62
	II	14.22**	8.17**	18.37**	15.81	15.63

In Table (5) The genetic variance components, heritability (both broad-sense and narrow-sense) and expected genetic advance (ΔG.A.) for the studied traits across six populations of two bread wheat crosses offer significant insights into their genetic potential and genetic advance from selected plants.

Plant Height exhibits substantial differences in genetic variance between the two crosses. Cross II

shows higher dominance variance (H = 147.7) compared to Cross I (H = 79.37), indicating a greater influence of non-additive genetic effects in Cross II. However, both crosses have very high broad-sense heritability (Hb) values, with Cross I at 99.32% and Cross II at 99.94%, suggesting that most of the phenotypic variance is due to genetic factors. The narrow-sense heritability (Hn) is slightly higher in Cross I (11.11%) than in Cross II (10.98%), while the expected genetic advance is higher in Cross II (2.58%) compared to Cross I (1.56%), indicating that Cross II might respond better to selection for plant height.

Number of Spikes per Plant shows similar dominance variance across both crosses (H = 8.95). The broad-sense heritability is high in both crosses (98.06%), with narrow-sense heritability and genetic advance also being comparable. Cross II shows a slightly higher expected genetic advance (40.41%) compared to Cross I (33.9%), suggesting a better potential for improvement through selection in Cross II.

Number of Kernels per Spike indicates substantial genetic variance in both crosses, with Cross II showing a higher additive variance (D = 14.89) and dominance variance (H = 5.01) compared to Cross I (D = 16, H = 8.01). Heritability in the broad sense is high for both (98.04% in Cross I and 90.53% in Cross II), but narrow-sense heritability is significantly higher in Cross II (44.73%) compared to Cross I (56.63%). This, combined with the higher genetic advance in Cross II (6.46%) compared to Cross I (3.41%), suggests that Cross II holds more promise for breeding programs.

Focused on increasing the number of kernels per spike.

Grain Weight per Spike reveals lower genetic variance overall, with Cross II showing slightly higher dominance variance (H = 0.12) than Cross I (H

= 0.40). The broad-sense heritability is high in both crosses (83.33% in Cross I and 94% in Cross II), but the expected genetic advance is notably higher in Cross II (44.55%) compared to Cross I (34.76%), indicating that Cross II may have a greater potential for genetic improvement in grain weight per spike. These results were in the same line with Sharshar and Esmail (2019) and **Elmassry et al., (2020).**

100-Kernel Weight shows very low genetic variance in both crosses, with slightly higher dominance variance in Cross II (H = 0.27) compared to Cross I (H = 0.07). Broad-sense heritability is relatively lower compared to other traits, particularly in Cross I (75%) compared to Cross II (94%). The narrow-sense heritability is also low, with Cross II showing slightly higher values. The expected genetic advance is higher in Cross II (18.87%) compared to Cross I (15.02%), again indicating a better response to selection in Cross II.

Grain Weight per Plant demonstrates substantial additive variance in Cross I (D = 46) compared to Cross II (D = 4.40). However, the broad-sense heritability is slightly higher in Cross II (97.75%) than in Cross I (93.33%), with similar narrow-sense heritability in both crosses. The expected genetic advance is higher in Cross I (26.29%) than in Cross II (21.45%), suggesting that Cross I might offer more opportunities for selection in improving grain weight per plant.

These findings underscore the varying genetic architectures and breeding potentials of the two wheat crosses for different traits, emphasizing the need for trait-specific strategies in wheat breeding programs (Singh and Gupta, (2021) and Johnson and Patel (2022)).

Table 5. Genetic variance components, broad (Hb) and narrow (Hn) sense heritability estimates and expected genetic advance (G.S. %) in the two crosses for all studied traits.

Traits	Crosses	Genetic variance			(H/D)1/2	Heritability		ΔG. A (%)
		D	H	E		Hb (%)	Hn (%)	
Plant height (cm)	I	10	79.37	0.16	2.8	99.32	11.11	1.56
	II	22.2	147.7	0.03	2.5	99.94	10.98	2.58
No. of spikes/plant	I	26	8.95	0.26	0.5	98.06	44.83	33.9
	II	26	8.95	0.26	0.6	98.06	44.83	40.41
No, Kernal	I	16	8.01	0.03	0.7	99.64	56.63	
/spike	II	14.89	5.01	0.80	0.6	90.53	44.73	6.46
Grain weight per spike (g)	I	0.8	0.40	0.10	0.7	83.33	40	34.76
	II	0.9	0.12	0.02	0.4	94	42	44.55
100 – grain weight (g)	I	0.07	0.3	0.03	0.5	75	38.46	15.02
	II	0.6	0.27	0.03	0.66	87	42	18.87
Grain weight /plant-1	I	46	20	2	0.6	93.33	40.35	21.45
	II	4.40	76	0.90	0.24	97.75	41.30	26.29

Figure (1) shows The SSR marker analysis for the Yr15 gene, which shows a band size of 180 bp, was

used to assess the presence of the Yr15 resistance gene across six populations derived from crosses between the monogenic line Yr15 and two wheat cultivars, Misr 2 and Gemmeiza 11. The image indicates that the 180 bp band, characteristic of the Yr15 gene, is consistently present in the Yr15 parent line and the resistant populations, including the F1, F2r, Bc1r, and Bc2 generations. The presence of this band across these generations confirms the successful introgression of the Yr15 gene into the progeny, demonstrating that the resistance trait has been effectively passed down through these generations. Conversely, in susceptible plants (represented by F2s and Bc1s populations), the absence or reduced intensity of this 180 bp band suggests either the lack of the Yr15 gene or a lower frequency of homozygous resistant plants in these populations. This pattern aligns with the expectation that resistance traits governed by a single gene, such as Yr15, should follow a Mendelian inheritance pattern, with resistant plants showing the presence of the specific marker band. The SSR marker thus serves as a reliable tool for tracking the presence of Yr15 in breeding programs aimed at enhancing yellow rust resistance in wheat (Sandhu *etal*.,2024), (Shahin *etal*.,2024).

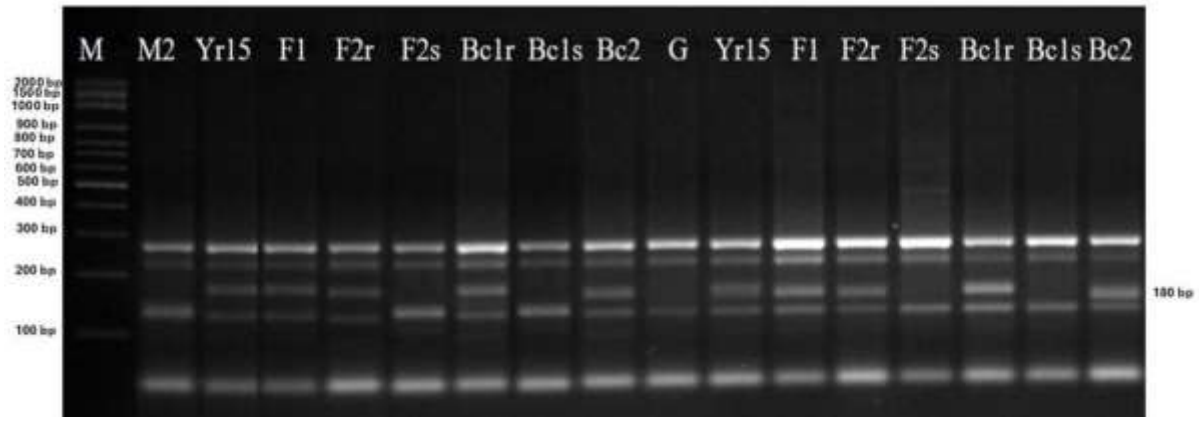

Fig 1: The SSR marker analysis for the Yr15 gene.

Table (6): Polymorphic and Monomorphic Band Distribution across Wheat Crosses Between Misr2 (M2) and YR15, and Gmiza11 and YR15

Band size	Misr2	Yr15	F1	F2r	F2s	Bc1r	Bc1s	Bc2	Gemmeiza 11	Yr15	F1	F2r	F2s	Bc1r	Bc1s	Bc2
270	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
225	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
180	0	1	1	1	0	1	0	1	0	1	1	1	0	1	0	1
125						1	1	1	1	1	1	1	1	1	1	1

The analysis of SSR markers in the crosses between Misr2 (M2) and YR15, as well as Gemmeiza 11 and YR15, reveals critical insights into the genetic diversity within these populations. The monomorphism observed in the 270 bp and 225 bp, 125 bands, suggests a conserved region across the samples. while the 180 bp band, which is polymorphic, highlights the genetic variability between the parents and their progeny.

In the Misr2 x YR15 cross, the 180 bp band was polymorphic, present in YR15 but absent in Misr2, and its segregation in F2, and backcross generations reflects typical Mendelian inheritance. The 180 bp band was present in most samples, while the monomorphic 270bp,225bp,125bp bands was consistently present across all populations. This stability in the monomorphic bands suggests a conserved genetic locus, potentially associated with Resistance traits.

In the Gemmeiza 11 x YR15 cross, similar patterns emerged, though Gemmeiza 11 lacked the 180 bp band, indicating genetic divergence at this locus. However, the 270bp,225bp, 125bp band remained conserved, showing its stability across different parental combinations.

The analysis revealed that 25% of the bands were polymorphic, demonstrating significant genetic diversity between the parental lines and their hybrids. In contrast, 75% of the bands were monomorphic, indicating that certain loci, such as the 270bp,225bp,125bp bands, are highly conserved and potentially essential for key biological functions across these populations.

This balance of polymorphism and monomorphism provides a solid foundation for genetic studies, as the polymorphic markers help identify diversity and inheritance patterns, while monomorphic markers may serve as indicators of essential or conserved

traits.

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تقييم تأثيرات الجينات والتأثيرات الجينية المضافة لمقاومة الصدأ ألأصفر في القمح المصري: رؤى حول جين Yr15باستخدام عالمات SSR

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لتحسين مقاومة الصدأ الأصفر في القمح المصري ، تم ادخال جين Yr15 في أصناف مصر–2 وجميزة11 باستخدام تقنيات التهجين التقليدية خلال موسمي النمو 2020/2019 الى 2022/2021 في محطة البحوث الزراعية بسخا. ركزت الدراسة بشكل أساسي على فهم تأثيرات الجينات والتأثيرات الجينية المضافة المتعلقة بصفات المحصول في هجينين: مصر2 x Yr15 (الهجين الأول) وجميزة11xYr15 (الهجين الثاني). أظهرت الهجن التي تحمل جين Yr15 مقاومة ميدانية قوية ضد الصدأ الأصفر ، مما يشير الى أن هذا الجين يسهم بشكل كبير في مقاومة المرض. تشير التباينات الجينية العالية والمكافئ الوراثي على المدي الواسع في هذه الهجن إلى أن الاختيار لمقاومة الصدأ الأصفر فعال للغاية في الأجيال الانعزالية. أظهرت نباتات الجيل الثاني للصنف الحساس للمرض والتي أدخل فيها الجين Yr15 أعلى تكرار للنباتات المقاومة، مما يؤكد فعالية الجين. توصي النتائج المتحصل عليها إلى ادخال جينYr15في برنامج تربية القمح الوطني في مصر لتعزيز مقاومة الصدأ الأصفر. بالإضافة إلى ذلك، تقدم نباتات الجيل الثاني مصدرًا جيدا قيمًا لتطوير تراكيب وراثية للقمح ذات إنتاجية عالية ومقاومة للصدأ الأصفر. كانت الخصائص الجزيئية من خلال تحليل علامات SSR ضرورية حيث حددت هذه العلامات جين Yr15 بفعالية نظرًا للتعدد المظهري العالي والسيادة المشتركة لها. أكدت أنماط حزم الحمض النووي المتميزة التكامل الناجح لجين Yr15 في السلالات الابوية والهجن. علاوة على ذلك، تسهل عالمات SSRالاختيار المبكر للصفات المرغوبة في مرحلة البادرة ، مما يقلل بشكل كبير من الحاجة إلى الفحص الظاهري الواسع ويسرع عملية التربية.