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Genetic Analysis of Yellow Rust Resistance in two Egyptian Wheats: Unveiling the Role of Yr8 Using SSR Markers

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Abstract

This study investigated gene effects and epistasis for yield traits in two wheat hybrids: Gemmeiza 11 x YR8 (Cross I) and Misr 2 x YR8 (Cross II), using six population means (P1, P2, F_1, F_2, BC_1, and BC_2). the experiment was conducted at Sakha Agricultural Research Station over the 2019/2020 to 2021/2022 seasons. The Yr8 yellow rust resistance gene was introduced into Egyptian wheat cultivars Gemmeiza-11 and Misr-2 through conventional crossing. Both hybrids with Yr8 showed strong field resistance. High genetic variance and broad-sense heritability estimates for these crosses suggest that effective selection for yellow rust resistance is feasible in the segregating generations. The highest frequencies of resistant F2 plants were found in hybrids combining Yr8 as compared with susceptible cultivars, demonstrating the gene's effectiveness. Thus, incorporating and pyramiding Yr8 into the national wheat breeding program is recommended to improve yellow rust resistance Egyptian wheat. Selected F2 plants provide valuable genetic variation for developing high-yielding, rust-resistant wheat germplasm. SSR marker analysis was pivotal for molecular characterization. Since SSR markers provide the effective in detecting the *Yr8* gene due to their high polymorphism. Distinct banding patterns confirmed successful *Yr8* gene introgression in parental lines and hybrids. This marker-assisted selection (MAS) approach facilitates early identification of resistant genotypes, enhancing breeding efficiency and accelerating the development of yellow rust-resistant wheat varieties. SSR markers' ability to detect desirable traits at the seedling stage reduces reliance on extensive phenotypic screening, thereby expediting the breeding process.

Key words: six populations, scaling test, genetic variances, yellow rust, Bread wheat, SSR marker, Yr8 gene, monogenic line

Introduction

Bread wheat (*Triticum aestivum* L.) is the most important cereal crop in the world and most importantly in Egypt. The global concern about the gap between food production and consumption has intensified the research on the genetics, and breeding of cereal crops *(*Ballesta *et al*., 2023). Stripe rust, caused by *Puccinia* f. sp. *striiformis* continue to threaten wheat production worldwide, Stripe rust infection may be developed at any moment throughout the plant's life cycle, from the one-leaf stage until the time of maturity (Mapuranga *et al.*, 2022). Utilized of six populations model to generation means analysis is a simple and useful method for computation of genetic effects for the quantitative traits and its greatest merit reside in the capability to measure the epistatic effects such as, additive × additive, additive × dominance and dominance × dominance types (Yassin *et al*., 2019 and Attri *et al*., 2021). Singh *et al*. (2004) and Devi *et al*. (2018) suggested that heterosis over better performing parent (heterobeltiosis) can be useful for determining true heterotic cross combinations. High heritability estimates associated with in high genetic advance for yield components in wheat offer better 1512 scope for selection of genotypes in early segregating generations *(*Singh and Chatrath 1992; Memon *et al.*, 2005). The heritability may indicate that certain morphological traits that influence grain yield in wheat are more heritable than yield itself (Fethi and Mohamed 2010) and it is a valuable tool when used in conjunction with other parameters in predicting genetic gain that follows the selection for that character. Plant breeders are interested in the estimation of gene effects in order to formulate the most advantageous breeding procedures for improvement of the attribute in question. Therefore, breeders need information about nature of gene action, heterosis, inbreeding depression, heritability and predicted genetic gain from selection for plant height, yield and yield components. The major factors, that must be considered, and which may limit progress in the analysis of quantitative genetic

variation are the number of genes involved, the type of gene action and the genotype-environment interaction (Erkul *et al*., 2010; Ansari *et al*., 2005). Based on the evaluated genetic parameters, selection in advanced generations might be effective for some grain yield traits, due to dominance and epistatic effects (Erkul *et al*., 2010). Recent advancements in molecular genetics have utilized SSR (Simple Sequence Repeat) markers to enhance the understanding of yellow rust resistance. SSR markers, due to their high polymorphism and genome-wide distribution, are instrumental in mapping resistance genes and developing resistant wheat varieties. Studies have demonstrated the efficacy of SSR markers in identifying quantitative trait loci (QTL) associated with yellow rust resistance, providing valuable insights for breeding programs aimed at improving disease resistance in wheat (Bansal *et al*., 2019; Wang *et al*., 2021). These markers facilitate the selection of resistant genotypes and help in tracking the inheritance of resistance traits across generations. The integration of SSR markers into wheat breeding strategies is thus pivotal for developing robust yellow rust-resistant varieties and ensuring food security. The research aims to develop wheat genotypes carrying specific yr effective genes, thereby equipping these varieties with strong resistance against yellow rust. This integration of resistance genes into cultivated wheat varieties is expected to provide an effective and sustainable solution to combating the harmful effects of yellow rust and ultimately maximize wheat production in Egypt.

Materials and Methods

Experimental site and plant materials

This study was conducted at the experimental farm of Sakha Agricultural Research Station in Egypt over the three wheat-growing seasons of 2019/2020, 2020/2021, and 2021/2022. The research involved two Egyptian bread wheat cultivars supplied by the Wheat Research Department, Field Crops Research Institute, Agricultural Research Center (ARC), Egypt, and one of yellow rust monogenic line obtained from the International Maize and Wheat Improvement Center (CIMMYT), Mexico (Table 1). The study includes two hybrids, namely MISR 2 x YR8 and GEMIZA 11 x YR8, which are referred to as the first and second crosses in the text, respectively.

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
GEMIZA11	BOW"S"/KVZ"S"//7C/SER182/3/GIZA168/SAKHA 61 GM7892-2GM-1GM-2GM-1GM-0GM	Egypt
YR8	Yr8/6*AOC	CIMMYT

Crossing:

During the 2019/2020 growing season, two Egyptian bread wheat cultivars one is susceptible and one is resistant to yellow rust were selected, along with Yr8 monogenic line for the study. To generate F1 hybrids, each of the cultivars was crossed with the resistant parents carrying the mono-gene Yr8. In the subsequent 2020/21 season, the F1 plants underwent self-pollination and were simultaneously backcrossed with each parent under controlled conditions to produce F2, BC1, and BC2 generations for each cross.

In the following growing season, 2021/2022, seeds from the six populations (P1, P2, F1, F2, BC1, and BC2) arising from the two crosses were sown in a randomized complete block design experiment with three replications, in November 20th, utilizing natural infection. The plants were arranged in rows 5.5 m long and 2e cm apart, ensuring a distance of 20 cm between individual plants within the row.

The plot sizes consisted of 13 rows for the F2 generation and 2 rows for each of BC1, BC2, P1, P2, and F1 populations. Cultural practices were maintained in accordance with standard wheat cultivation methods. Data were systematically collected from the six populations in each cross to evaluate all the traits (No. of Spike/plant, Plant height (cm), Grain yield/plant (g), No. kernel /spike, Weight of kernel/spike (g) and 100 Kernel weight(g)).

Genetic and statistical analysis

All genetic analyses were conducted using generation means, and scaling tests (A, B, and C) were applied according to Mather *and* Jinks (1982) and Ibrahim *et al*. (2023) to evaluate the presence of non-allelic interactions as follows:

$A = 2\,\bar{B} - \bar{P} - \bar{F}$

$B = 2\,\bar{B}2 - \bar{P}2 - \bar{F}1$

$C = 4\,\bar{F}2 - 2\,\bar{F}1 - \bar{P}1 - \bar{P}2$

The genetic model parameters (m, a, h, aa, ad, and dd) were based on the frameworks established by Jinks and Jones (1958), Hayman (1958), and Ibrahim *et al*. (2023). where m = mean

a = additive effect = $\bar{B}1 - \bar{B}2$

h = dominance effect = $\bar{F}1 - 4\,\bar{F}2 - ½\,\bar{P}1 - ½\,\bar{P}2 + 2\,B\bar{c}1 + 2\,B\bar{c}2$

aa = additive × additive gene interaction = 2 B$\bar{c}$1 + 2B$\bar{c}$2 − 4 $\bar{F}$2
ad = additive × dominance =B$\bar{c}$1 − ½ $\bar{P}$1 − B$\bar{c}$2 + ½ $\bar{P}$2
dd = dominance × dominance = $\bar{P1}$ + $\bar{P2}$ + 2 $\bar{F1}$ + 4 $\bar{F2}$ − 4 $\bar{Bc}$1 − 4 $\bar{Bc}$2
The genetic variance components were calculated using the F2 variance formulas outlined by Mather and Jinks (1982) as follows:
E (environmental variance) = ⅓ (VP1 + VP2 + VF1)
D (additive variance) = 4 VF2 – 2 (VBC1 + VBC2)
H (dominance variance) = 4 (VF2 – ½ VD – VE)
The significance of the genetic components was tested using the t-test, where t
= effect / (variance effect)1/2.

Heterosis:

Estimates of heterosis (%) were calculated as the percentage deviation of the F1 mean performance from either the mid-parent or the better parent, according to El Hanafi et al., 2022 as follows:
Heterosis from the mid − parent % (M. P) = ($\bar{F}_1$ − $\bar{M}$P) / $\bar{M}$P) × 100
Heterosis from the better − parent % (BP) = ($\bar{F_1}$ − $\bar{B}$P) /$\bar{B}$P) × 100
Inbreeding Depression (I. D. %)
Its values measured from the following equation:
I. D % = ($\bar{F}$1 − $\bar{F}$2 / $\bar{F}$1) × 100
Variances of I. D deviation = V$\bar{F}$1 + V$\bar{F}$2
T: I. D = $\bar{F}$1 + $\bar{F}$2 / (V. I. D)0.5
Phenotypic and genotypic coefficients of variability were calculated according to the methods described by Burton (1952) and Mishra *et al*. (2024).

PCV = ($\sqrt{VP}$ /$\bar{X}$) × 100

GCV = ($\sqrt{VG}$ /$\bar{X}$) × 100

The average degree of dominance (ā)
The average degree of dominance (ā): was calculated by the formula presented by Mather and Jinks (1982):
$\bar{a}$ = (H/D)1/2
Complete dominance is considered when ā = ± 1.0, partial dominance is indicated when fall between > 0.0 and < ± 1.0, while over- dominance is considered if lies the ratio exceeded ± 1.0. if the degree of dominance value is equal to zero, it indicates the absence of dominance. The positive and negative signs indicate the direction of dominance.
Heritability
Heritability in broad sense (h^2b)
Heritability in the broad sense (h^2b) was estimated using the formula provided by Mather and Jinks (1982):
h2 b %= (VG / VP) × 100
Heritability in narrow sense (h^2n)
It was estimated using the formula presented by Mather and Jinks (1982):
h2n % = (½ D /VP) × 100
Expected gain from selection (G.S)
The expected gain from selecting (G.S) was calculated according to Allard (1960) and Javed *et al* 2024:
G.S % = [1/2 D /vF2] × 100
Molecular analysis part

a) Genomic DNA extraction

Genomic DNA extraction was performed on samples from both resistant and susceptible plant populations, following the procedure outlined by Zhang et al. (1995). Fresh leaves (100 mg) from individual lines were processed using a modified SDS extraction method. To eliminate contaminating RNA, 10 µL/mL of RNase was added to the extracted DNA, which was then incubated at 37 ºC for 45 minutes.
For the extraction process, the ground wheat leaves were transferred into sterile 1.5 mL microfuge tubes. To each tube, a DNA extraction buffer comprising 200 mM Tris-HCl (pH 8.5), 250 mM NaCl, 25 mM EDTA, 0.5% SDS, and 1% PVP was added to the homogenized samples. Six microliters of RNaseA (20 µg/mL in water) were included, and the tubes were incubated in a water bath at 65 °C for 15 minutes, with gentle shaking every 5 minutes to ensure thorough mixing.
For protein precipitation, 160 µL of 3 M sodium acetate (pH 5.3) was introduced along with an equal volume of chloroform: isoamyl alcohol (24:1). The mixture was gently inverted to mix, followed by centrifugation at 15,000×g for 8 minutes at room temperature. The supernatant was carefully extracted into a new tube and precipitated by adding an equal volume of cold isopropanol at 4 °C. The mixture was thoroughly mixed to promote the formation of filamentous DNA and allowed to incubate for 10 minutes at room temperature.
To collect the genomic DNA, centrifugation was performed at 8,900×g for 10 minutes, resulting in a pellet that was washed twice using 75% ethanol at ambient temperature and subsequently centrifuged at 5,700×g for 2 minutes. The final DNA pellet was dried under vacuum using a SensoQuest labcycler (*SensoQuest* GmbH Germany) at 37 °C for 5 minutes, and the purified DNA was ready for PCR amplification and electrophoretic separation of products.

b) PCR reaction and conditions

The preparation of each PCR reaction (25 µL) utilized specific simple sequence repeat (SSR) primers designed for the Yr8 gene. The reaction mixture consisted of 50 mM KCl, 10 mM Tris-HCl (pH 8.8), 1.5 mM MgCl2, and 200 µM of each dNTP. Additionally, 200 µM of both the forward (YR8, Xgwm157 F: GTCGTCGCGGTAAGCTTG) and reverse (R: GAGTGAACACACGAGGC) primers were incorporated, along with 1.0 unit of Taq polymerase (Promega) and 40-60 ng of genomic DNA. For SSR markers, the annealing temperature adhered to the established protocols of Röder *et al.*

(1998) and Saal and Wricke (1999). Following a thorough assessment of the DNA's quality and quantity, amplification was executed using an SensoQuest labcycler to explore genetic polymorphism among different genotypes bearing 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 both forward and reverse primers, 5 µL of 10× PCR buffer, and 0.5 µL each of BSA and PVP. A tailored PCR amplification protocol was applied specifically for the Yr8 gene-linked primer, anticipating an amplicon size of 120 bp. The resulting amplicons were separated on a 1.5% agarose gel and visualized using the GELDOC BIORAD XR+, post-staining with Ethidium Bromide (EtBr).

Results and Discussion:

Generation means of the six populations differed significantly for most studied yield traits, Table (2) indicating the presence of genetic variability for these traits in the studied materials and revealing that level of the differences between generations' means could be subjected to statistical-genetic analyses. The results summarized in Table (2) highlight the mean performance and variance for six populations (P1, P2, F1, F2, BC1, and BC2) across three different crosses for various traits. Notably, Misr 2 exhibits superior plant height (110.3cm) and grain yield (40g) compared to YR8 which had 92 cm and 39 g for both traits, respectively. while Gemmiza 11 outperforms in grain yield (45.39 g) and kernel weight (2.5 g) but has a shorter plant height (104.3 cm) than Misr 2. Both crosses generally display enhanced traits in the F1 generation compared to their parents, indicating hybrid vigor, Table (2).

Table 2. Means ($\bar{x}$) 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, kernel /spike	Weight of kernel /spike (g)	100 Kernel weight(g)
Misr 2	P1	$\bar{x}$	15.5±0.09	110.3±0.03	40.0±0.06	52.7±0.02	1.9±0.04	4.0±0.01
		S2	0.26	0.02	0.12	0.02	0.05	0.01
YR8	P2	$\bar{x}$	10.4±0.10	95.2±0.03	39.0±0.022	40.2±0.03	1.7±0.08	3.7±0.03
		S2	0.31	0.02	0.01	0.03	0.21	0.03
	F1	$\bar{x}$	20.5±0.09	100.5±0.032	47.7±0.12	70±0.03	2.7±0.06	4.6±0.03
		S2	0.26	0.03	0.36	0.04	0.10	0.03
Cross 1 (Misr2 x	F2	$\bar{x}$	18.9±0.47	105.6±0.60	35.3±0.33	67.7±0.3	2.0±0.06	3.9±0.05
YR8)		S2	27.14	43.36	13.33	9.83	0.48	0.40
	Bc1	$\bar{x}$	24.0±0.63	103.6±0.63	46.5±0.42	69.7±0.4	2.1±0.08	4.4±0.07
		S2	23.93	23.33	10.40	8.88	0.44	0.30
	Bc2	$\bar{x}$	22.1±0.61	101.7±0.72	44.8±0.49	68.9±0.22	1.9±0.06	4.0±0.06
		S2	22.27	30.97	9.59	2.99	0.28	0.20
Gemmiza 11	P1	$\bar{x}$	12.7±0.03	104.3±0.03	45.3±0.03	57.5±0.02	2.5±0.02	4.7±0.07
		S2	0.02	0.03	0.02	0.02	0.02	0.13
YR8	P2	$\bar{x}$	10.4±0.10	95.2±0.03	39.0±0.0217	40.2±0.03	2.0±0.04	3.7±0.03
		S2	0.31	0.02	0.03	0.03	0.06	0.03
	F1	$\bar{x}$	14.5±0.09	108.7±0.03	48.3±0.03	67.5±0.03	3.4±0.08	5.3±0.08
		S2	0.26	0.04	0.04	0.02	0.17	0.18
Cross 2	F2	$\bar{x}$	12.8±0.19	111.4±0.32	42.7±0.08	64.0±0.3	3.1±0.06	5.0±0.06
(Gemmiza 11x		S2	4.23	12.31	9.83	9.50	0.41	0.40
YR8)	Bc1	$\bar{x}$	14.9±0.25	106.6±0.41	47.0±0.08	60.3±0.3	3.0±0.07	4.9±0.08
		S2	3.91	9.94	8.88	6.95	0.35	0.38

In Cross 1, the F1 mean surpasses the parental means in traits such as spike number, grain yield, 100 kernel weight, number of kernels per spike, and kernel weight per spike, suggesting over-dominance in these traits. Significant deviations of the F1 mean from the mid-parent value imply non-additive gene action. However, for plant height, the F1 mean falls between the P1 and P2 means, indicating partial dominance. The variance (S2) is relatively low across most parameters, reflecting stable trait expression, though significant differences were observed among most genotypes for the measured traits.

Overall, the F1 and backcross populations show markedly higher mean performances than the best parent in most traits, indicating a strong heterotic effect. Additionally, the variance in F2 and backcross

populations is higher for all traits compared to P1, P2, and F1, suggesting a significant impact of environmental factors on trait expression. These findings align with previous studies by Kalhoro *et al*. (2015).

The observed variability in subsequent generations, particularly in F2 and backcrosses, is likely due to genetic segregation and recombination. F2 populations show means closer to one parent for traits such as spike number, 100 kernel weight, plant height, and kernel weight per spike, indicating additive and dominance effects, while traits like kernel weight and grain yield suggest additive effects. The pronounced variance in F2, especially in Cross 1, indicates greater genetic diversity, which could benefit to the breeding programs.

In Backcross 1 (BC1) and Backcross 2 (BC2), most traits are closer to the Misr 2 parent, with values intermediate between the two parents. Cross 2's F1 generation generally exhibits higher values for spike number, grain yield, and 100 kernel weight compared to its parents, with higher variance indicating diverse expressions. F2 shows significant variance, particularly in grain yield. The backcross populations in Cross 2 tend to be closer to Gemmiza 11, though some instability in traits is indicated by the observed variance.

In conclusion, Cross 1 (Misr 2 x YR8) seemed to be promising for traits like grain yield and spike number, while Cross 2 (Gemmiza 11 x YR8) could be advantageous for 100 kernel weight and grain yield, although careful selection and breeding are necessary due to the observed variability. such data suggests that hybrid crosses have strong potential for trait improvement, but managing genetic variability and selection is key to stabilizing desirable characteristics in future generations. Meanwhile data in Table (3) shown the scaling test (A, B, C) and gene action parameters (m, a, d, aa, ad, dd) for various plant traits across two crosses, providing valuable insights into the genetic architecture of these traits. The significant values in the scaling tests indicate the presence of non-allelic interactions, or epistasis, in traits such as plant height, number of spikes per plant, number of grains per spike, and grain weight per spike.

The gene effects, calculated using the Gamble procedure, reveal the contributions of additive (a), dominance (d), and interaction effects (aa, ad, dd) to each trait. For instance, in Cross I, plant height shows a strong additive effect (105.6**), with additional contributions from dominance and interaction effects, highlighting the complexity of gene interactions. Traits like grain weight per plant and the number of grains per spike show significant additive and non-additive effects, suggesting that selection based on these parameters could effectively improve these traits.

The scaling test results further emphasize the presence of epistasis, particularly when the additive-dominance model alone cannot fully explain the genetic variation. When the scaling test values deviate significantly from zero, it suggests complex interactions, which must be considered when interpreting gene effects. For example, significant additive effects suggest that selection could improve these traits in subsequent generations, while significant dominance or epistatic effects imply the importance of hybrid vigor or specific gene combinations.

In both crosses (MISR2 x YR8 and MISR2 x YR15), the significant scaling tests confirm that the six-parameter model is appropriate for explaining the gene action underlying these traits. Notably, additive gene effects (a) are positive and significant for traits such as grain yield per plant 1.67**,2.00** in cross 1and 2 respectively, grain weight per spike 0.25*,0,34** in cross 1and 2 respectively, plant height 1.90**,1.47** in cross 1and 2 respectively, number of spikes per plant 1.88* in cross 1, and 100-grain weight 0,3**7 in cross 1. These findings suggest that using a pedigree selection program could be particularly effective for improving these traits.

Dominance effects (d) are also significant, especially for grain yield per plant, grain weight per spike, number of spikes, number of grains per spike, and 100-grain weight, emphasizing the critical role of dominance in the inheritance of these traits. The significance of both additive and dominance components suggests that selection may be effective in both early and late generations.

Additionally, significant additive x additive (aa) epistatic effects were detected for several traits, including the number of spikes, number of grains per spike, 100-grain weight, and grain yield, particularly in Cross I. Similarly, dominance x dominance (dd) and additive x dominance (ad) effects were significant for traits like 100-grain weight and grain weight per plant. These results highlight the importance of duplicate epistatic gene effects in the inheritance of these traits, complicating the use of epistasis in breeding programs.

Overall, the dominance and dominance x dominance effects are more influential than additive x additive (aa) effects in the expression of most traits across both crosses. These findings, consistent with previous research by Sheikh *et al.* (2009); Yassin *et al.* (2019) and Raffo et al. (2022), underscore the complexity of genetic interactions and the importance of carefully planned selection strategies, particularly in later generations, to effectively harness these genetic effects for breeding purposes.

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 (m)	Additive (a)	Dominance (d)	Add. X Add. (aa)	Add. X Dom. (ad)	Dom.x Dom. (dd)
Plant height (cm)	I	-3.647	7.63**	16.053**	105.6**	1.90*	-14.3	-12.07	-5.64	8.08
	II	0.250	6.39**	28.903**	111.4**	1.47**	-13.4	-22.27	-3.07	15.63**
No. of spikes/plant	I	12.000**	13.37**	8.800**	18.9**	1.88*	24.1**	16.57**	-0.68	-41.93
	II	2.600**	3.80**	-0.867	12.8**	0.57	10.2**	7.27**	-0.60	-13.67
No, grains /spike	I	16.419**	27.31**	37.398**	67.7**	0.81	30.3**	6.33**	-5.45	-50.07
	II	-4.477	11.51**	23.320**	64.0**	0.67	2.4	-16.29	-7.99	9.25**
Grain weight per spike (g)	I	-0.363	-0.58	-0.932	2.0**	0.25*	0.9*	-0.01	0.11	0.95
	II	0.080	-0.11	0.948**	3.1**	0.34**	0.2	-0.97	0.09	1.00**
100 – grain weight (g)	I	0.109	-0.29	-1.296	3.9**	0.37**	1.9**	1.12**	0.20*	-0.94
	II	-0.337	0.53**	1.100**	5.0**	0.10	0.2	-0.91	-0.43	0.71
Grain weight /plant^{-1}	I	5.339*	2.94*	-33.040	35.3**	1.67**	49.5**	41.32**	1.20*	-49.61
	II	0.300	2.58**	-10.273	42.7**	2.00**	19.3**	13.15**	-1.14	-16.03

Where * and ** Significant and highly significant at 0.05 and 0.01 levels of probability, respectively.

Table 4 provides an insightful analysis of heterosis (%), inbreeding depression (I.D.%), and the phenotypic (PCV) and genotypic (GCV) coefficients of variation for plant height and grain yield component traits in two wheat hybrids. These metrics reveal the genetic potential and variability within these crosses, offering valuable information for breeding strategies.

Heterosis, also known as hybrid vigor, is a critical measure indicating how the performance of hybrids compares to their parent lines. High positive heterosis percentages for key traits suggest significant hybrid vigor, meaning that these crosses could yield superior offspring, especially for traits related to yield and stress resistance. This highlights the potential of these hybrids to outperform their parent lines, making them valuable candidates for breeding programs. However, heterotic effects may change in the F2 and later generation. More effect of the environment could alter heterotic effects.

Inbreeding depression percentages (I.D.%) serve as a crucial countermeasure, revealing the decline in vigor or performance when hybrids are selfed. High I.D.% values indicate a substantial reduction in hybrid vigor in subsequent generations, emphasizing the need to maintain heterozygosity to sustain optimal performance mean over, heterozygosity can not be maintained in highly selected plants like wheat crop

The phenotypic (PCV) and genotypic (GCV) coefficients of variation provide further insight into the variability of these traits. High PCV and GCV values suggest considerable variation, which is promising for selection. However, when PCV significantly exceeds GCV, it indicates that environmental factors play a substantial role in trait expression, potentially complicating selection efforts. Traits with high GCV, moderate I.D.%, and positive heterosis are ideal for breeding programs, as they offer genetic potential with resilience to inbreeding effects, increasing the likelihood of achieving lasting genetic improvements in wheat.

The data reveal highly significant and positive heterotic effects for all studied traits in both crosses, except for plant height in Cross 1, where negative and non-significant heterosis was observed—likely due to the internal cancellation of heterosis components. This suggests that the direction of dominance favored the better-performing parent, and the significant heterotic effects could be attributed to dominance and dominance × dominance interactions. These findings align with previous studies, reinforcing the value of heterosis in identifying superior hybrid combinations according to Begna (2021).

In terms of inbreeding depression, significant positive values were noted for all traits in both crosses, except for plant height, which exhibited negative and significant inbreeding depression. This pattern is logical, as the expression of heterosis in F1 hybrids is often followed by a reduction in F2 performance due to increased homozygosity. The reduction in non-additive genetic components is a typical consequence of inbreeding depression, consistent with previous research.

Overall, Table (4) highlights the importance of understanding both genetic and environmental influences on trait variation. The data suggest that while high heterosis offers a path to enhancing traits, careful management of inbreeding and environmental factors is essential for sustained improvement.

The data on wheat hybrids reveal significant variations in heterosis, inbreeding depression, and phenotypic (PCV) and genotypic (GCV) coefficients of variation across the studied traits, offering valuable insights for breeding programs. Notable positive heterosis is observed in yield-related traits such as the number of spikes per plant (58.51% in Cross I) and grain weight per spike (48.19% in Cross I), indicating that hybrid vigor greatly enhances these traits. Conversely, traits like plant height in Cross I exhibit negative heterosis, should a reduction in plant height. Inbreeding depression negatively affects traits such as the number of spikes per plant (7.72% in Cross I) and grain weight per spike (25.01% in Cross I), Table (4) highlighting the importance of maintaining heterozygosity to optimize yield. However, these can't be maintained in wheat. The PCV and GCV values reflect genetic variability, with high values in traits like grain weight per spike (PCV: 34.57%, GCV: 30.22%) suggesting strong potential for selection. In contrast, traits like grain weight per plant in Cross II show low variability, limiting their breeding potential. Our finding agreed with Zaazaa (2017).

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	-2.22	-8.90	-5.13**	6.23	6.23
	II	8.93**	4.19**	-2.55**	3.15	3.15
No. of spikes/plant	I	58.51**	32.26**	7.72**	27.54	27.40
	II	25.72**	14.17**	11.72**	16.07	15.66
No, Kernal /Spike	I	51.51**	33.51**	3.71**	4.63	4.62
	II	38.21**	17.39**	5.19**	4.82	4.81
Grain weight per spike (g)	I	48.19**	37.27**	25.01**	34.57	30.22
	II	50.33**	35.60**	9.75**	20.99	18.07
100 – grain weight (g)	I	20.84**	15.51**	15.61**	16.13	15.63
	II	26.62**	12.33**	5.33**	12.58	10.27
Grain weight /plant-1	I	20.76**	19.34**	25.92**	10.34	10.25
	II	14.56**	6.62**	11.67**	2.12	2.10

Where * and ** Significant and highly significant at 0.05 and 0.01 levels of probability, respectively. m = mean, a: additive, d: dominance, aa: additive × additive, ad: additive × dominance, dd: dominance × dominance effects.

Table (5) presents the average degree of dominance, showing values greater than one for most traits, except for plant height and 100-grain weight in Cross I, and plant height, number of grains per spike, and grain yield per plant in Cross II. This indicates the presence of over-dominance toward the better parent, suggesting early selection through a will prepared designed experiment that could improve these traits. When the degree of dominance is less than one, it confirms partial dominance, as seen in traits like the number of spikes per plant and grain weight per spike.

Heritability estimates further illuminate the genetic landscape, with broad-sense heritability (Hb) being consistently high across traits, indicating strong genetic control. However, narrow-sense heritability (Hn) varies, suggesting that non-additive genetic factors and some environmental factors play a significant role in some traits. The comparison between broad and narrow-sense heritability highlights the equal importance of both additive and non-additive effects in the genetic control of these traits. The values were consistently high for most traits (above 90%), indicating that genetic factors largely control the expression of these traits.

High narrow-sense heritability values indicate that selection may be more effective for improving traits in early segregating generations. The number kernel per spike trait showed a high narrow sense value (56.53) indicating the presence of additive gene effect. Conversely, low to medium narrow-sense heritability across most traits suggests that environmental and non-additive effects have a larger impact than additive genetic effects. The narrow-sense heritability varied significantly. Traits like plant height and number of spikes per plant were lower Hn values (e.g., 34.35% for plant height in cross I and 15.33% for spikes per plant in cross I). This suggests a significant influence of non-additive genetic factors (e.g., dominance and epistasis).

The expected genetic advance from selection is also detailed in, Table (5), with the highest gains observed for grain weight per spike and 100-grain weight (29.99% and 21.70%, respectively) in Cross I. These high genetic advances, coupled with high narrow-sense heritability, suggest that selection in these populations could be particularly effective in early generations.

Overall, the data emphasizes the importance of focusing on traits with high narrow-sense heritability for successful breeding, as these are more responsive to selection. The significant genetic variance observed, particularly in traits with over-dominance, underscores the potential benefits of heterozygosity

for improving performance, especially in yield-related traits. For long-term improvement, breeders should prioritize traits with high genetic potential and responsiveness to selection to maximize yield gains.

Table 5. Genetic variance, broad (Hb) and narrow (Hn) sense heritability 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	64.82846	43.69	0.02	0.8209458	99.93	34.35	8.16
	II	16.468423	16.22	0.03	0.9923209	99.71	38.94	3.68
No. of spikes/plant	I	16.130423	75.18	0.28	2.1588883	99.05	15.33	14.32
	II	1.1114609	13.91	0.20	3.5372422	93.88	6.51	3.69
No, Kernal /spike	I	12.379989	13.14	0.02	1.0303957	99.78	34.58	5.49
	II	15.596423	8.01	0.03	0.716735	99.64	56.63	6.42
Grain weight per spike (g)	I	0.4725389	0.49	0.12	1.0174507	80.03	29.65	29.99
	II	0.428223	0.46	0.08	1.0327331	57.63	29.18	19.07
100 – grain weight (g)	I	0.6136719	0.28	0.02	0.6739083	92.29	43.98	21.70
	II	0.2990461	0.54	0.12	1.3422322	53.86	21.64	8.22
Grain weight /plant-1	I	13.354899	25.97	0.17	1.3943795	97.29	25.82	9.06
	II	1.3892986	0.42	0.02	0.5530299	97.47	37.78	3.15

The values were equal, or more than unity referred to over-dominance while the values were less than unity referred to partial dominance gene effect.

Data of Specific Simple Sequence Repeat (SSR) presented in, Figure (1) show cases the SSR marker analysis of the *Yr8* yellow rust resistance gene in various wheat cultivars and their crosses, providing key insights into the genetic foundation of disease resistance in these genotypes. The SSR marker linked to the Yr8 gene serves as a molecular tool to confirm the presence of this resistance gene in the cultivars and their offspring. The banding patterns observed in the gel electrophoresis reveal which individuals carry the Yr8 gene, with resistant cultivars and crosses displaying a specific band associated with the SSR marker linked to *Yr8*.

The clear presence of the SSR marker in parent cultivars and selected progeny indicates the successful incorporation of the *Yr8* gene through conventional breeding methods. This marker-assisted selection (MAS) technique allows breeders to effectively track the inheritance of resistance genes, thus accelerating the breeding process by identifying resistant lines early in development. The absence of the marker in some progeny suggests expected segregation of the resistance gene within hybrid populations according to Jamil *et al.* (2020). This figure is therefore essential in validating the success of the breeding program in integrating the Yr8 gene into new wheat lines and demonstrates the effectiveness of SSR markers in selecting for disease resistance traits in wheat.

The SSR marker analysis illustrated in the figure plays a critical role in the molecular characterization of disease resistance. Due to their high polymorphism and co-dominant nature, SSR markers are powerful tools for detecting specific resistance genes like *Yr8* in wheat. The distinct banding patterns confirm the presence of the *Yr8* gene in certain parental lines and their hybrids, affirming successful gene introgression through breeding efforts. This MAS approach is particularly valuable as it allows for the early identification of resistant genotypes, enhancing the efficiency of breeding programs focused on developing yellow rust-resistant wheat varieties.

The presence or absence of the SSR marker in different progeny indicates genetic segregation of resistance genes, which is typical in hybrid populations, and highlights the genetic diversity within the crosses. As demonstrated in this figure, SSR markers significantly contribute to accelerating the development of resistant cultivars by enabling the selection of desirable traits at the seedling stage, reducing the need for extensive phenotypic screening, and speeding up the overall breeding process according to Jiang and Zhang (2020).

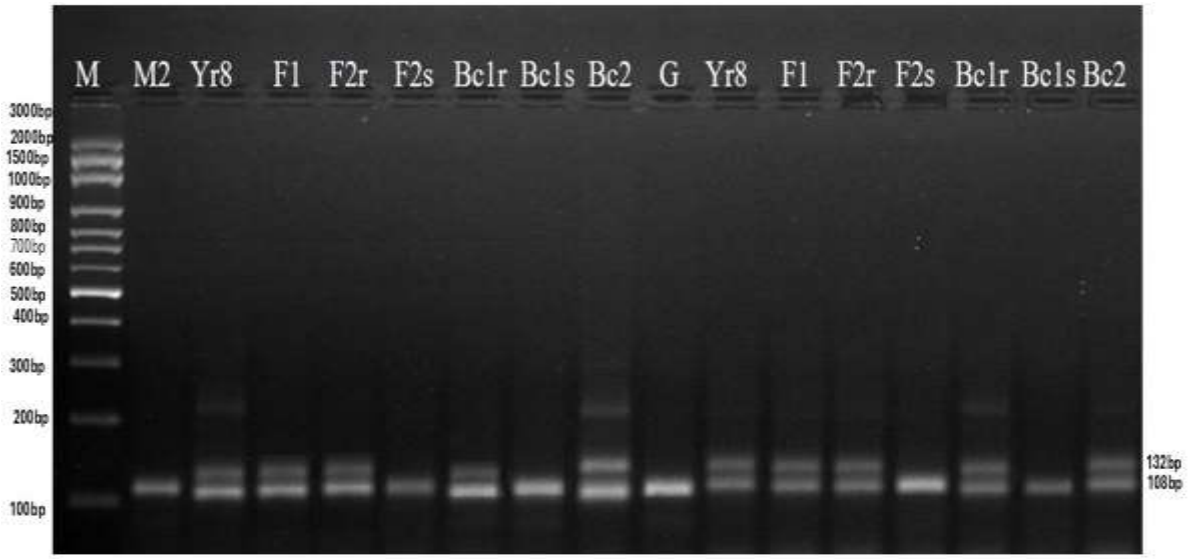

Fig 1: Polymorphic and Monomorphic Band Distribution Across Wheat Crosses Between Misr2 (M2) and YR8, and Gmiza11 and YR8
r: Resistance s: susceptible

The analysis of SSR markers in the crosses between Misr2 (M2) and YR8, as well as Gmmeiza11 and YR8, reveals critical insights into the genetic diversity within these populations. The polymorphism observed in the 200 bp and 132 bp bands highlights the genetic variability between the parents and their progeny, while the 108 bp band, which is monomorphic, suggests a conserved region across the samples.

In the Misr2 x YR8 cross, the 200 bp band was polymorphic, present in YR8 but absent in Misr2, and F1, F2, and backcross generations reflects typical Mendelian inheritance. The 132 bp band, shared by both parents, was present in most samples, while the monomorphic 108 bp band was consistently present across all populations. This stability in the 108 bp band suggests a conserved genetic locus, potentially associated with yellow rust resistance gene.

In the Gmmeiza11 x YR8 cross, similar patterns emerged, though G lacked the 132 bp band, indicating genetic divergence at this locus. However, the 108 bp band remained conserved, showing its stability across different parental combinations.

The analysis revealed that 66.67% of the bands were polymorphic, demonstrating significant genetic diversity between the parental lines and their hybrids. This high level of polymorphism is beneficial for studying trait inheritance and selection in wheat breeding programs. In contrast, 33.33% of the bands were monomorphic, indicating that certain loci, such as the 108 bp band, 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 polymorphic markers help identify diversity and inheritance patterns, while monomorphic markers may serve as indicators of essential or conserved traits.

1. **Total Number of Bands**: 3 (200 bp, 132 bp, 108 bp)
2. **Polymorphic Bands**: 2 (200 bp, 132 bp)
3. **Monomorphic Bands**: 1 (108 bp)

Percentage of Polymorphism

$$\text{Percentage of Polymorphism} = \left(\frac{\text{Polymorphic Bands}}{\text{Total Number of Bands}}\right) \times 100 = \left(\frac{2}{3}\right) \times 100 = 66.67\%$$

Percentage of Monomorphism

$$\text{Percentage of Monomorphism} = \left(\frac{\text{Monomorphic Bands}}{\text{Total Number of Bands}}\right) \times 100 = \left(\frac{1}{3}\right) \times 100 = 33.33\%$$

Conclusion

Cross 1 (Misr 2 x YR8) shows great promise for improving traits such as grain yield and spike number, while Cross 2 (Gemmiza 11 x YR8) offers potential benefits for 100-kernel weight and grain yield, though careful selection and breeding are crucial due to the variability observed. The findings highlight the strong potential of hybrid crosses for trait enhancement, emphasizing the need for meticulous management of genetic variability and selection to stabilize desirable traits in future generations. Dominance and dominance x dominance effects play a more significant role than additive x additive (aa) effects in the expression of most traits across both crosses, reinforcing the complexity of genetic interactions and the importance of strategic selection, particularly in later generations, to fully leverage these genetic effects.

Table 4 underscores the need to understand both genetic and environmental influences on trait variation. While high heterosis offers a path to enhancing traits, careful management of inbreeding and environmental factors is vital for sustained improvement. Breeding strategies should aim to maximize heterosis while minimizing inbreeding depression to secure long-term genetic gains in wheat. Emphasizing traits with high narrow-sense heritability is crucial for successful breeding, as these traits are more responsive to selection. The observed genetic variance, particularly in traits with over-dominance, highlights the advantages of heterozygosity for improving performance, especially in yield-related traits. For long-term success, breeders should focus on traits with high genetic potential and responsiveness to selection to optimize yield gains.

Additionally, the presence or absence of the SSR marker in different progeny indicates genetic segregation typical of hybrid populations, highlighting the genetic diversity within the crosses. SSR markers play a pivotal role in accelerating the development of resistant cultivars by facilitating the early selection of desirable traits, reducing the need for extensive phenotypic screening, and speeding up the breeding process.

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التحليل الجيني لمقاومة صدأ القمح الأصفر في صنفين من القمح المصري: الكشف عن دور الجين Yr8 باستخدام علاماتSSR

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تناولت هذه الدراسة تأثيرات الجينات والتفاعل الجيني لصفات المحصول في هجيني قمح: جميزا 11) Yr8 × التهجين (I ومصر 2 Yr8 ×)التهجين(II ، باستخدام متوسطات ستة عشائر P1 ، P2، F1، F2، BC1، BC2. أجريت الدراسة في محطة البحوث الزراعية سخا الزراعية خلال مواسم 2020/2019 إلى 2022/2021، حيث تم إدخال جين مقاومة الصدأ الأصفر *Yr8* إلى أصناف القمح المصري مصر–2 وجميزا–11 من خلال التهجين التقليدي. أظهرت كلا الهجينين مع *Yr8* مقاومة قوية في الحقل المفتوح. تشير التقديرات العالية للتباين الوراثي والمكافئ الوراثي الواسع النطاق لهذه التهجينات إلى أن الانتقاء الفعال لمقاومة الصدأ الأصفر ممكن في الأجيال الانعزالية. وُجدت أعلى نسبة من النباتات المقاومة في الجيل F_2 ضمن الهجين الذي يجمع *Yr8* مع أصناف قابلة للإصابة، مما يثبت فعالية الجين. وبالتالي، يُوصى بإدماج جين Yr8 في برنامج التربية الوطني للقمح لتحسين مقاومة الصدأ الأصفر، حيث توفر نباتات الجيل الثاني المنتخبة تنوعًا وراثيًا ثمينًا لتطوير أصناف قمح عالية الإنتاج ومقاومة للصدأ. كان تحليل علامات SSR أساسيًا للتوصيف الجزيئي، حيث أثبتت علامات SSR فعاليتها في الكشف عن الجين Yr8بفضل تعدد الأشكال العالي والتغاير المشارك. أكدت أنماط التلوين المميزة نجاح إدخال الجين Yr8 في الخطوط الأبوية والهجن. وتسهم هذه الطريقة في اختيار العلامات المساعدة (MAS) في تحديد الجينات المقاومة مبكرًا، مما يعزز كفاءة التربية ويسرع من تطوير أصناف القمح المقاومة للصدأ الأصفر. حيث استغلال قدرة علامات SSR على اكتشاف الصفات المرغوبة في مرحلة الشتلات تقلل من الاعتماد على الفحص الظاهري المكثف، مما يسرع عملية التربية.