



Genetics studies using triadic test cross analysis for yield attributing traits in some rice genotypes



Elseidy, El-sayed H.¹, Abo-Yousef, Mahmoud I.², Elnagar, Osama A.², Talha, Ibrahim A.² and Bahgt, Mohamed M.^{3*}

¹Agronomy Science Department, Faculty of Agriculture, Tanta University, Egypt

²Rice Research and training center, Field Crops Research Institute, Agriculture Research Center, Egypt

³Agronomy department, Faculty of agriculture, Kafrelsheikh University, Egypt

THE ADDITIVE and dominance components determine the exploit method through pedigree to select the desirable genotypes in advanced generations of segregating populations, so, the experiment conducted at the experimental farm of Rice Research department, Sakha, Agriculture Research Station, Kafre El-Sheikh Egypt, during three 2020, 2021 and 2022 season, to evaluate the genetic parameters included type of gene action and phenotypic correlation for morphological and yield traits through top cross analysis. The genetic materials including three testers (P1, P2 and F1) Giza 177, GSRIR2-9-L1-L3R2 and their F₁ hybrid, (Giza 177 x GSRIR2-9-L1-L3R2) and fifteen promising lines of rice (lines) were crossed to produce forty five hybrid combinations through top cross analysis. The results revealed that, there were highly significant difference among the crosses evaluated for all traits studied, indicating presence of considerable variability among the rice accession. Rice crosses, Giza 177 x GSRIR2-9-L1-L3R2 and MJ5460-4 x GSRIR2-9-L1-L3R2 recorded the desirable values for yield contribution. Total epistasis influences were partitioned, showing that both [i] and [j + l] type interactions were significant, with a prevalent influence of [j + l] type interactions on these traits. Moreover, the MJ5460-4 line was the greatest contributed significant portion to the total epistasis for all the studied traits, so, the additive and dominance effects have significantly equal importance for all the traits. Finally, all genetic parameters controlling inheritance most of characters under investigation, so we can develop new high yielding rice lines in late generations depending on hybridization and recurrent selection.

Keywords: Triadic test cross, Rice genotypes, additive, dominance, epistasis, yield component.

Introduction

Rice (*Oryza sativa* L.) is the grain foodstuff which frames a vital place among three billion individuals eating rice around the globe Before commercial release, plant breeders typically evaluate the performance of the new varieties across various environments to assess their stability and consistency Aswidinnor et al 2023). It is a nutritious cereal crop, provides 20 percent of the calories and 15 percent of the protein consumed by world's population Veeresha et al. (2015). Stability analysis has been used by many researchers to decide whether the performance of the genotype is satisfactory? Stability and adaptation studies are useful for releasing agenotype to cultivate in wide range of environments (Shrestha et al. 2020).

In Egypt, rice is considered one of the most important cereal crops, contributing about 20 % of the total grain consumption and also as export crop. The area rice cultivated area is about 0.483 million hectares and the annual production of this area more than 4.227 million tons of paddy rice (RRTC 2023). Rice production must be increased to 40%

for all rice ecosystems even under different stress area to achieve the food security by 2025.

To meet the demand of the growing population, development of new high-yielding hybrids and improvement of lines/varieties along with a stable agricultural climate is a major challenge. One of the main limitations for low yield is insufficient understanding of gene action that governing yield and most of the yield components (quantitative traits), which determine choice that should be followed to improve them with the aim of obtaining higher yield. While estimating genetic components of variance for a metrical trait from second degree statistics, three difficulties inevitably arise. While estimating genetic components of variance for a metric trait from second degree statistics, three difficulties inevitably arise. First, non-allelic interactions are assumed to be absent. This assumption may be correct for some traits but not for others. Therefore, these analyses rarely provide a valid test of this assumption. Second, the screening of dominance components always have much larger standard errors than the corresponding additive component (ElAbd et al 2009). Third,

*Corresponding author email: mohammed_bahgat25@agr.kfs.edu.eg

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additive and dominance components are differentially affected by linkage and distribution of linkage in the parents and can only be compared in the unlikely event that the population sample is linkage equilibrium.

A good inheritable model is one that which enables the breeder to obtain accurate and unbiased estimates of all components of genetic variance. Determine an effective breeding method largely depends on understanding the genetic scheme controlling the traits to be selected.

A good genetic model is that which enables the breeder to obtain accurate and unbiased estimates of all the components of genetic variation. Treble test cross (TTC) design proposed by Kearsey and Jinks (1968), a modification of the "North Carolina III Design" use it widely because it gives an accurate detection of the presence of epistasis, regardless of the alleles frequency, inbreeding level and creates balance in the population.

The top cross design was developed by Kearsey and Jinks (1968) as an extension of the North Carolina Mating Design III (Comstock and Robinson 1952), which was recently expanded and modified by Jinks *et al.* (1969), and Jinks and Perkins (1970). The primary purpose of the crossover of the triadic test to unambiguously describe the epistasis for quantitatively inherited traits, and assuming that the L1 and L2 are very high and low selection lines (Kearsey and Jinks 1968), it also provides independent tests for the presence of additive and dominance components of genetic variance are equally subtle in the absence of epistasis (Jinks and Perkins 1970). TTC analysis (Kearsey and Jinks 1968) gives estimates on epistatic variation, further estimating the additive and dominance components of variation when epistasis is absent. The (hypotheticals) assumptions for a top cross analysis aren't demanding, and the model is independent of genetic linkage mating system and allelic frequency (Kearsey and Jinks 1968). This work aimed to study the gene action for yield and the characteristics of yield through triple test cross and developing breeding strategies to develop high-yielding varieties

1. Materials and Methods

1.1. Experimental site:

The present investigation was conducted at the farm of the Sakha, Kafr El-sheikh, Egypt during the three successive 2020, 2021 and 2022 seasons

1.2. Plant materials:

Two rice genotypes, Giza 177 (P_1) and GSRIR2-9-L1-L3R2 (P_2) designated as L1 and L2, respectively, were used as test genotypes.

Two rice accessions with various growth and yield characteristics and hybridized in a combination of Giza 177 x GSRIR2-9-L1-L3R2 in summer 2020 season. The resulting F_1 was the third tester

designated as L_3 . Fifteen promising lines, MJ5460-1, MJ5460-2, MJ5460-3, MJ5460-4, MJ5460-5, MJ5460-6, MJ5460-7, MJ5460-8, MJ5460-9, MJ5460-10, MJ5460-11, MJ5460-12, MJ5460-13, MJ5460-14 and MJ5460-15 were crossed with three testers (L_1 , L_2 and L_3) in next year summer 2021 season. The testers used a male lines in the entire triadic test cross combinations.

The experiment thus consisted of 45 hybrid combinations i.e. 15 L_{1i} , 15 L_{2i} and 15 L_{3i} families of a Triadic test cross (T.T.C.) design. At the same time, Giza 177 (L_1) and GSRIR2-9-L1-L3R2 (L_2) were obtained and crossed to produce F_1 hybrid (L_3) during the same season.

The materials consisting of 3 testers, 15 lines, and 45 hybrid combinations were in an experimental randomized complete block design with three replicates in 2022 rice growing season. Each replicate consisted of one row of F_1 hybrid among two rows of each parental line; the row was five meters long, 20 cm apart, and contained 25 plants. Maintain the distance between plants and within the rows at 20 cm respectively. All technical recommendations for rice cultivation were applied of **RRTC 2021** season. Data of 10 plants were randomly recorded from each replicate for the following traits: plant height (cm), flag leaf angle (O), number of total grains per panicle, grain yield/plant (g), harvest index% and grain shape (mm) as recommended by the Standard Evaluation System (SES) of **IRRI (2008)**.

1.3. Statical analysis

1.3.1. Analysis of variance

Mean squares analysis of variance was performed following the system described by Singh and Chaudhary (1999) for estimating the significance of treatments and partitioning the treatment effect so as to determine the significance differences between genotypes include that hybrid, parents, lines, testers, $P_1 + P_2$ vs. F_1 , P_1 vs P_2 , lines vs. testers and hybrids vs. parents for each character using the TTC system.

1.3.2. Test for epistasis:

Epistasis detection was performed according to Singh and Chaudhary (1999). The significance test of the difference ($L_1j + L_2j - 2L_3j$ (j = genotypes), provides information about the presence or absence of epistasis. Therefore $L_1j + L_2j - 2L_3j$ was calculated for each line (genotype) and each replicate (replication) and then tested., which consists of 4 values for each genotype) and Total epistasis was calculated for 4 degrees of freedom as uncorrected genotype (lines) squared sums [$S((L_1j + L_2j - 2L_3j)^2)/n$ over the total number of replicates C.F. correction factor = $[S (L_1j + L_2j - 2L_3j)]^2 / n$ mainly measures additive i type for one degree of freedom and genotypes corrected sum of squares [$S ((L_1j + L_2j - 2L_3j)^2 / n - c.f.)$ basically measures the type j + l (additive x dominance and x dominance) for 3 degrees of freedom.

The sum of squares associated with the interaction of epistasis with blocks (replicates) was calculated as the difference between the sum of squares and the type of epistasis (total epistasis – total epistasis/type i epistasis/type j + l). Each of the three types of epistasis was tested against their respective reaction to the blocks. But before testing individual epistasis, the reaction of homogeneity was first tested. Since there were only two variances (i x block and j + l x block) homogeneity was tested as follows:

$F(2, 14) = \text{Mean square of interaction of type i} / \text{Mean square of interaction of type j and i}$

When interaction of homogeneity variances was not significant, i-type and j + l-type epistasis were also tested against the pooled error, i.e., the total interaction of block x.

1.3.3. Individual genotypic epistasis

The individual contribution of each line to total epistasis was estimated and its significance tested according to Kitata et al (1976) for those traits for which total epistasis was significant. The mean value $(S L_{1j} - L_{2j} - 2L_{3j}) / r$ (where r is the number of frequencies) for each genotype for a trait was tested using a t-test with 8 degrees of freedom as follows: $t = \text{Mean}/\text{S.E.}$ where s. = (mean square error/frequency) $^{1/2}$

1.3.4. Additive-dominance model

The traits where total epistasis effects weren't detected by either test, an additive-dominance model was fitted to the data as outlined by the **Kearsey and Jinks (1968) and Jinks et al., (1969)**.

1.3.5. Estimation of additive variance component (D)

The sum of $L_{1j} + L_{2j}$ for each genotype was calculated replication accurately and showed to analysis of variance as follows:

Table 1. The form of mean square for analysis of variance to estimate the additive components of the genetic variance.

Source of Variance	df	MS	M.S. Expected
Replications	r-1	MS_r	
Genotypes sum ($L_{1j}-L_{2j}$)	g-1	MS_s	$\sigma_e^2 + 2r \sigma_s^2$
Error	(r-1)(g-1)	MS_e	σ_e^2

Where r = Replications; g = Genotypes; MS_r , MS_s , MS_e = Mean squares of replications, genotypes (sums) and error, respectively; σ_e^2 and σ_s^2 = Expected mean square of error and genotypes (sums).

The observed mean squares were substituted into the equations as follows:

$$\sigma_s^2 = (MS_s - MS_e)/2r$$

$$\sigma_s = (1/4)^D$$

$$D = 4(MS_s - MS_e)/2r$$

1.3.6. Estimation of dominance component (H)

The difference in $L_{1j} - L_{2j}$ for each genotype was calculated precisely and analysis of variance

showed as follows;

Table 2. The form of analysis of variance to estimate the dominance components of the genetic variance.

Source of Variance	df	MS	MS Expected
Replication	r-1	MS_r	
Genotypes difference ($L_{1j}-L_{2j}$)	g-1	MS_d	$\sigma_e^2 + 2r \sigma_d^2$
Error	(r-1)(g-1)	MS_e	σ_e^2

Where r = Replication; g = Genotypes; MS_r , MS_d , MS_e = Mean squares of replication, genotype (differences) and error, respectively; σ_e^2 and σ_d^2 = Expected MS of error and genotypes (differences).

The observed mean squares were substituted into the equations as follows:

$$\sigma_d^2 = (MS_d - MS_e)/2r$$

$$\sigma_d = (1/4)H$$

$$H = 4(MS_d - MS_e)/2r$$

1.3.7. Average degree of dominance

Average degree of dominance was calculated as $(H/D)^{1/2}$, where H and D were the dominance and additive variance components respectively.

1.3.8. Direction of dominance (rs_d)

The direction of dominance was determined by calculating the linear correlation coefficient rs_d between the sum of ($L_{1j} + L_{2j}$) and genetic differences ($L_{1j} - L_{2j}$) for all genotypes. Significant positive and negative correlations indicate a trend toward decreasing and increasing attribute values, respectively (**Jencks et al., 1969**).

All cross-test calculations for the three-way test were performed using the COSTAT-C package (Michigan State University and Agricultural University of Norway).

2. Results

2.1. Analysis of variance

Table 3 shows the mean square analysis of variance effects for different morphological, agronomic and physical traits of rice accessions. The results showed that there were highly statistically significant differences between the rice genotypes for the studied traits, which indicates the presence of overall differences between these accessions.

It was noticed that parents, lines, hybrids and testers for the studied characters which indicates the presence of great variation between the accessions. Mean squares of significant P_1 versus P_2 and $P_1 + P_2$ versus intercepts for plant height, flag leaf angle, number of total grains/panicle, grain yield/plant, harvest index % and grain shape. Highly differences between L_1 and L_2 led to the expression of higher average performance for F_1 (L_3) as indicated by the large contrast due to $P_1 + P_2$ vs. crosses.

2.2. Mean performance of parents and their F₁ hybrid combination:

The data in Table 4 and Figure 1 showed that mean of lines and indicate that there is strong genetic variation to improve plant height, flag leaf angle, number of total grains / panicle, grain yield /plant, harvest index, grain shape, which are important in breeding rice are showed in Fig 1, among the lines, the lines MJ5460-9, MJ5460-6, MJ5460-14, MJ5460-15 and MJ5460-10 was recorded the desirable values for plant height (85.32cm), angle of flag leaf (11.28 °), total grain number (248.39

grains), grain yield/ plant (57.43), harvest index (60.00%) and grain shape (2.08mm). For testers, the rice variety Giza 177 recorded the desired values for plant height (105.27), the line GSRIR2-9-L1-L3R2 recorded the desired values for flag leaf angle (14.41°), harvest index% (52.55%) and grain shape (2.36mm), while, the hybrid Giza 177 x GSRIR2-9-L1-L3R2 recorded the desirable values for total grains number (245.21 grains), grain yield / plant (50.47 g) and harvest index% (55.79%), respectively.

Table 3. Mean squares for various vegetative and yield their associated traits in rice.

S.O.V	Df	The studied traits					
		Plant height(cm)	Flag leaf angle (°)	No. of Total grains / panicle	Grain yield / plant (gm)	Harvest index (%)	Grain shape (mm)
Replication	2	0.07	0.19	1.84	0.17	0.06	0.02
Treatments	62	1087.49**	48.19**	10842.39**	171.44**	107.14**	0.04**
Hybrids	44	960.25**	29.99**	8282.59**	57.73**	107.58**	0.03**
Parents	17	431.89**	49.64**	3976.09**	137.32**	111.61**	0.04**
Lines	14	185.76**	13.82**	3225.73**	156.41**	69.43**	0.02**
Testers	2	611.44**	173.21**	11038.07**	44.12**	238.56**	0.16**
L ₁ +L ₂ vs hybrids	1	1205.24	296.95**	17156.93**	54.67**	462.79**	0.02**
L ₁ vs L ₂	1	17.65**	49.48**	4919.21**	33.56**	14.32	0.29**
Lines vs Testers	1	3518.59**	303.97**	357.14**	56.49**	448.22**	0.12**
Hybrids vs Parents	1	17831.18**	824.63**	240200.69**	5754.78**	11.34	0.08**
Error	124	1.23	0.27	12.35	1.87	7.96	0.00

Table 4. Mean performance of genotypes (lines and testers) for plant height, flag leaf angle, number of total grains/ panicle, grain yield plant⁻¹, harvest index and grain shape during 2021 and 2022 growing season.

Entries	The studied characters					
	Plant height (cm)	Flag leaf angle (°)	No. of Total grains / panicle	Grain yield / plant (g)	Harvest index (%)	Grain shape (mm)
Lines						
MJ5460-1	89.27	16.12	133.23	45.58	51.81	2.03
MJ5460-2	94.53	13.99	122.29	43.67	49.49	1.85
MJ5460-3	99.23	15.50	169.19	55.41	55.56	1.97
MJ5460-4	102.00	13.21	189.55	37.53	44.58	1.92
MJ5460-5	100.41	20.20	198.29	47.55	53.03	2.08
MJ5460-6	85.53	11.28	177.93	54.08	56.39	1.92
MJ5460-7	93.99	13.17	206.52	39.47	45.51	1.87
MJ5460-8	88.17	16.48	222.71	37.52	48.87	2.04
MJ5460-9	85.32	12.73	230.70	54.83	57.42	2.01
MJ5460-10	89.54	14.46	181.05	56.14	60.00	2.08
MJ5460-11	83.02	15.65	195.17	52.31	59.74	1.86
MJ5460-12	92.91	14.42	199.06	56.41	56.77	2.02
MJ5460-13	92.47	14.15	191.65	55.17	52.56	2.02
MJ5460-14	113.70	16.50	248.39	52.83	56.13	1.96
MJ5460-15	92.48	16.79	189.78	57.43	57.06	2.04
Mean	93.50	14.98	190.37	49.73	53.66	1.98
Tester						
Giza 177	105.27	20.15	123.96	42.88	49.46	1.92
GSR	108.70	14.41	181.23	47.61	52.55	2.36
Giza 177 x GSR (F ₁)	131.53	29.47	245.21	50.47	55.79	2.03
Mean	115.16	21.34	183.47	46.98	45.93	2.11

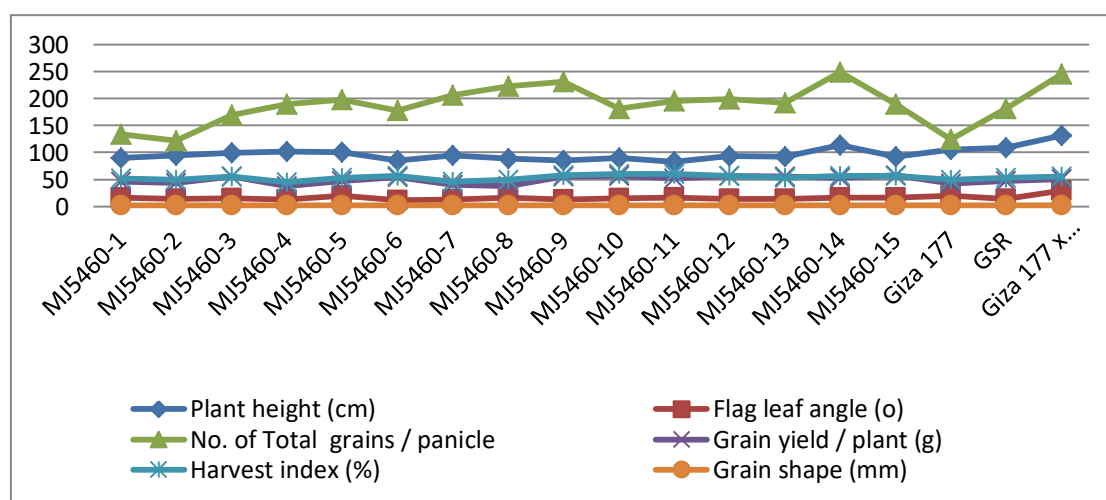


Fig. 1. Illustrated Mean performance of parental lines for plant height, flag leaf angle, number of total grains/ panicle, grain yield plant⁻¹, harvest index and grain shape during 2021 season.

Table 5. Mean performance of F₁ (triadic test cross) for plant height, flag leaf angle, number of total grains/ panicle, grain yield plant⁻¹, harvest index and grain shape during 2022 growing season.

Hybrid combinations	Plant height	Flag leaf angle	No. of Total grains / panicle	Grain yield / plant (g)	Harvest index	Grain shape
MJ5460-1 x Giza 177	108.65	25.78	182.18	58.91	58.13	2.01
MJ5460-1 x GSR	128.41	20.75	256.61	62.89	49.48	2.12
MJ5460-1 x F ₁	140.46	23.28	282.70	66.14	50.97	1.98
MJ5460-2 x Giza 177	117.37	30.16	207.13	63.98	57.41	1.98
MJ5460-2x GSR	123.58	16.96	253.33	65.08	41.69	2.20
MJ5460-2 x F ₁	105.62	19.92	316.27	69.12	60.58	1.95
MJ5460-3 x Giza 177	93.13	18.83	262.10	63.97	60.02	1.96
MJ5460-3 x GSR	151.67	21.49	398.99	68.15	54.68	2.18
MJ5460-3 x F ₁	138.32	20.75	302.73	67.56	52.40	2.05
MJ5460-4 x Giza 177	118.27	24.24	234.80	67.09	57.75	2.02
MJ5460-4 x GSR	147.56	22.35	355.10	72.15	52.50	2.09
MJ5460-4 x F ₁	116.65	22.14	193.85	65.35	53.82	1.90
MJ5460-5 x Giza 177	105.93	23.75	228.75	54.17	56.82	2.10
MJ5460-5 x GSR	130.23	20.79	274.57	56.13	50.79	2.20
MJ5460-5 x F ₁	122.64	21.01	237.05	58.48	39.35	2.06
MJ5460-6 x Giza 177	89.39	20.94	244.93	58.38	54.84	1.84
MJ5460-6 x GSR	139.59	19.30	341.12	55.89	46.02	2.00
MJ5460-6 x F ₁	130.70	21.25	276.03	64.73	51.41	1.88
MJ5460-7 x Giza 177	95.51	20.78	236.10	58.35	58.90	2.06
MJ5460-7 x GSR	141.78	16.74	345.13	64.84	37.12	2.02
MJ5460-7 x F ₁	103.50	16.43	241.33	60.76	52.32	2.02
MJ5460-8 x Giza 177	96.52	18.61	248.78	57.79	56.65	1.90
MJ5460-8 x GSR	139.46	17.68	330.20	58.73	53.30	1.93
MJ5460-8 x F ₁	114.60	22.64	277.60	68.54	56.38	2.10
MJ5460-9 x Giza 177	98.41	16.56	236.97	58.94	55.96	1.92
MJ5460-9 x GSR	128.48	15.50	314.99	57.69	55.98	2.04
MJ5460-9 x F ₁	108.31	27.41	270.30	66.53	57.02	2.21
MJ5460-10 x Giza 177	96.34	16.71	214.38	57.34	53.99	1.94
MJ5460-10 x GSR	127.49	19.02	327.96	59.63	55.57	2.05
MJ5460-10x F ₁	111.87	17.49	259.87	63.01	45.42	2.04
MJ5460-11x Giza 177	103.62	20.70	225.17	54.27	55.46	1.92
MJ5460-11x GSR	127.25	21.30	350.92	56.68	55.43	2.08
MJ5460-11x F ₁	106.48	16.67	203.83	61.95	39.50	2.10
MJ5460-12x Giza 177	91.47	25.05	264.07	54.29	59.23	2.06
MJ5460-12x GSR	163.59	26.14	296.13	64.04	39.81	2.04
MJ5460-12x F ₁	122.40	22.60	265.43	60.17	59.24	2.07
MJ5460-13x Giza 177	103.33	17.85	149.71	62.95	56.17	2.10
MJ5460-13x GSR	127.48	21.67	293.30	59.96	50.83	2.16
MJ5460-13x F ₁	114.20	19.86	267.10	62.54	53.32	2.01
MJ5460-14x Giza 177	99.53	20.19	305.89	57.79	61.88	1.94
MJ5460-14x GSR	133.64	22.27	334.70	59.45	51.79	2.20
MJ5460-14x F ₁	122.55	21.10	263.33	64.33	55.14	1.94
MJ5460-15x Giza 177	98.12	18.95	169.81	59.25	56.26	2.03
MJ5460-15x GSR	139.24	19.28	282.76	57.63	51.91	2.21
MJ5460-15x F ₁	114.36	16.93	241.90	61.24	47.94	2.37
Mean	118.62	20.66	268.13	61.49	52.92	2.04

As for hybrid combinations MJ5460-5 x Giza 177 recorded the desired values for plant height (89.39cm), while, the cross MJ5460-9 x GSRIR2-9-L1-L3R2 recorded the desirable value for flag leaf angle (15.50°), the cross MJ5460-3 x GSRIR2-9-L1-L3R2 gave the highest value for number of total grain/ panicle 398.99 grains) and the hybrid combination MJ5460-4 x GSRIR2-9-L1-L3R2 gave the highest value for grain yield/ plant (72.15 g).

On the other side, the cross; MJ5460-14 x Giza 177 recorded the desirable values for harvest index % (61.88%), while, the hybrid combination MJ5460-15 x F₁(Giza 177 x GSR) gave the desirable value for grain shape (2.37mm) as shown in Table (5) and Fig 2, so, rice breeders should be using these genotypes as donors factors to develop new rice varieties in breeding programs.

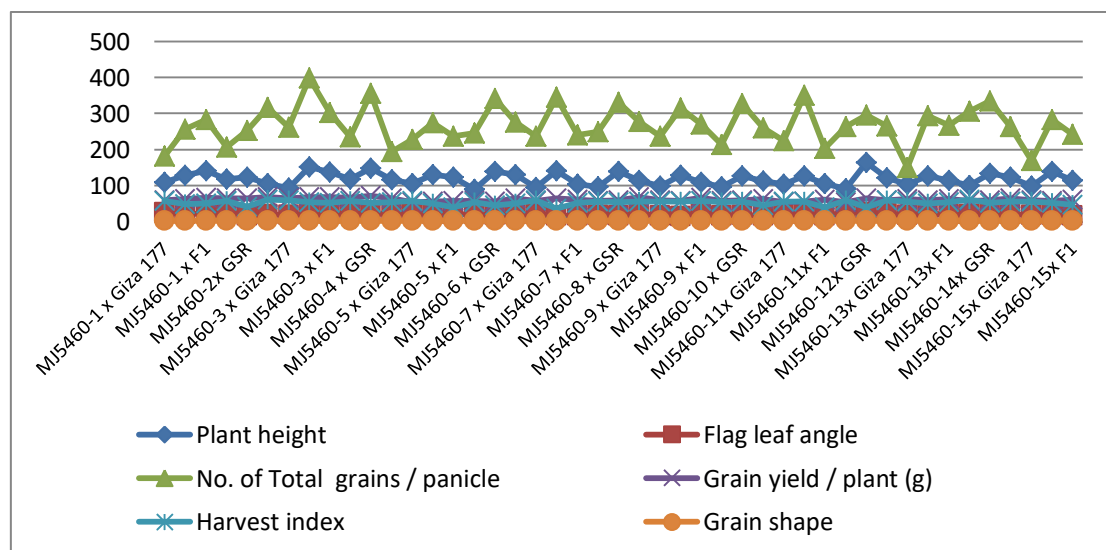


Fig. 2. Illustrated Mean performance of F₁ (triadic test cross) for plant height, flag leaf angle, number of total grains/ panicle, grain yield plant⁻¹, harvest index and grain shape during 2022 season.

2.3. Detection of epistasis:

Epistasis is the interaction between alleles of different genes, i.e. non-allelic interaction. Most mating designs usually adopted in the rice breeding programs assume the absence of epistasis. Thus, ignoring this effect led to missing information about part of the effects of genes and epistasis as well as estimates of genetic components, such as; Additional variance and dominance will be biased. Thus, triple test analysis indicates the relative importance of each component in the inheritance of a particular trait and helps the breeder follow alternative

breeding procedures.

Analysis of variance to test the presence of epistasis in the inheritance of the studied traits is showed in (Table 6). The mean square of the deviations L1i + L2i – 2L3i showed high significance on all samples for all studied traits. Further subdividing the epistasis into its component parts revealed that the variation of additive × additive (i) epistasis type was of great importance for grain/plant production. The variance of added dominance x and dominance x dominance of the type (J+I) was highly significant for the studied traits.

Table 6. Analysis of variance to detect epistasis for yield and their associated traits in rice accessions.

S.O.V	Df	Plant height (cm)	Flag leaf angle (°)	No. of Total grains / panicle	Grain yield / plant (g)	Harvest index (%)	Grain shape (mm)
I type epistasis	1	77.697**	0.374	27074.00**	2623.11**	644.03**	0.000
j+I type epistasis	14	1650.56**	176.20**	31075.72**	169.83**	694.86**	0.213**
Total epistasis	15	1545.70**	164.47**	30808.94**	333.39**	691.49**	0.199**
I type epistasis x block	3	19.032	1.155	37.980	25.441	6.858	0.010
j+I type epistasisx block	42	5.012	0.383	39.641	6.882	28.488	0.006
Total epistasisx block	45	5.947	0.435	39.530	8.119	27.046	0.006

Table 7 shows the visual deviations of individual lines for plant height, flag leaf angle, total grains/flower clusters, grain yield/plant, harvest index and grain shape, to illustrate the direction of dominance and relative magnitudes and to identify the lines, which interacted with L1 and L2 to

produce significant deviations. Moreover, line MJ5460-4 had the most significant contribution to the total epistasis of all studied traits, and it also accounted for the largest positive portion of the total epistasis for flag leaf angle, total grain/panicle, grain yield/plant, and grain shape, respectively.

MJ5460-1 rice line was the major contributors of negative epistasis portion to the total epistasis for plant height. In addition, line MJ5460-2 and MJ5460-11 were the largest contributors to positive epistasis portion to the total epistasis for angle of flag leaf, harvest index% and grain shape. The effect of lines on the non-allelic interaction of these

traits indicated that the occurrence of epistasis is determined to some extent by the lines used in the study. Therefore, multiple lines and very high versus low (L_1 and L_2) tests should be used in such studies that aim to detect epistasis and to estimate the dominant and additive component of variation with equal accuracy by the TTC technique.

Table 7. Epistasis deviations of individual rice genotypes exhibiting significant differences for grain yield and their associated traits in rice.

S.O.V	Plant height	Flag leaf angle	No. of Total grains / panicle	Grain yield/ plant	Harvest index	Grain shape
MJ5460-1	-43.85**	-0.03	-126.61**	-10.49**	5.67*	0.18**
MJ5460-2	29.71**	7.29**	-172.07**	-9.17**	-22.06**	0.28**
MJ5460-3	-31.83**	-1.18**	55.62**	-3.01**	9.89**	0.04
MJ5460-4	32.54**	2.30**	202.20**	8.54**	2.60	0.30**
MJ5460-5	-9.11**	2.51**	29.23**	-6.66**	28.92**	0.17**
MJ5460-6	-32.42**	-2.27**	33.99**	-15.18**	-1.96	0.09**
MJ5460-7	30.29**	4.66**	98.57**	1.67	-8.63**	0.05*
MJ5460-8	6.77**	-8.98**	23.78**	-20.57**	-2.81	-0.37**
MJ5460-9	10.27**	-22.76**	11.36**	-16.43**	-2.11	-0.45**
MJ5460-10	0.09	0.74	22.60**	-9.05**	18.71**	-0.09**
MJ5460-11	17.90**	8.65**	168.42**	-12.94**	31.89**	-0.19**
MJ5460-12	10.25**	5.99**	29.33**	-2.02	-19.43**	-0.04
MJ5460-13	2.40**	-0.20	-91.19**	-2.18*	0.37	0.25**
MJ5460-14	-11.93**	0.27	113.92**	-11.43**	3.40	0.25**
MJ5460-15	8.64**	4.38**	-31.23**	-5.60**	12.28**	-0.49**

2.4. Detection and estimation of components of additive and dominant genetic variation:

1- Estimating the variance components of genetic parameters:

Estimates of additive variance (D), dominance (non-additive) (H), degree of dominance (H/D)^{1/2}, and direction of dominance ($rs.d$) are shown in Table 8. The additive and dominant effects are of equal significance for the studied characters. The higher magnitude of additive variance for flag leaf angle, number of total grains/ panicle, weight grain yield/ plant and shape indicates the presence of favorable alleles in the testers and their cumulative effect in the expression of these traits which can be improved by pedigree method of selection. Estimating degree of dominance (H/D)^{1/2} 2 showed that the effect of the over dominance gene in relation to plant height and harvest index% indicating the high influence of

dominance components. While the flag leaf angle, number of total grains/panicle, weight of grain yield/plant, and grain shape showed a value less than one unit of dominance, which indicates partial or incomplete dominance.

2- Direction of dominance:

If (r) is negative and significant, then an increase in the type of gene is dominant and vice versa. It was also shown that the correlation coefficients between the sum and the differences were negative for plant height, the number of total grains/panicle, weight of grain yield/plant, harvest index %, and grain shape (mm). As for the remaining traits (for traits that show the absence of epistasis), showed positive correlation coefficients of sums and differences and both directions were insignificant.

Table 8. Estimates of genetic parameters for studied traits showing non-significant epistasis in rice accessions.

Genetic parameter	Plant height (cm)	Flag leaf angle (°)	No. of Total grains / panicle	Grain yield/ plant (gm)	Harvest index (%)	Grain shape (mm)
D	470.3	105.4	19716	245.84	93.176	0.0734
H	1106.6	69.209	5749.3	42.691	143.22	0.0274
$(H/D)^{1/2}$	1.534	0.810	0.540	0.417	1.240	0.611
$rs.d$	-0.274	0.332	-0.035	-0.299	-0.796	-0.140

2.5. Cluster analysis

Clustering genotypes (line and testers) based on similarity of quantitative characteristics, produced two large groups (Fig. 3). The first group included fifteen genetic combinations and divided into two subgroups. The first subgroup included two rice genotype MJ5460-14 and the hybrid combination Giza 177 x GSRIR2-9-L1-L3R2 which strain in number of total grains/ panicle, weight of grain yield / plant and harvest index% and grain shape (mm). While, the second sub-group included thirteen rice genotypes which the second subgroup divided to two sub sub group, the first sub sub group included three genotypes namely the lines MJ5460-7, MJ5460-8

and MJ5460-9 which are similar in weight of grain yield / plant (gm) and harvest index%, while, the second sub-sub group included ten genotypes which similar in angle of flag leaf and harvest index%.

The second large group included three rice genotypes MJ5460-1, MJ5460-2 and Giza 177, these genotypes strain in grain yield/ plant, harvest index% and size of grain. These results explained genotype in cluster I will provide genotypes with desirable characters for breeding program. Also considering promising genotypes of cluster II, III, IIII and IV may provide superior desirable segregates for developing high yielding genotypes.

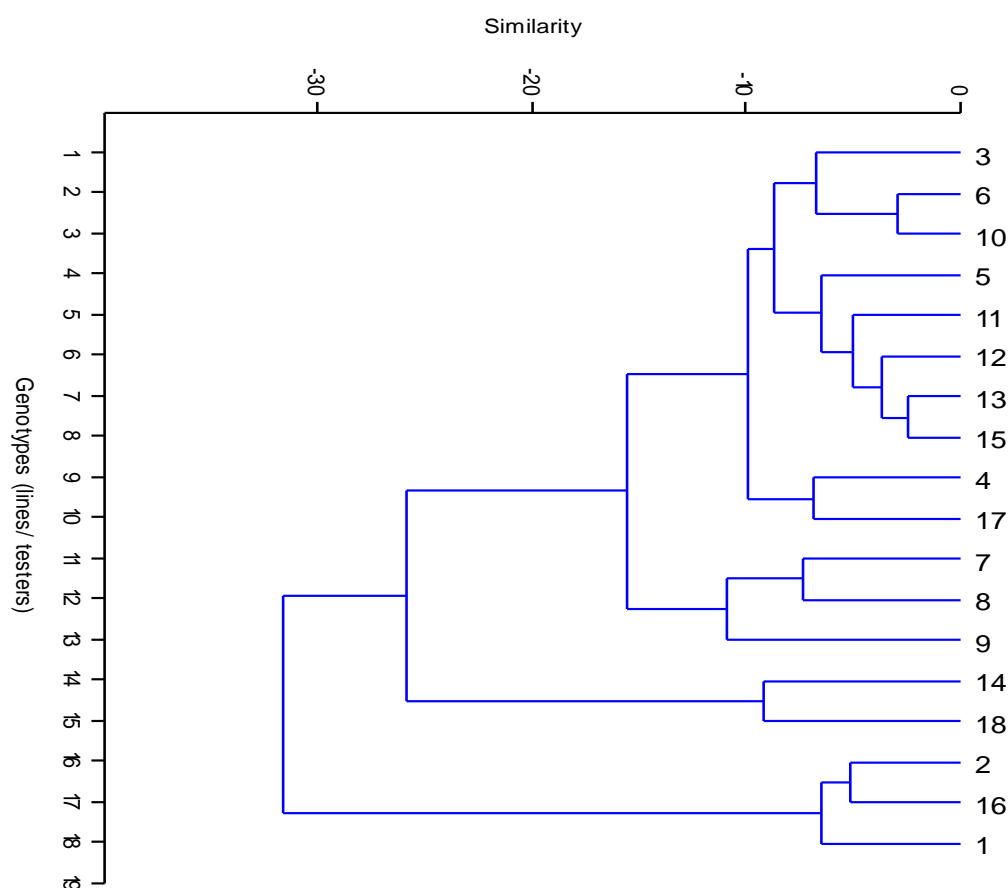


Fig. 3. Cluster analysis between parents (lines / testers) for yield and its component traits.

Concerning (Triadic) crosses forty five crosses rice genotypes evaluated in this study were grouped into two main groups using clustering analysis based on the differences between the crosses and contribution of the traits evaluated (Figure 4). Number of genotypes per cluster varied from 6 to 12 cross (Fig.

4). Cluster 1 was the largest and contained 12 cross rice genotypes which strain in flag leaf angle, number of total grains/ panicle and weight of grain yield/ plant. While the smallest cluster contain six crosses which strain in grain yield/ plant (gm), harvest index% and grain shape.

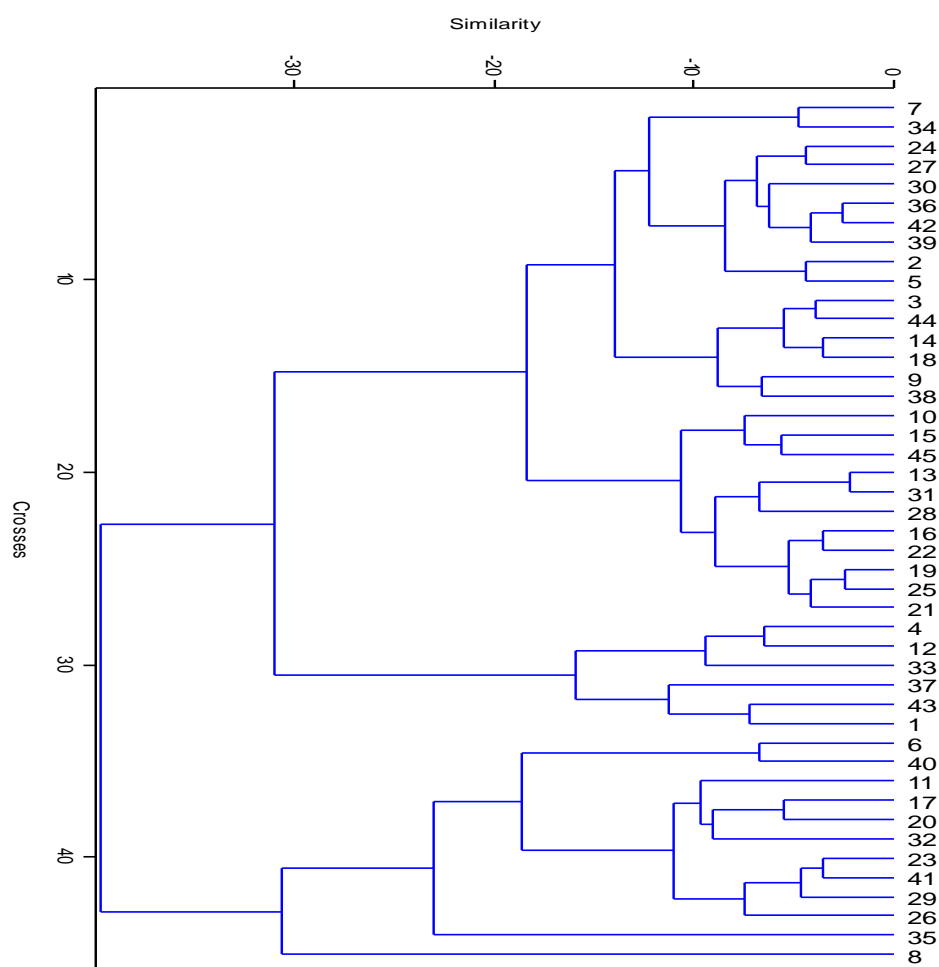


Fig. 4. Cluster analysis between crosses (TTC) for yield and its component traits.

2.6. Phenotypic correlation

The correlation coefficient's between the studied traits plant height, angle of flag leaf, number of total grains / panicle, harvest index% and size of grain is an important factor, especially in economic and complex character as yield. **Steel and Torrie (1984)** stated that correlations are measures of the intensity of association among traits. The selection for one

character results in progress for all characters that are positively correlated and retrogress for traits that are negatively correlated. In general, the phenotypic correlation results indicated that yield per plant correlated positively and significantly with plant height, flag leaf angle and number of total grains / panicle as shown in Table 9. These results support the results of Girish *et al.* (2006).

Table 8. Phenotypic correlation coefficients between vegetative and yield and its component traits of some rice genotype during 2022 season.

Traits	PH	FLA	NTG	GY	HI	GS
PH	1.00					
FLA	0.473**	1.00				
NTG	0.713**	0.332**	1.00			
GY	0.495**	0.435**	0.540**	1.00		
HI	-0.391**	0.155	-0.070	0.098	1.00	
GS	0.331**	0.148	0.229	0.209	-0.161	1.00

3. Discussion

4.1 Analysis of variance:

There is a large amount of genetic variation for genotypes including lines, testers and hybrids as shown in (Table 3) due to the presence of highly significant differences between the studied traits. Significant differences between parents clearly revealed that L1 and L2 testers were modeling high challenges versus low choices and would provide an estimate of additive contrast and dominance with equal accuracy, as reported by **Kersey and Jenks (1968)**. Therefore, inadequacy of testers cannot be ruled out.

Because two testers showed very large differences for each trait, they would provide accurate estimates of additive variance and dominance as reported by **Kearsey & Jinks (1968)** and **El-Abd *et al.*, (2009a)**. High significant differences were also recorded for all traits between the genotypes, including: Parents, lines, labs, crosses, P1+P2 vs. crosses, lines vs. hybrids and hybrids vs. parents. These results are in generally agreed with those obtained by **Hussain *et al.* (2014)** who detected very significant differences between genotypes, hybrids, parents, and hybrids vs parents with respect to yield and its contributing traits in TTC rice lines, suggesting the presence of genetic variation between hybrids and parents.

They also found significant differences between parents vs. hybrids. On the same time, parents (lines and testers) used in this study were divergent. Therefore, the breeder can exploit this variation through the breeding program.

4.2. Mean performance of parents and their F₁ hybrid combination:

The previous genotypes achieved also to make a big change in yield weight when estimated which reflection the impact of additive and additive x additive gene action for controlling and inheriting the recent studied growth and yield traits and its fruitful role for enhancing and increasing yield in rice entries under Egyptian conditions as shown in Tables 4 and 5). These results have been reported by **Asnake and Mohammed 2017, Balat *et al.*, 2018 and Abo-Youssef *et al.*, (2023)**.

4.3. Detection of epistasis:

The j and i type epistasis detected for the studied traits as shown in (Table 6) are non-directional and unfixable by selection under self-fertilization in rice, and therefore would not be suitable for developing new promising lines for these traits, although it could be used in developing hybrids (**Subraman and Rangasamy, 1989**).

The larger magnitude of i type epistasis compared to j and i types has a special significance in rice, being a self-fertilized crop in which a directional linear and fixable component (i type epistasis) of genetic variation can be more easily exploited for increasing grain yield plant⁻¹ through standard hybridization and selection procedures. The j and i type epistasis

are non-directional and unfixable by selection under self-fertilization and therefore would not be suitable for developing pure lines for plant height, flag leaf angle, number of total grains/bubs, harvest index % and grain shape, however it can be used for developing hybrids According to **Kiani *et al.* (2013), Koli *et al.*, 2014 and Dawwam *et al.*, (2015)**.

The i type epistasis was found much larger in magnitude than j+ i type of epistasis indicating the predominant role of I type non- allelic interaction controlling these traits. The results revealed that i and j+ i types of epistasis were in similar with those obtained by **Saleem *et al.*, (2005), Muhanmad. *et al.* (2009), Hassan *et al.*, (2013) and Hanifei *et al.*, (2021)**.

In another study obtained that the effect of additive variance is greater than dominance variance ($D > H1$) for length and shape of rice. Higher magnitude of additive variance (D) indicated the greater role of additive gene action, while variance of non-additive (dominant) indicated the greater importance of non-additive gene action (dominant). **Quamruzzaman *et al.*, (2020)**.

The presence of asymmetric interactions for economic trait may play an important role in genetic in plant breeding. Type i of epistasis represents fixable epistasis, while types j + i shows an unfixable portion of genetic variations **Mather (1949)**. The results indicated the presence of i and j + i types of epistasis is most traits and hence in this case recurrent selection technique is suggested.

Generally, partitioning of the epistasis gene effects into its components would indicate that the (i) type (additive x additive) was found to be much larger in magnitude than additive x dominance and dominance x dominance (J+L). so, for grain yield/ plant the epistatic types indicating that, non-fixable component of epistasis was less important than fixable one in the inheritance of such traits. The presence or absence of epistasis may depend on the environment in which the plant material was assessed and may not always be related to the genetic potential of a genotype, the components of variance changed to different degrees with changing environments.

The (i) type of epistasis was also been found to be more important than (J+L) types in rice by **Hussain *et al.*, (2014)** for number of panicle/ plant, grain yield / plant and grain quality, while **Morad (2012)** revealed significant (J+L) type of epistasis for most yield traits and was large in magnitude than (i) type.

Detection and estimation of additive and dominance genetic variance components:

Since, epistasis plays an important role in controlling all traits studied, although estimates of additive and dominant genetic variance will be biased. Thus, ignoring this effect resulted in missing information about epistasis and estimates of genetic components would be biased. Thus, the triadic test cross analysis indicates their relative importance in

the inheritance of a particular traits and help breeder to follow alternative breeding procedures.

The effect of lines on non-allelic interactions for the studied characters indicated that the appearance of epistasis is determined to some extent by the lines employed for the study (Table 7). The large number of lines used in these studies may fail to detect non-allelic gene action, which is actually part of the genetic system (**Burton, 1968; Ketata et al., 1976**). The optimal experimental size required to detect epistasis by TTC depends largely on the genetic diversity in the tester parents (**Pooni et al., 1980**). Therefore, several lines and high vs. low testers (L1 and L2) should be used in such studies that aim to detect of epistasis and to estimate the additive and dominant component of variance with equal accuracy by the TTC technique.

Estimation of additive and dominant genetic components appeared to have been biased by epistasis to unknown extent. The presence of epistasis leads to a kind of discrepancy in the relative importance of the additive and dominance components of genetic variation in these materials under study. Although the triple testcross analysis provides a reliable estimate of such components, even if epistasis is present, it must be compared the efficiency of this approach with other biometrical procedure. The predominance of additive and non-additive (dominant) gene action for yield and yield component in rice was reported by **Ram et al. (2007) El-Abd et al., (2009)**.

The degree of dominance (h^2_D)^{1/2} was less than unity for all studied traits, which indicates the role of partial in the inheritance of these traits and ensuring that, most genes in self-pollinating crops, are homozygous and the over-dominance is sometimes. Genetic advance in genetic system with over-dominance and epistasis are slower than when gene effects are purely additive or partially dominance. These results confirm with the finding of **Ram et al. (2007) and (Esmail 2007)**.

However, correlation coefficients for the remaining traits were non-significant indicating that genes with positive and/or negative effects were evenly distributed among the genotypes including in this study (umbidirectional dominance). In this regard, **Esmail (2007)** found significant positive F value for 100 grain weight and insignificant value for the rest of the characteristics. While **Dawwam et al., (2015)** found a significant positive F value for main spike length, no. kernels/main spike and main spike yield and negative for spike length in the F_1 under normal condition. However, **Marwa, M. El-Nahas (2015)** found significant positive F value for no. spike / plant. The non-allelic interactions shown for crop and its constituent traits can be manipulated through the technique of recurrent selection to improve these traits. Recurrent selection for non-allelic inherited traits in rice has also been proposed (**Vijayakumar et al. (1996), Saleem et al. (2005) and Saleem et al.**

(2010). Since non- additive (dominance) gene effects were insignificant for yield and its component exception seed setting%, Simple treatments in later generations may not contribute significantly to improving these traits. Additive components in these traits can be successfully exploited by the pedigree method of additive genetic action effects in the first generations of segregated populations. To exploit all types of genetic effects, a two-parent approach and/or recurrent selection may be practical to improve grain production and components of rice lines in advanced generations as suggested by **Khattak et al. (2001)**.

Generally, from the results, may be concluded that, the traits under study are complex inherited and most type of gene effect were significant but additive coupled with the additive x additive as well as additive x dominance were higher in magnitude than dominance effect with some exception. Thus, selection procedures based on the accumulation of additive effects will be successful in improving most of traits under investigation. So, the maximize selection advance, procedures known to be effective in shifting gene frequency i.e. recurrent selection when additive and non-additive gene affects are concerned would be preferred.

4.4. Cluster analysis

This procedure, using cluster analysis based on Euclidean distance, was applied to elucidate relative genetic distances and genetic diversity within a given germplasm. Morphological characters have been utilized successfully to estimate of genetic diversity because they provide a simple way to measure genetic diversity (**Fufa et al., 2005**). Analysis of genetic diversity in rice germplasm helps in classifying and identifying genotypes with the potential to utilize them to achieve a specific breeding goal (**Mohammadi and Prasanna, 2003**).

Cluster analysis isolates genotypes into groups that exhibit high within group homogeneity and high between groups' heterogeneity. Finally, the breeders wish to increase genetic diversity among rice cultivars, while at the same time maintaining the combinations of desirable agronomic and quality characteristics found in existing economic varieties. Developing such a combination would be difficult, as the introduction of new genetic material would be expected to disturb genetic complex responsible for the desired traits. Using hybridization between disparate varieties could be a way to achieve both goals.

4.5. Phenotypic correlation:

Grain yield is a complicated trait and greatly influenced by many genetic factors and environmental conditions. In breeding method, direct selection for yield as such could be false. Effective selection relies on information on the genetic variation and association of morpho-agronomic traits with grain yield. Genotypic correlation studies showed that, the association of

various traits with grain yield.

Plant breeders must be concerned with the totality of economic traits and not just one trait. So, the change occurring in one letter is along the proportional change in the other. The reliability of the genetic components estimated from TTC makes its calculation more reliable.

Generally, the existence of additive, dominant and epistatic genetic correlations were found between some characters. At the same time the great part of traits revealed non-significant genetic correlations and confirmed that triple test cross mating system was useful in break up undesirable linkage to obtain new recombinant lines. In this connection, **Menshawy (2008)** and **Morad (2012)** found the efficiency of top cross for obtaining new recombinant lines in wheat.

4. Conclusion

From this study, it became clear that epistasis is an essential part of the genetic system that controls the genetic expression of the studied traits, including plant height, angle of flag leaf, number of total filled grains panicle⁻¹ and grain shape. The additive components in these characters can be successfully exploited through pedigree method of selection because of the significant contribution of additive gene effects in early generations. While, the effects of dominant gene were important for harvest index, recurrent selection may be practical for developing high yielding rice lines in late generations development from present TTC population.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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