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Assessment of genetic diversity, combining ability and photosynthetic parameters of some rice genotypes under two sowing dates

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Abstract

This investigation was conducted at Agriculture Research station, Sakha, Kafr El-sheikh, Egypt during 2019 and 2020 rice seasons to study genetic diversity, combining ability and photosynthetic parameters in rice. A line x tester model was used in this study where five rice varieties were crossed with three testers. Four primers were used to estimate the genetic diversity of the studied paternal lines and the values of major allele frequency ranged from 0.62 to 0.75 and averaging 0.69. Genetic diversity values ranged from 0.37 to 0.47 and averaging 0.42. The PIC values ranged from 0.43 to 0.61 and averaging 0.52. The GD dendrogram classified the studied rice genotypes into two main clusters: The first cluster included Giza 178 and Sakha 108, while the second cluster included the other genotypes. Genetic distance ranged from 0.147 to 0.886 and the highest genetic distance was observed between Giza 182 and Sakha 178. Highly significant differences were detected among genotypes and their partitions for all studied traits. The highest mean values were detected for the crosses Sakha 107 x Sakha 101 for earliness as well as Sakha 108 x Sakha 101 and Giza 182 x Giza 179 for grain yield plant^{-1}. The mean squares due to SCA were much higher than those of GCA for all studied traits, indicating that the studied traits were governed by non-additive gene action. The parent Sakha 107 expressed the highest significant and negative gca effects for days to 50% flowering. Parent Sakha 108 seemed to be the best general combiner for grain yield plant^{-1}. The most desirable SCA effects for days to 50% flowering were detected for the cross Sakha 108 x Sakha 101, while the cross Giza 182 x Giza 179 expressed the most desirable SCA effects for grain yield plant^{-1}. The most desirable mid-parent and better-parent heterosis values were detected for the cross Sakha 107 x Sakha 101 for days to 50% heading and the cross Sakha 108 x Sakha 101 for grain yield plant^{-1}. The cross Sakha 108 x Sakha 101 expressed the highest mean values of cuvette temperature, leaf diffusive, net assimilation rate and stomatal conductance, while the cross Sakha 107 x Giza 179 gave the best values for transpiration rate.

Key words: Rice, genetic diversity, Combining ability, photosynthetic parameters.

1. Introduction

Rice (*Oryza sativa,* L.) is one of the most important food crop for about half of the population in the world [1]. In Egypt, it represents the second important cereal crop after wheat with a cultivated area of 1.074 million fed. in 2021 season (Including water-saving rice strains such as dry rice and rice grown on relatively high salinity water) produced 4.83 million tons of grain yields/ fed (3.38 million tons of milled rice) [2]. The importance of this crop in Egypt comes from its rank as a staple food after wheat; being a land reclamation crop and regarded as a social crop where farmers have a lot of work and gain money during the growing season.

The successful plant breeding programme depends mainly upon the choice of most appropriate parents for hybridization process. Combining ability is an effective tool for rice breeders since it provides some important genetical information about the studied traits. The line × tester analysis method is widely used to determine general combining ability (GCA) and specific combining ability (SCA) effects and helps in selecting favorable parents and hybrids. General combining ability is a function of additive gene action, while specific combining ability is a function of non-additive genetic variance. Additive genetic variance was reported to be important in the inheritance of rice grain yield and other related traits[3, 4].On the other hand, non-additive was reported to be more important in governing earliness and grain yield plant^{-1} [5,6,7,8,9,10]. Meanwhile, both types of combining ability were influenced by planting dates for earliness, yield and most of its attributed traits [11].

Determination of the level of genetic diversity in different field crops is of great importance for selecting the most suitable parents and maximum utilization of heterosis [12]. Genetic diversity is very effectively technology used in characterization and analyzing genetic materials as compared to morphological traits. It requires small number of samples not affected by environment fluctuations, and does not require large equipment [13]. Several molecular markers such as REFLP, PCR, SSR can be used effectively in genetic diversity study. However, SSR markers are codominant, highly polymorphic, informative, easily analyzed, and not expensive [14]. Several investigators used molecular markers in analyzing the quantitative traits in rice [15,16,17, 18].

Several photosynthetic traits such as cuvette temperature, quantum sensing, leaf diffusive resistance, leaf transpiration rate, stomatal conductance and net assimilation rate are good indicators when selecting desirable rice genotypes with higher yield productivity. Limochi and

Eskandari [19] evaluated the effect of planting dates on stomata performance and yield of rice genotypes and found that planting date and varieties significantly affected all anatomical properties including stomata area, stomata diameter and stomata number. Early planting resulted in reducing the area and diameter of stomata and also grain yield, while the maximum value of the recent properties was observed in late planting. The variability among rice entries were studied by several researchers [20,21,22,23,24, 25]. The main objective of this work was to study genetic diversity, combining ability and photosynthetic traits in some top crosses of rice under different planting dates.

2. MATERIALS AND METHODS

Plant Materials:

A set of eight rice varieties were selected for this study and divided into five lines *viz.*, Giza 178, Sakha 106, Sakha 107, Sakha 108 and Giza 182 as well as three testers *viz.*, Sakha 101, Giza 177 and Giza 179. Grains of the parental lines were obtained from the genetic stock of the Rice Research and Training Center (RRTC), Egypt. The names, pedigree, origin and type of included varieties are shown in Table (1).

Table (1): Names, pedigree, origin and type of included varieties.

NO.	Parent	Genotypes	Pedigree	Origin	Type
1	Parental lines	Giza 178	(Giza175 / Milyang 49)	Egypt	Indica / Japonica
2		Sakha 106	(Giza 177 / Hexi 30)	Egypt	Japonica
3		Sakha 107	(Giza 177/BL1)	Egypt	Japonica
4		Sakha 108	(Sakha101/ HR5824-B-3-2-3// Sakha 101)	Egypt	Japonica
5		Giza 182	(Giza 181 / IR39422-161-1-3// Giza 181)	Egypt	Indica
6	Testers	Sakha 101	(Giza 176 / Milyang 79)	Egypt	Japonica
7		Giza 177	(Giza 171/ Yomji No.1 // Pi No. 4)	Egypt	Japonica
8		Giza 179	(GZ 1368-S-5-4 /GZ 6296-12-1-2)	Egypt	Indica / Japonica

Field Experiment:

In 2019 growing season, the grains of eight parents were sown in 15 May. After thirty days old seedlings of each parent were individually transplanted in the permanent field in two rows, 5 meters long and 20 x 20 cm apart among plants and rows. At flowering time during this season, the five lines were crossed with the three testers to produce 15 F_1 grains using bulk emasculation method according to Butany [26] by using hot water (42-44 °C for 10 min).

In 2020 summer season, the hybrid seeds of 15 crosses with their parents (lines and testers) were planted at two sowing dates in the nursery on 15 and 30 May. After 30 days the seedling of the parental lines with their crosses were transplanted in an experiment using randomized complete block design with three replications as individual plant/hill with plant spacing 20 x 20 cm and 5m long for each row. All agricultural practices were made according to rice recommendations. Data were recorded for number of days to 50% heading, plant height, chlorophyll content, 1000-grain weight and grain yield/ plant. Photosynthetic traits included: cuvette temperature, quantum sensing, leaf diffusive resistance, leaf transpiration rate, stomatal conductance and net assimilation rate. These parameters were estimated using a portable porometer “steady-state porometer, LICOR, LI-1600, Lincoln, NE, USA” designed for assessing the steady-state Co_2 and H_2O exchange degrees of plant leaves [27,28].

Genomic DNA extraction and PCR amplification:

Genomic DNA was extracted from seedlings of the 8 parental genotypes using the modified CTAB method [29]. The integrity of DNA was verified on 1% agarose gel using gel electrophoresis image (Gel Doc. Bio-Rad). The DNA purity and concentration were calculated at absorbance ratio of A260/A280 using BioTek Epoch2 microplate reader (Thermo Scientific).

Polymerase chain reaction (PCR) was conducted in 25 µl reaction volume containing 0.2 µM of each primer with concentration of 10 pM, 200 µM of dNTPs mix, 2.5 µL of 10× PCR reaction buffer, 1.5 µM MgCl2, 2 units of Promega Taq DNA polymerase, 2 µL of template DNA and the final volume was adjusted with sterilized double distilled water [30]. Annealing temperature varies for individual marker and the remaining PCR thermo profile is as follows: Initial denaturation step of 95 ˚C for 3 min followed by 35 cycles at 95 ˚C for 50 s, annealing temperature was calculated for each primer, then for 1 min with an extension of 72 ˚C for 1 min followed by final extension temperature at 72 ˚C for 5 min. The products of PCR were stored at −20 ˚C and verified on 2% agarose gel electrophoresis image using GeneRuler 1 kb DNA ladder, then visualized using gel documentation system (Gel Doc. BioRad). Four primers namely, RM569, RM271, RM184 and RM596 were used in this study (Table 2).

Table (2) SSR heading date primers for rice genotypes.

No.	Marker	Forward	Reverse	AT
1.	RM569	GACATTCTCGCTTGCTCCTC	TGTCCCCTCTAAAACCCTCC	54
2.	RM271	TCAGATCTACAATTCCATCC	TCGGTGAGACCTAGAGAGCC	50
3.	RM184	ATCCCATTCGCCAAAACCGGCC	TGACACTTGGAGAGCGGTGTGG	60
4.	RM596	ATCTACACGGACGAATTGCC	AGAAGCTTCAGCCTCTGCAG	53

Data Analysis:

The analysis of variance for all studied traits under two planting dates as well as combined data were performed according to Steel and Torrie [31]. General and specific combining analysis were estimated for days to 50% heading, plant height, chlorophyll content, 1000- grain weight as well as grain yield plant^{-1} according to line x tester model [32]. Heterosis percentage relative to mid- parent and better parent for all studied traits were estimated according to Mather and Jinks [33] and Mather [34]. The amplified bands were scored for each SSR marker based on the presence or absence of bands. The scores were obtained in the form of matrix with (1) for the presence and (0) for the absence of a bands for each genotype. Polymorphic information content values were calculated for each SSR marker by using the formula described by Anderson [35]. Matrix was analyzed using the PAST, ver. 1.90 [36]. The data matrix were used to calculate genetic similarity based on Jaccard's similarity coefficients , and dendrogram displaying relationships among 8 rice genotypes was constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method.

Results and Discussion

The results of this work will be presented and discussed for genetic diversity, combining ability, heterosis and photosynthetic for all the studied traits as follows:

Genetic diversity study using SSR markers:

In this study four primer pairs were investigated and revealed polymorphic among the eight studied rice genotypes (Table 3 and Fig. 1). A total number of seven alleles were ranged from one (RM184) to two (RM569, RM271 and RM596) with an averaging 2.33 alleles/locus. The values of major allele frequency ranged from 0.62 (RM569 and RM596) to 0.75 (RM271 and RM184) with an averaging 0.69. The values of gene diversity ranged from 0.37 (RM271) to 0.47 (RM596) with an averaging 0.42. The value of polymorphic information content (PIC) ranged from 0.43 (RM184) to 0.61 (RM569) and averaging 0.52. Such results indicated the dissimilarity among studied varieties. Similar results have been reported by [15,16,17, 18].

The dendrogram classified the studied rice genotypes into two main clusters: The first cluster included Giza 178 and Sakha 108. While, the second cluster included two sub clusters, one included Giza 177 and Giza 182 while the other genotypes grouped together (Fig. 2). Genetic distance estimates based on SSR method ranged from 0.147 to 0.886 averaging 0.516 (Table 4). The lowest genetic distance was obtained between Sakha 101 and Sakha 179, while the highest genetic distance was observed between Giza 182 and Sakha 178. These results will help rice breeder when selecting parents for effective breeding program. Such results refer to nature of studied primers which may be related to grain yield per plant for the genotypes under study.

Table (3): Number of alleles, major allele frequency, gene diversity and polymorphic information content (PIC) of the four SSR markers used in this study.

Marker	Ch.*	Size Range (bp)	Repeat Type	No. of Alleles	Major Allele Frequency	Gene Diversity	PIC**
RM569	6	120-130	(GA)15	2	0.62	0.46	0.61
RM271	6	100-120	(CT)44	2	0.75	0.37	0.44
RM184	10	100	(GA) 15	1	0.75	0.37	0.43
RM596	10	100-160	(GA)15	2	0. 62	0.47	0.60
Average						**0.42**	**0.52**

***Ch. Refers to chromosome . **PIC refers to Polymorphic Information Content.**

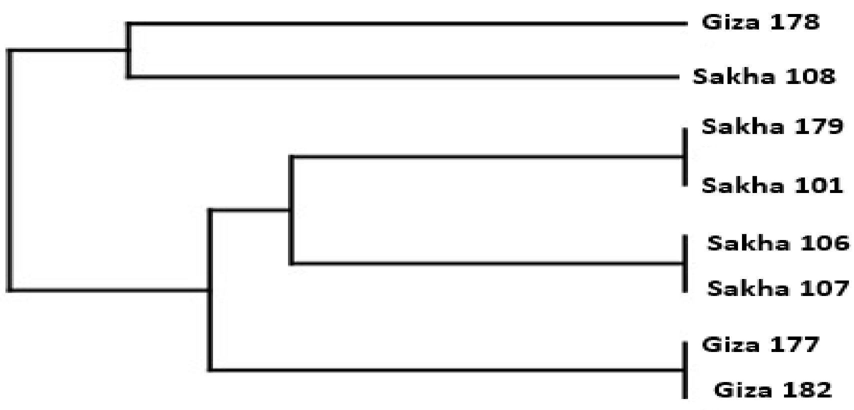

Fig. (1) Dendrogram of the eight rice varieties constructed from SSR data using (PAST) program.

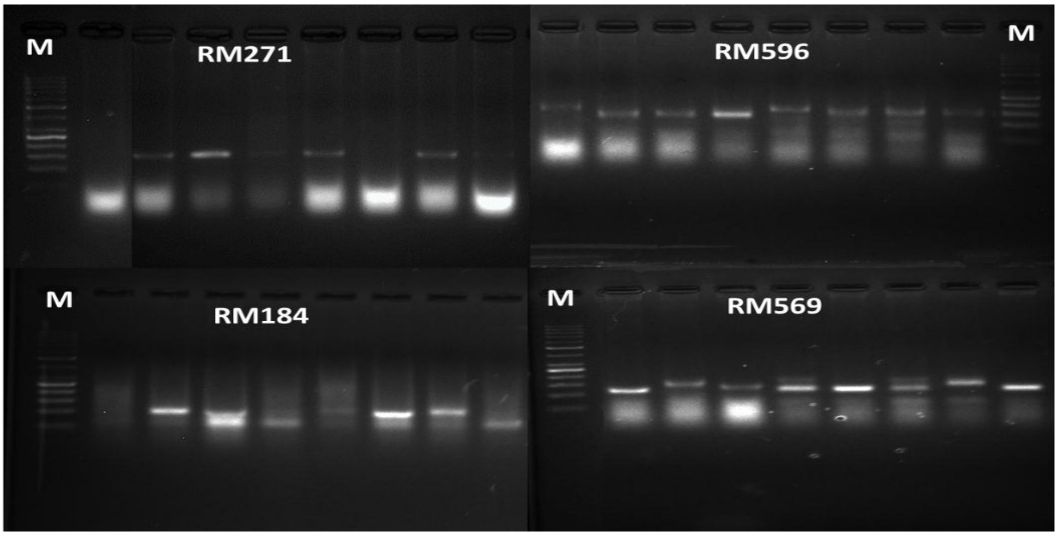

Fig. (2) PCR amplifications of the investigated SSR markers with the eight varieties. M denotes to 100 bp DNA ladder.

Table (4) Matrix of genetic distance, constructed from simple sequence repeat data, for the eight varieties of rice.

parent	Sakha101	Giza177	Sakha179	Giza178	Sakha106	Sakha107	Sakha108	Giza182
Sakha101	**-**	**0.5٠٠**	**0.147**	**0.648**	**0.28٠٠**	**0.645**	**0.642**	**0.382**
Giza177		**-**	**0.512**	**0.523**	**0.554**	**0.501**	**0.532**	**0.5٠٠**
Sakha179			**-**	**0.637**	**0.266**	**0.634**	**0.643**	**0.378**
Giza178				**-**	**0.769**	**0.639**	**0.652**	**0.886**
Sakha106					**-**	**0.276**	**0.777**	**0.250**
Sakha107						**-**	**0.635**	**0.381**
Sakha108							**-**	**0.377**
Giza182								**-**

Analysis of variance and mean performance

Analysis of variance for days to 50% flowering, plant height, chlorophyll content, 1000- grain weight and grain yield plant^{-1} in each planting date and combined over both planting dates are presented in Table (5). Results indicated that mean squares due to planting dates were highly significant for all traits with higher mean values of early sowing being much higher than that of late sowing (Table 6). Such results are expected and may be due to the prevailed favorable temperature and day length leading to better vegetative growth, yield of rice plant and its components. Mean squares due to genotypes, parents, crosses and parent vs crosses were highly significant for all traits in each planting date and the combined dates. Such results indicated the wide diversity between the parental materials used in the present study. Also, significant mean squares between genotypes and their partitions form one side and planting dates from the other side for all studied traits revealed that these genotypes ranked differently as a response to the change of planting dates. These results are in harmony with the findings of other researchers [6,7,8,9, 10].

Mean performances of all genotypes concerning days to 50% flowering, plant height, chlorophyll content, 1000 grain weight and grain yield plant^{-1} at each planting date and over planting dates are presented in Table (6). The parent Giza 179 expressed the highest significant and desirable mean values for days to 50% flowering and grain yield plant^{-1} at both and across planting dates. Parent Sakha 101 was the best for plant height in the 1**st**, 2**nd** planting dates and combined data as compared to other studied parents. Regarding to chlorophyll content, parent Sakha 107 exhibited the highest significant mean values in early, late sowing and combined analyses. Parent Sakha 108 was the best for 1000 grain weight under both sowing dates and combined data as compared to other parents.

The crosses Sakha 107 x Sakha 101 (in early sowing) and Sakha 107 x Giza 179 (in early and late sowing) did not significantly differ from Giza 179 for days to 50% flowering. Therefore, these two crosses are prospective for rice breeding program regarding breeding towards earlines. For plant height, the best mean values were detected for the crosses Giza 178 x Sakha 101 in early, late sowing and combined data. The cross Giza 182 x Giza 179 expressed the most desirable mean values for chlorophyll content and ranked the second best for grain yield plant^{-1} under both planting dates and combined data. The cross Sakha 108 x Sakha 101, exhibited the most desirable values for 1000 grain weight and grain yield plant^{-1} under the two sowing dates and combined over them (Table 5). In conclusion, the crosses Sakha 107 x Sakha 101 and Sakha 107 x Giza 179 (for earliness) as well as Sakha 108 x Sakha 101 and Giza 182 x Giza 179 (for grain yield plant^{-1}) are prospective in future rice breeding programs.

Combining ability analysis

Analysis of variance for general (GCA) and specific (SCA) combining abilities for days to 50% flowering, plant height, chlorophyll content, 1000 grain weight and grain yield plant^{-1} at early, late and combined planting dates analyses are presented in Table (5).

The mean squares due to SCA were much higher than those of GCA for all studied traits, indicating that the studied traits were mainly governed by non-additive gene action. The genetic variance have been reported to be mostly due to non-additive gene action for the studied traits [5,6,7,8,9, 10]. However**,** Dharwal and Gowayed [3,4] reported that additive gene action was predominant in governing earliness and grain yield plant^{-1} in rice. Also, the value of δ^2 SCA x sowing date were much higher than those of δ^2 GCA x sowing date, revealing that the magnitude of non-additive types of gene action was more influenced than additive gene action from one planting date to another. These results were fully agreement with those reported by [11].

Estimates of GCA effects for all studied traits under both planting dates and combined data are presented in Table (7). The parent Sakha 107 expressed the highest significant and negative GCA effects for days to 50% flowering under both planting date, as well as, combined data. Giza 182 seemed to be the best general combiner for plant height in early and late planting dates and combined data. Parent Sakha 106 expressed the most desirable GCA effects for chlorophyll content and 1000 grain weight under both planting dates and combined analyses. Parent Sakha 108 seemed to be the best general combiner for grain yield plant^{-1} since it expressed the highest significant and positive GCA effects under both planting dates and combined over them (Table 7).

Out of the tester lines, the tester Giza 177 expressed the highest significant and negative GCA values for days to 50% flowering in early, late sowing date and combined data as well as plant height at late sowing. The tester Giza 179 seemed to be the best general combiner for plant height in early sowing, chlorophyll content and 1000 grain weight in early, late sowing and combined data as well as grain yield plant^{-1} at late planting. The tester Sakha 101 was the best general combiner for grain yield plant^{-1} at early planting and combined data (Table 7).

Specific combining ability effects for all studied traits under both planting dates and combined over them are presented in Table (8). For days to 50% flowering, six, six and seven crosses expressed significant and negative effects for days to 50% flowering in early, late planting and combined data, respectively. However, the most desirable effects for this trait were detected for the cross Sakha 108 x Sakha 101 in all environments. For plant height, eight, five and eight crosses had desirable GCA effects in early, late planting and combined data, respectively. The cross Giza 178 x Sakha 101 was the best among all crosses since it had the most desirable effects for plant height. Regarding chlorophyll content, six crosses (in early sowing), six crosses (in late sowing) and five crosses in the combined data expressed significant and positive SCA effects. However, the cross Giza 182 x Giza 179 was the best since it had the highest significant and positive effects in all environment. For 1000 grain weight, eight, eight and eight crosses exhibited significant and positive SCA effects in the 1^{st}, 2^{nd} environments and combined analyses, respectively. However, the cross Giza 182 x Giza 179 was the best for this trait. Regarding grain yield $plant^{-1}$, eight, seven and six crosses gave significant and positive SCA effect for early, late sowing and combined data, respectively. Again, the cross Giza 182 x Giza 179 expressed the most desirable SCA effects in all environments. In general, the two crosses Sakha 108 x Sakha 101 and Giza 182 x Giza 179 are of prime importance regarding earliness and yield potentiality in rice breeding programs.

Heterosis

Heterosis percentage relative to both mid parent and better parent for all studied traits under early, late planting and combined data are presented in Tables (9 and 10). Results indicated that the most desirable heterotic effects relative to mid parent were detected for the crosses: Sakha 107 x Sakha 101 for days to 50% flowering, under early, late planting and combined data and grain yield $plant^{-1}$ at early planting date. The most desirable heterotic effects relative to mid-parent for plant height were detected for the crosses Giza 182 x Giza 177 at early planting and Giza 178 x Sakha 101 at late planting and combined data. The cross Giza 182 x Giza 179 expressed the mot desirable mid-parent heterosis for chlorophyll content and 1000 grain weight under early, late planting and combined data. For grain yield $plant^{-1}$, the best heterotic effects relative to mid parent were detected for the cross Sakha 108 x Sakha 101 at late planting and combined data recording 31.38% and 29.51%, respectively (Table 9).

Regarding heterotic effects relative to better parent similar trend observed in case of mid-parent heterosis was detected where the best values for days to 50% flowering were detected for the Sakha 107 x Sakha 101 under early, late planting and combined data and grain yield $plant^{-1}$ at early planting date (Table 10). The cross Giza 178 x Sakha 101 expressed the most desirable better-parent heterosis effects for plant height under late planting and combined data. The most desirable better-parent heterosis for chlorophyll content was detected for the cross Sakha 106 x Giza 177 under both planting dates and combined data. The cross Giza 182 x Giza 179 gave the best heterotic effects relative to mid parent for 1000 grain wight under all environments. For grain yield $plant^{-1}$, the most desirable heterotic effects relative to better parent were detected for the crosses Sakha 107 x Sakha 101 at early planting and Sakha 108 x Sakha 101 at late planting and combined data recording 28.11% and 24.47%, respectively (Table 10). These results agreed with the data reported by several investigators [6,9,37,38,39, 40].

Photosynthetic traits

Six photosynthetic parameters namely, cuvette temperature, quantum sensor, leaf diffusive resistance, leaf transpiration rate, stomatal conductance and net assimilation rate were estimated and are presented in Figures (3-8). Generally, the mean values of photosynthetic traits in early planting were much higher than those of late planting for all studied genotypes. The cross Sakha 108 x Sakha 101 had the highest mean values for cuvette temperature followed by the crosses Giza 182 x Giza 179 and Giza 178 x Giza 179 at early, late planting and combined data (Fig. 3). For quantum sensing, the most desirable mean values were detected for the genotype Giza 177 followed by the cross Sakha 108 x Sakha 101 and Giza 182 x Giza 179 under both planting dates and combined analyses (Fig. 4). The best mean values for leaf diffusive resistance were detected for the cross Giza 178 x Giza 177 followed by the cross Sakha 108 x Sakha 101 and Giza 182 x Giza 179 under both planting dates (Fig. 5). For leaf transpiration rate, the cross Sakha 107 x Giza 179 gave the highest mean values followed by the crosses Sakha 107 x Giza 177 and Sakha 106 x Sakha 101 (Fig. 6). The cross Sakha 108 x Sakha 101 gave the most desirable mean value for stomatal conductance at early, late planting and combined analyses recording 0.1, .09 and 0.1, respectively (Fig. 7). For net assimilation rate, the best mean values were detected for the crosses Sakha 108 x Sakha 101 followed by Giza 182 x Giza 179 giving values of 37.08, 36.89 in the combined analysis, respectively (Fig. 8). The variability among rice genotypes regarding photosynthetic traits had been reported by [20,21,22,23,24, 25].

Finally, the three crosses *viz*, Sakha 108 x Sakha 101, Giza 182 x Giza 179 and Giza 178 x Giza 179 seemed to be the best among studied crosses regarding photosynthetic characters and they may be helpful in future rice breeding programs.

Table (5) Mean squares of line x tester analysis for all studied traits in the two sowing dates during 2020 season and their combined analyses.

S.O.V.	DF		Days to 50% flowering			Plant height(cm)			Chlorophyll content			1000- grain weight (g)			Grain yield / plant (g)		
	Single	Comb.	Early	Late	Comb.	Early	Late	Comb.	Early	Late	Comb.	Early	Late	Comb.	Early	Late	Comb.
Sowing dates		1			1818.8**			1985.65**			126.602**			59.57**			416.17**
Reps/ S	2	4	4.05	14.79	9.48	0.04	2.6	1.32	1.84	1.65	1.75	1.05	0.93	0.97	0.05	4.25	2.15
Genotypes	22	22	116.43**	126.14**	238.67**	57.97**	56.22**	87.04**	9.06**	9.93**	18.71**	16.86**	15.31**	31.36**	116.72**	123.65**	236.02**
Parents (P)	7	7	115.14**	109.79**	220.71**	53.65**	50.96**	63.41**	10.05**	21.09**	19.89**	16.05**	17.93**	33.97**	37.37**	47.66**	63.84**
Crosses (Cr)	14	14	117.76**	128.16**	242.23**	47.11**	53.56**	94.6**	5.55**	5.35**	10.63**	16.25**	13.63**	28.71**	151.18**	168.87**	314.8**
P *vs*. Cr	1	1	106.96**	212.38**	310.5**	240.32**	130.25**	362.28**	51.58**	26.81**	76.38**	31.14**	20.84**	37.99**	189.65**	66.48**	240.34**
Lines (L)	4	4	170.17**	214.84**	380.15**	62.24**	108.86**	165.35**	4.86**	6.76**	11.38**	16.48**	23.02**	38.94**	81.3**	73.63**	149.86**
Testers (T)	2	2	56.64**	43.82**	94.24**	21.92**	11.37**	14.32**	7.32**	7.74**	14.79**	35.52**	12.84**	44.45**	92.53**	59.9**	146.02**
Line x Tester	8	8	106.83**	105.9**	210.27**	45.84**	36.45**	79.29**	5.41**	4.04**	9.22**	11.32**	9.13**	19.66**	200.79**	243.74**	439.46**
Genotype x S		22			4.67**			5.51**			1.1**			1.22**			3.35**
Crosses x S		14			3.68**			6.07**			0.24**			1.17**			5.26**
Lines x S		4			4.86**			5.75**			0.24**			0.56**			5.07**
Testers x S		2			6.21**			18.97**			0.27**			3.91**			6.41**
Line x Tester		8			2.46**			3.01**			0.23**			0.79**			5.07**
Parents x S		7			4.28***			3.85**			2.7**			1.07**			7.19**
P *vs*. Cr x S		1			8.95**			8.38**			0.92**			2.98**			15.78**
Error	44	88	1.08	0.93	1.1	1.03	1.05	1.04	0.03	0.05	0.04	0.2	0.22	0.21	0.75	1.22	0.99
δ2 GCA			0.39	0.79	0.57	0.04	0.60	0.35	0.08	0.05	0.07	0.17	0.16	0.16	1.75	2.65	2.20
δ2 SCA			35.25	34.95	34.88	14.94	11.80	13.04	1.80	1.33	1.53	3.71	2.97	3.24	66.68	80.84	73.08
δ2 GCA x S					0.26			0.78			0.04			0.12			0.06
δ2 SCA x S					0.49			0.86			0.06			0.19			1.36

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

Table (6) Mean performance of all genotypes for all studied traits in the two sowing dates during 2020 season and their combined analyses.

Genotypes	Days to 50% flowering (day)			Plant height(cm)			Chlorophyll content (SPAD)			1000- grain weight (g)			Grain yield / plant (g)		
	Early	Late	Comb.	Early	Late	Comb.	Early	Late	Comb.	Early	Late	Comb.	Early	Late	Comb.
Giza 178	101.33	92.67	97.00	105.33	100.45	102.75	41.57	38.88	40.23	22.35	21.58	21.96	46.22	42.50	44.35
Sakha 106	96.33	87.33	91.67	103.35	97.67	100.55	42.60	39.50	41.05	28.67	28.31	28.49	42.42	37.70	40.10
Sakha 107	92.67	84.67	88.67	107.88	98.65	103.25	44.50	42.17	43.33	27.33	25.53	26.43	41.33	37.80	39.55
Sakha 108	100.33	90.67	95.33	102.2	92.00	96.25	38.80	36.67	37.73	29.22	28.44	28.88	48.67	43.32	46.00
Giza 182	95.00	88.67	91.67	99.35	92.45	95.88	40.90	38.47	39.68	25.57	23.47	24.52	44.15	40.37	42.25
Sakha 101	110.67	102.33	106.67	96.00	89.33	92.80	43.80	41.23	42.52	29.10	28.08	28.67	43.67	41.15	42.65
Giza 177	95.33	90.67	93.00	98.67	92.15	95.88	42.60	40.37	41.48	27.33	26.42	26.88	40.35	36.40	38.86
Giza 179	91.33	82.33	86.67	96.33	90.45	93.35	43.50	41.50	42.50	28.33	27.76	28.05	49.77	45.57	47.78
Giza 178 x Sakha 101	105.33	98.33	101.67	98.33	91.35	94.84	44.30	41.67	42.98	27.67	26.34	27.05	45.25	38.88	42.15
Giza 178 x Giza 177	100.33	94.33	97.33	107.55	97.15	102.35	42.80	40.47	41.63	23.77	22.86	23.26	46.67	43.25	44.95
Giza 178 x Giza 179	97.11	92.00	94.67	105.30	98.85	102.15	44.50	42.77	43.63	28.25	27.11	27.68	57.60	54.15	55.86
Sakha 106 x Sakha 101	97.33	88.67	93.00	110.15	101.62	105.88	45.70	43.17	44.43	30.67	29.74	30.15	54.77	49.57	52.18
Sakha 106 x Giza 177	97.67	91.33	94.33	104.94	96.71	100.83	45.50	43.44	44.48	28.33	27.57	27.95	43.67	40.17	41.91
Sakha 106 x Giza 179	104.45	91.33	97.67	103.48	98.18	100.83	43.88	42.17	43.02	30.57	28.33	29.45	51.33	45.22	48.27
Sakha 107 x Sakha 101	92.54	86.67	89.61	115.56	107.58	111.57	44.20	42.53	43.37	27.00	26.20	26.60	54.39	50.96	52.67
Sakha 107 x Giza 177	94.00	86.67	90.33	106.00	97.41	101.70	44.20	42.27	43.23	27.67	26.64	27.18	44.76	41.33	43.10
Sakha 107 x Giza 179	93.00	83.67	88.34	106.56	100.83	103.70	45.50	43.33	44.42	31.10	27.82	29.61	49.50	46.35	47.95
Sakha 108 x Sakha 101	99.67	94.00	96.67	104.52	93.69	99.11	43.50	41.17	42.33	31.67	30.41	31.15	59.24	55.53	57.25
Sakha 108 x Giza 177	103.67	97.67	100.67	108.77	94.65	101.71	43.70	42.30	43.00	27.67	26.70	27.20	47.66	43.27	45.55
Sakha 108 x Giza 179	111.33	106.33	108.67	101.53	95.20	98.37	44.20	42.47	43.33	28.67	27.7	28.15	39.80	37.60	38.70
Giza 182 x Sakha 101	113.00	107.00	110.00	99.54	91.45	95.48	42.10	39.73	40.92	25.43	24.73	25.08	37.46	35.02	36.24
Giza 182 x Giza 177	96.00	90.00	93.00	103.67	93.75	98.80	41.20	39.17	40.18	28.67	27.37	28.02	36.47	33.03	34.75
Giza 182 x Giza 179	94.67	88.33	91.33	104.33	96.77	100.55	46.20	43.45	44.82	31.66	30.40	31.00	57.61	54.16	55.87
Over all mean	99.27	92.03	95.57	104.06	96.03	100.00	43.47	41.26	42.36	28.12	26.93	27.54	47.08	43.19	45.17
L.S.D 0.05	1.22	1.43	1.08	1.46	1.68	1.19	0.25	0.38	0.28	0.73	0.77	0.68	1.42	1.82	1.57
L.S.D 0.01	1.64	1.91	1.44	1.96	2.24	1.59	0.33	0.51	0.38	0.98	1.03	0.91	1.90	2.43	2.10

Table (7) Estimates of GCA effects (g_i) of parental lines and testers for all studied traits in the two sowing dates during 2020 season and their combined analyses.

Genotypes	Days to 50% flowering (day)			Plant height(cm)			Chlorophyll content (SPAD)			1000- grain weight (g)			Grain yield / plant (g)		
	Early	Late	Comb.	Early	Late	Comb.	Early	Late	Comb.	Early	Late	Comb.	Early	Late	Comb.
Lines :															
Giza 178	0.99**	1.75**	1.37**	-0.84**	-1.23**	-1.03**	-0.23**	-0.37**	-0.3**	-2.2**	-2.55**	-2.37**	1.77**	-0.11	0.83*
Sakha 106	-0.12	-2.7**	-1.41**	0.63**	1.84**	1.23**	0.93**	0.93**	0.93**	1.21**	1.33**	1.27**	-1.95**	-1.63**	-1.79**
Sakha 107	-7.04**	-7.21**	-7.13**	3.8**	4.93**	4.37**	0.53**	0.7**	0.61**	0.03	-0.35*	-0.16	0.33	0.86**	0.59
Sakha 108	4.92**	6.19**	5.56**	-0.62*	-2.5**	-1.56**	-0.3**	-0.03	-0.16**	1.02**	1.32**	1.17**	3.74**	4.24**	3.99**
Giza 182	1.25**	1.97**	1.61**	-2.97**	-3.04**	-3.01**	-0.93**	-1.23**	-1.08**	-0.06	0.25	0.09	-3.89**	-3.36**	-3.62**
LSD 0.05	0.50	0.60	0.42	0.60	0.69	0.49	0.10	0.17	0.12	0.30	0.33	0.28	0.58	0.74	0.63
LSD 0.01	0.67	0.80	0.56	0.80	0.92	0.65	0.14	0.22	0.16	0.40	0.45	0.38	0.78	0.99	0.85
Testers															
Sakha 101	1.69**	1.79**	1.74**	0.66**	0.13	0.39**	-0.14**	-0.35**	-0.25**	-0.16	0.24	0.04	1.89**	0.82**	1.36**
Giza 177	-1.6**	-1.14**	-1.37**	0.67**	-1.08**	-0.21	-0.62**	-0.47**	-0.54**	-1.45**	-1.02**	-1.24**	-2.81**	-2.27**	-2.55**
Giza 179	-0.09	-0.65**	-0.37*	-1.33**	0.95**	-0.18	0.76**	0.82**	0.79**	1.61**	0.78**	1.2**	0.92**	1.45**	1.19**
L.S.D 0.05	0.39	0.47	0.32	0.46	0.54	0.38	0.08	0.13	0.09	0.23	0.26	0.22	0.45	0.58	0.50
L.S.D 0.01	0.52	0.62	0.43	0.62	0.71	0.50	0.11	0.17	0.12	0.31	0.35	0.29	0.60	0.77	0.66

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

Table (8) Estimates of SCA effects of cross combinations for days to 50% flowering, plant height(cm) and chlorophyll content, 1000 grain- weight and grain yield plant^{-1} at the two sowing dates during 2020 season and their combined analyses.

Cross combinations	Days to 50% flowering (day)			Plant height(cm)			Chlorophyll content (SPAD reading)			1000- grain weight (g)			Grain yield / plant (g)		
	Early	Late	Comb.	Early	Late	Comb.	Early	Late	Comb.	Early	Late	Comb.	Early	Late	Comb.
Giza 178 x Sakha 101	2.72**	1.65**	2.19**	-4.06**	-4.54**	-4.29**	0.56**	0.38**	0.48**	1.53**	1.02**	1.27**	-6.49**	-10.27**	-8.38**
Giza 178 x Giza 177	1.01*	0.59	0.80*	2.16**	2.44**	2.30**	-0.45**	-0.69**	-0.57**	-2.16**	-1.70**	-1.91**	-0.35	1.54*	0.59
Giza 178 x Giza 179	-3.73**	-2.24**	-2.98**	1.89**	2.10**	1.99**	-0.12	0.31*	0.09	0.62*	0.68*	0.65*	6.85**	8.74**	7.79**
Sakha 106 x Sakha 101	-4.17**	-3.57**	-3.87**	3.29**	2.66**	2.98**	0.81**	0.57**	0.70**	0.56*	1.00**	0.78**	4.65**	5.28**	4.97**
Sakha 106 x Giza 177	-0.55	2.03**	0.74**	-1.91**	-1.05	-1.48**	1.09**	1.01**	1.05**	0.88**	1.15**	1.02**	1.03*	0.72	0.88
Sakha 106 x Giza 179	4.72**	1.54**	3.13**	-1.38*	-1.61**	-1.50**	-1.90**	-1.59**	-1.75**	-1.44**	-2.15**	-1.80**	-5.70**	-6.01**	-5.85**
Sakha 107 x Sakha 101	-1.91**	-1.05**	-1.48**	5.53**	5.51**	5.52**	-0.29**	0.18	-0.06	-1.94**	-1.87**	-1.90**	4.12**	5.18**	4.65**
Sakha 107 x Giza 177	2.71**	1.88**	2.30**	-4.05**	-3.45**	-3.75**	0.19*	0.03	0.11	1.38**	1.00**	1.19**	1.19*	0.65	0.92
Sakha 107 x Giza 179	-0.8	-0.83	-0.82**	-1.49**	-2.06**	-1.77**	0.11	-0.20	-0.05	0.56*	0.86**	0.71**	-5.30**	-5.84**	-5.57**
Sakha 108 x Sakha 101	-6.76**	-7.13**	-6.94**	-1.07*	-0.95	-1.01*	-0.16	-0.46**	-0.31**	1.75**	1.49**	1.62**	5.32**	6.34**	5.83**
Sakha 108 x Giza 177	0.41	-0.53	-0.06	3.16**	1.22*	2.19**	0.52**	0.80**	0.66**	0.40	0.29	0.34	3.02**	2.51**	2.76**
Sakha 108 x Giza 179	6.35**	7.65**	7.00**	-2.09**	-0.27	-1.18**	-0.36**	-0.34*	-0.35**	-2.15**	-1.77**	-1.97**	-8.34**	-8.85**	-8.59**
Giza 182 x Sakha 101	10.13**	10.11**	10.11**	-3.70**	-2.68**	-3.19**	-0.93**	-0.69**	-0.81**	-1.90**	-1.64**	-1.78**	-7.60**	-6.53**	-7.07**
Giza 182 x Giza 177	-3.59**	-3.97**	-3.78**	0.64	0.84	0.74	-1.35**	-1.14**	-1.24**	-0.50	-0.75**	-0.62**	-4.89**	-5.42**	-5.15**
Giza 182 x Giza 179	-6.54**	-6.13**	-6.34**	3.07**	1.84**	2.45**	2.28**	1.83**	2.05**	2.41**	2.39**	2.40**	12.49**	11.96**	12.22**
LSD Sij 0.05	0.87	1.04	0.72	1.04	1.20	0.84	0.18	0.29	0.20	0.51	0.53	0.46	1.01	1.27	1.10
LSD Sij 0.01	1.16	1.39	0.97	1.38	1.60	1.13	0.24	0.38	0.27	0.68	0.70	0.62	1.34	1.69	1.47
LSD sij-skl 0.05	1.22	1.47	1.02	1.46	1.69	1.19	0.25	0.41	0.29	0.72	0.74	0.65	1.42	1.79	1.55
LSD sij-skl 0.01	1.63	1.96	1.35	1.95	2.26	1.58	0.23	0.55	0.38	0.96	0.99	0.86	1.90	2.39	2.05

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

Table (9) Heterosis relative to mid -parents for all studied traits at two sowing dates during 2020 season and their combined analyses.

Cross combinations	Days to 50% flowering (day)			Plant height(cm)			Chlorophyll content (SPAD)			1000- grain weight (g)			Grain yield / plant (g)		
	Early	Late	Comb.	Early	Late	Comb.	Early	Late	Comb.	Early	Late	Comb.	Early	Late	Comb.
Giza 178 x Sakha 101	-0.63	0.85	0.08	-3.31	-3.72**	-3.50**	3.79**	4.01**	3.90**	16.09**	12.95**	14.55**	-3.15**	-20.38**	-9.91**
Giza 178 x Giza 177	2.03**	2.91**	2.46**	4.93**	1.07	3.06**	1.70**	2.12**	1.91**	-4.73**	-4.75**	-4.74**	7.32**	7.43**	7.37**
Giza 178 x Giza 179	0.81	0.38	0.59	4.42**	3.74**	4.09**	4.62**	6.41**	5.49**	12.58**	10.22**	11.44**	19.18**	22.23**	20.64**
Sakha 106 x Sakha 101	-5.96**	-6.50**	-6.22**	10.50**	8.49**	9.53**	5.79**	6.94**	6.34**	5.74**	3.54**	4.64**	22.96**	23.17**	23.06**
Sakha 106 x Giza 177	1.91**	2.62**	2.25**	3.38**	1.92**	2.68**	6.81**	5.89**	6.36**	1.18**	0.76**	0.98**	7.73**	8.90**	8.29**
Sakha 106 x Giza 179	11.31**	7.66**	9.58**	3.65**	4.39**	4.01**	1.93**	1.61**	1.77**	7.25**	1.07**	4.18**	-10.14**	-8.57**	-9.58**
Sakha 107 x Sakha 101	-8.85**	-7.31**	-8.11**	13.36**	14.26**	13.79**	0.11	-1.05**	-0.46**	-4.71**	-4.50**	-4.60**	28.01**	29.50**	28.51**
Sakha 107 x Giza 177	-2.77**	-3.70**	-3.23**	2.13**	1.76**	1.94**	1.49**	2.42**	1.95**	1.22**	2.57**	1.88**	14.58**	15.18**	14.87**
Sakha 107 x Giza 179	-1.69**	-4.04**	-2.85**	4.36**	6.65**	5.64**	3.41**	3.59**	3.50**	12.81**	14.71**	13.74**	-3.62**	-2.60**	-3.18**
Sakha 108 x Sakha 101	-5.42**	-2.59**	-4.07**	6.93**	3.72**	5.39**	5.33**	5.69**	5.50**	8.57**	6.25**	7.42**	27.80**	31.38**	29.51**
Sakha 108 x Giza 177	5.96**	7.72**	6.81**	9.22**	3.38**	6.43**	7.37**	6.73**	7.05**	-1.78**	-1.45**	-1.61**	16.89**	20.30**	18.53**
Sakha 108 x Giza 179	15.94**	22.93**	19.26**	3.70**	4.94**	4.29**	7.41**	5.95**	6.69**	3.49**	3.37**	3.43**	-10.04**	-7.88**	-9.02**
Giza 182 x Sakha 101	9.89**	12.04**	10.92**	-1.93**	-2.97**	-2.44	-0.59**	-0.29**	-1.98**	-0.45**	-3.31**	-5.84**	-12.27**	-14.08**	-13.14**
Giza 182 x Giza 177	0.88	-2.17**	-0.62	-4.42**	-2.42**	-3.16*	-1.32**	-0.63**	-0.99**	-8.38**	-6.98**	-7.77**	-13.49**	-15.06**	-14.24**
Giza 182 x Giza 179	1.49**	-1.85**	-0.15	6.65**	2.52**	4.62**	9.48**	8.63**	9.07**	17.50**	17.37**	18.64**	18.12**	26.01**	24.18**

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

Table (10): Heterosis relative to better -parents for all studied traits at two sowing dates during 2020 season and their combined analyses.

Cross combinations	Days to 50% flowering (day)			Plant height(cm)			Chlorophyll content (SPAD)			1000- grain weight (g)			Grain yield / plant (g)		
	Early	Late	Comb.	Early	Late	Comb.	Early	Late	Comb.	Early	Late	Comb.	Early	Late	Comb.
Giza 178 x Sakha 101	-4.82**	-3.91**	-4.38**	-7.59**	-8.73**	-8.20**	1.14**	1.05**	1.12**	-5.68**	-10.19**	-7.94**	-3.07**	-19.83**	-11.11**
Giza 178 x Giza 177	-0.99	1.80	0.34	2.10**	-2.97**	-0.37	0.47**	0.25	0.36*	-13.41**	-16.38**	-14.90**	0.58	0.39	0.19
Giza 178 x Giza 179	-4.17**	-0.72	-2.52**	-0.04	-1.28	-0.64	2.30**	3.05**	2.67**	8.22**	5.52**	6.61**	15.21**	18.87**	16.96**
Sakha 106 x Sakha 101	-12.05**	-13.36**	-12.68**	6.57**	4.05**	5.34**	4.34**	4.69**	4.51**	4.55**	5.42**	5.53**	20.61**	17.99**	19.34**
Sakha 106 x Giza 177	1.38*	0.74	1.61*	1.54*	-2.97**	0.32	6.81**	7.68**	7.23**	-1.17**	-2.60**	-1.88**	5.56**	8.46**	6.94**
Sakha 106 x Giza 179	8.42**	4.58**	6.60**	0.13	0.52	0.32	0.87**	1.61**	1.23**	6.62**	1.66	3.38**	-17.33**	-16.83**	-17.09**
Sakha 107 x Sakha 101	-16.27**	-15.31**	-15.81**	7.12**	9.06**	8.04**	-0.67**	0.87**	0.8	-7.95**	-6.44**	-7.21**	24.56**	23.79**	24.19**
Sakha 107 x Giza 177	-1.40**	-4.41**	-2.87**	-1.75*	-2.26**	-1.52*	-0.67**	0.24	-0.23	1.22**	-0.86	-0.65	13.18**	14.58**	13.85**
Sakha 107 x Giza 179	-0.72	-0.26	-0.50	-1.22	2.21*	0.42	2.25**	2.77	2.50**	8.82**	6.12**	7.50**	-11.99**	-10.97**	-11.51**
Sakha 108 x Sakha 101	-9.84**	-8.14**	-9.02**	5.05**	2.96**	4.05**	-0.68**	-0.16	-0.43**	7.95**	3.41**	5.68**	21.23**	28.11**	24.47**
Sakha 108 x Giza 177	3.32**	7.72**	5.41**	9.13**	2.76**	6.07**	2.58**	4.79**	3.66**	-4.60**	-3.84**	-4.23**	6.85**	12.10**	9.32**
Sakha 108 x Giza 179	10.74**	17.28**	13.84**	2.05**	-1.18	2.11**	1.61**	2.33**	1.96**	2.30**	3.36**	2.82**	-11.24**	-10.15**	-10.72**
Giza 182 x Sakha 101	2.11**	4.56**	3.29**	3.22	-4.77**	-0.77	-3.88**	-3.64**	-3.76**	-13.30**	-11.68**	-12.49**	-12.60**	-14.92**	-13.38**
Giza 182 x Giza 177	0.70	-0.74	-0.09	-4.24**	-5.96**	-5.01**	-3.29**	-2.97**	-3.13**	-11.46**	-12.18**	-11.81**	-17.12**	-18.17**	-17.62**
Giza 182 x Giza 179	-0.47	-0.38	-0.42	5.05**	0.45	4.38**	6.21**	4.66**	5.42**	11.76**	9.51**	10.65**	15.16**	18.82**	16.91**

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

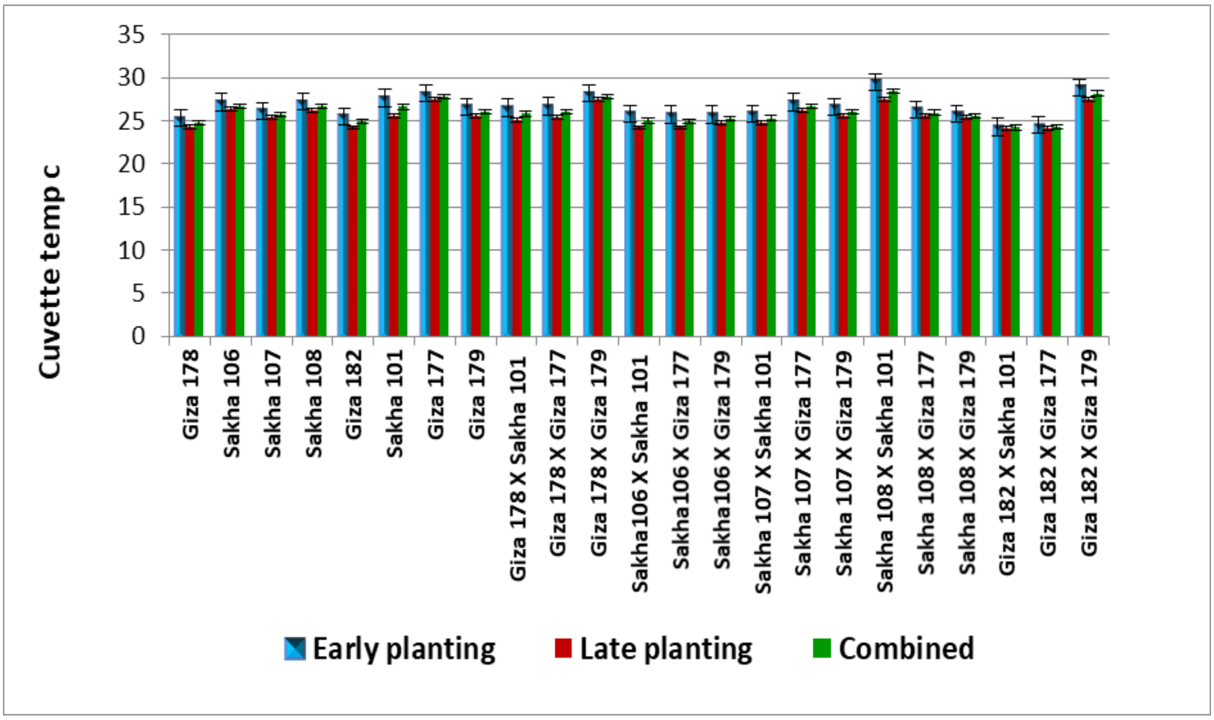

Fig. (3) Effect of planting date on rice genotypes for cuvette temperature.

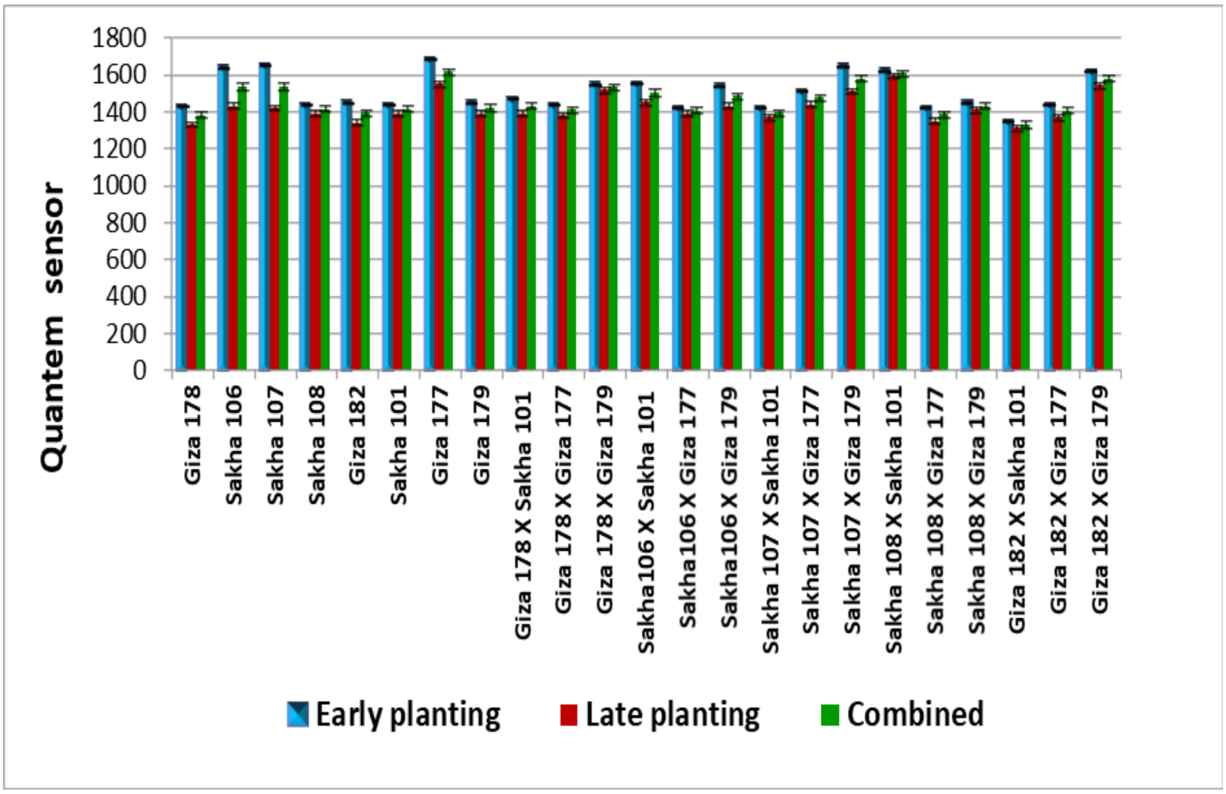

Fig. (4) Effect of planting date on rice genotypes for quantum sensor.

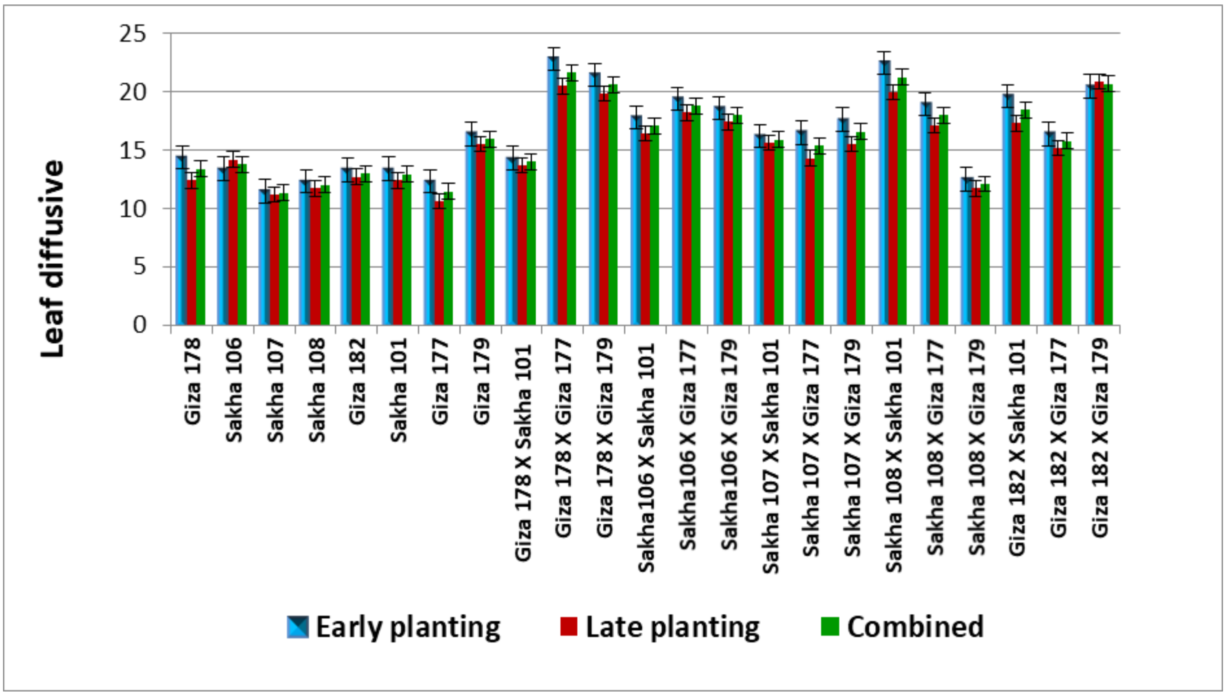

Fig. (5) Effect of planting date on rice genotypes for leaf diffusive resistance.

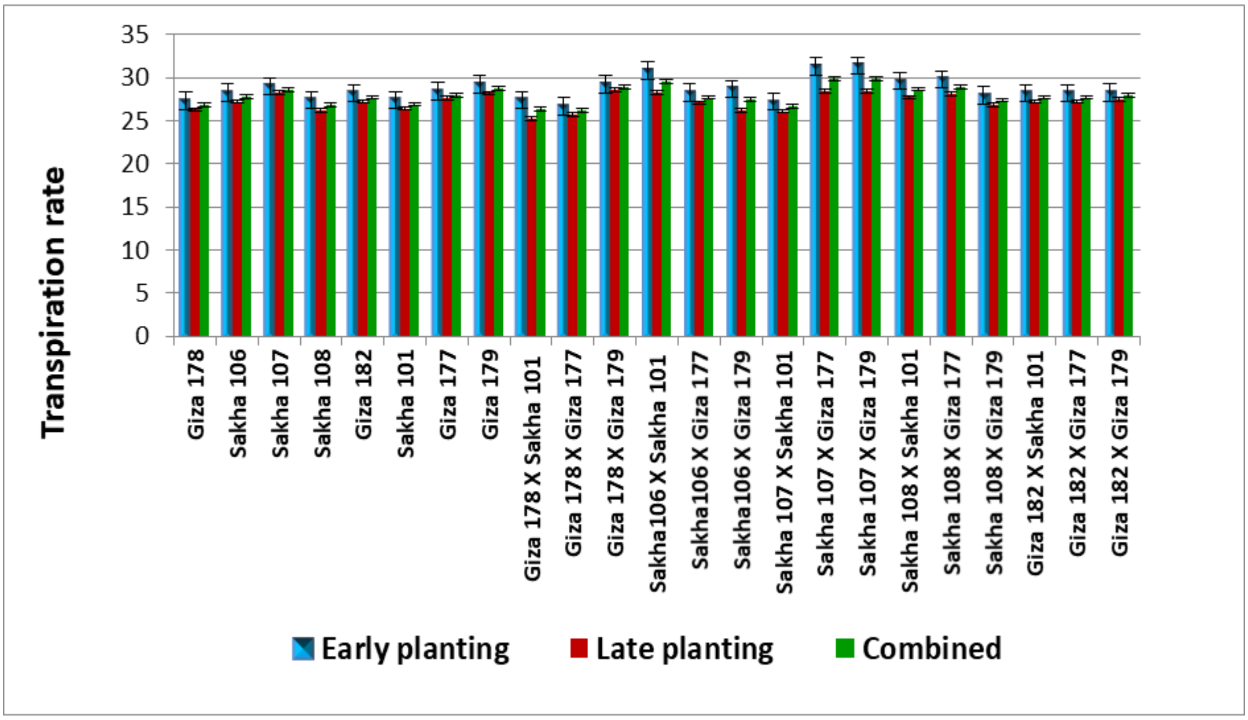

Fig. (6) Effect of planting date on rice genotypes for leaf transpiration rate.

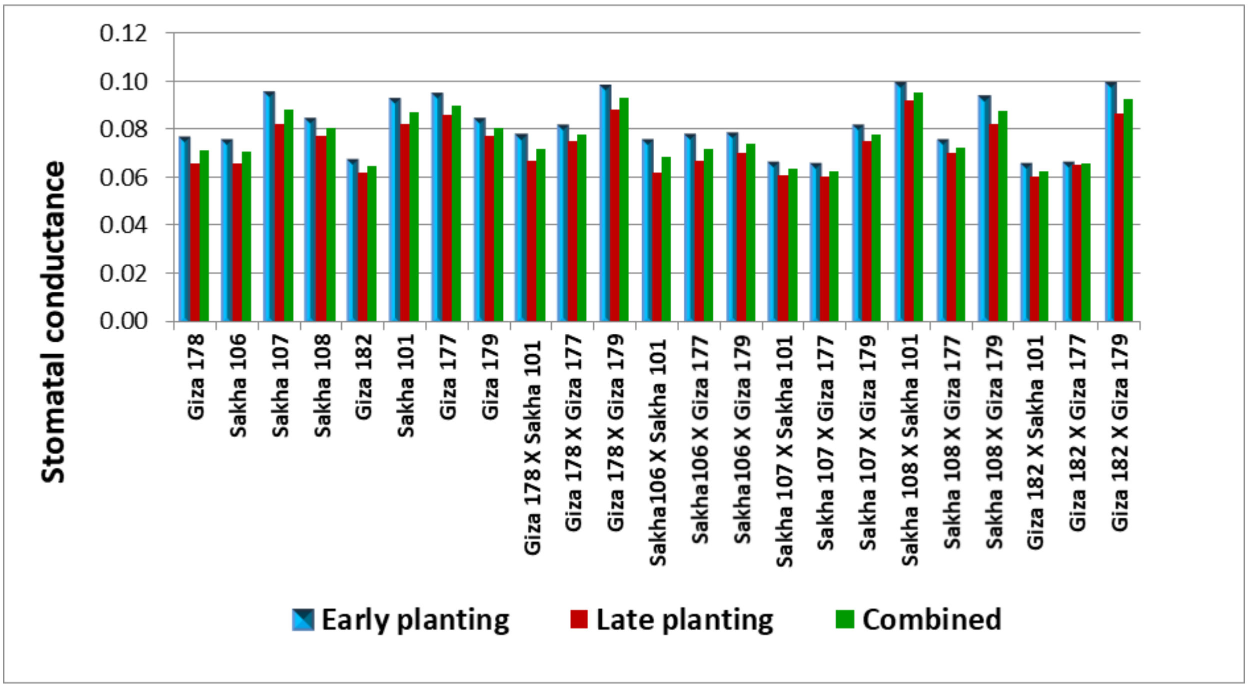

Fig. (7) Effect of planting date on rice genotypes for stomatal conductance.

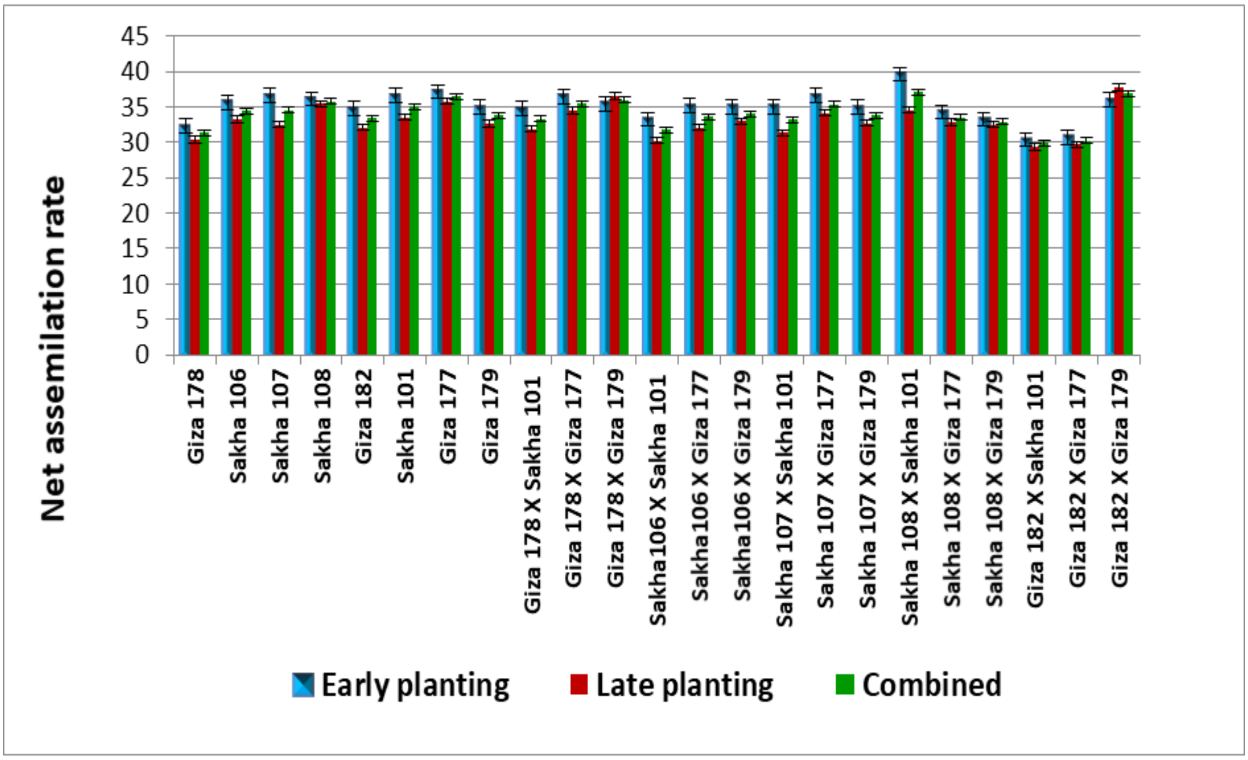

Fig. (8) Effect of planting date on rice genotypes for net assimilation rate.

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