Original article

Heterosis and combining ability for floral and yield characters in rice using cytoplasmic male sterility system

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ABSTRACT

Developing high-yielding rice genotypes is decisive to ensure global food security with current population growth and the threat of environmental pressures. Cytoplasmic male sterility (CMS) system provides a valuable approach for commercial exploitation of heterosis and producing high-yielding and quality hybrid rice. Three CMS lines and ten diverse restorers were crossed using line × tester mating design. The obtained thirty F_1 hybrids and their thirteen parents were evaluated. Yield traits as well as certain floral traits characters that influence the efficiency of crossing and hybrid seed production as the duration of floret opening (min), stigma exsertion (mm), stigma length (mm), opening floret angle, and anther length (mm) were assessed. Highly significant variations were detected among parents, crosses, and parents *vs.* crosses for all the studied traits. The CMS line L2 and the restorer T5 were determined as good combiners for stigma exsertion, stigma length, opining floret angle, and duration of floret opening. Besides, the hybrids L1 × T1, L1 × T3, L2 × T2, L2 × T5, L3 × T4, L3 × T5, and L3 × T9 exhibited positive SCA effects for most floral traits. Moreover, the CMS lines L1 and L3 as well as the restorers T1, T2, T3, T6, and T9 were identified as good general combiners for grain yield and certain related traits. The hybrids L1 × T1, L1 × T5, L1 × T7, L2 × T3, L2 × T4, L2 × T5, L2 × T10, L3 × T1, L3 × T2, and L3 × T6 displayed positive SCA effects for grain yield and one or more of its attributes. Both additive and non-additive gene effects were involved in the governing inheritance of all evaluated traits. The biochemical variations among the certain evaluated genotypes were further studied. The esterase and peroxidase isozymes were applied for verifying the genetic diversity at the protein level among the used CMS lines, restorers, and their crosses. All the applied isozymes displayed polymorphism for the parents and their crosses. The banding pattern and intensity differences provided accurate results on the reliable variability among the tested genotypes.

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1. Introduction

Rice (*Oryza sativa* L.) is a globally essential cereal food crop for over half the world's population (Muthayya et al., 2014; El Sayed et al., 2021). It has valuable nutritional benefits with high contents of carbohydrates, minerals, calories, protein, and vitamins (Kun et al., 2013). Its cultivated area is almost 162 million hectares that produce about 756 million tonnes (FAOSTAT, 2021). This production should be increased to cope with continuing population growth and the threat of environmental pressures (Chang et al., 2016). The commercialization of F_1 hybrid vigor derived by crossing genetically different parents offers a choice to increase rice

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yield potential above the present ceiling (Khush and Gupta, 2013; Huang et al., 2014; Vaz Mondo et al., 2016). Rice is a self-pollinated crop; thus, the great impediment is producing hybrid seeds for commercial hybrid production. The cytoplasmic male sterility (CMS) system is governed by cytoplasmic and nuclear genes that generates male-sterile plants are unable to produce functional pollen which prevents self-fertilization and enhances genetic diversity (Singh et al., 2015). The CMS female lines are crossed by appropriate fertile restorers and produce hybrid seeds in self-pollinating crop species like rice. Accordingly, the CMS system provides a valuable approach for commercial exploitation of hybrid technology and producing high-yielding hybrid rice (El-Namaky, 2018; Liao et al., 2021). The hybrid rice based on the CMS framework has prompted an increment of grain yield by over 20% compared with the conventional rice cultivars (Abebrese et al., 2018; Toriyama et al., 2019).

Large-scale hybrid rice is limited due to its low capacity of hybrid seed production and high seed cost. To cope with these constraints, it is essential to enhance the outcrossing ability of CMS lines (El-Namaky, 2018). The outcrossing of CMS lines is greatly increased by improved floral traits such as; longer duration of floret opening, wider angle of floret opening, longer stigma, and higher stigma exsertion (Anis et al., 2019). Consequently, the floral traits of CMS lines are vital aspects of the hybrid rice breeding program. Furthermore, yield traits are essential for ascertaining heterosis for grain yield (El-Namaky, 2018).

The line × tester analysis is widely employed to investigate general (GCA) and specific combining ability (SCA) effects (Kamara et al., 2021a). Combining ability analysis facilitates selecting the best parents for crossing and identifying promising cross recombinants (Kempthorne, 1957). The GCA and SCA variances are utilized to identify the role of additive and non-additive gene effects concerned with the expression of targeted traits (Kamara et al., 2021b). The GCA signifies additive gene effects, whereas SCA indicates the deviation of hybrid performance from the used parents, and it is related to non-additive gene effects (Bradshaw, 2017; Parimala et al., 2018; Salem et al., 2020). Moreover, line × tester analysis determines gene action that is responsible for the expression of the studied traits (Mutimaamba et al., 2020).

The isozymes could be used as a biochemical marker to detect the variations at the protein level among CMS lines and restorers as well as their crosses (El Shamey et al., 2016; Kuwer et al., 2018). Changes in the electrophoretic mobility of enzymes provide an advantageous method of evaluating genetic diversity. The esterase and peroxidase isozymes are effective biochemical techniques that reflect the substructure differences at the protein level among

the genotypes (Roy and Mandal, 2005; Singh et al., 2009a). These isozymes have been commonly employed as a valuable tool to investigate the genetic diversity at the protein level in different species (Swapna, 2002; Jain et al., 2006; Singh et al., 2009b; Dasgupta et al., 2010; Majumder et al., 2012).

Cytoplasmic male sterility could be exploited to develop new high-yielding and quality hybrids in rice. Accordingly, crossing CMS lines by diverse restorers could provide promising high-yielding hybrids to cope with the rapidly growing global population and threat of environmental pressures. The present study was designed to (i) estimate combining ability effects of the CMS lines, restorers, and their corresponding hybrids, (ii) identify high-yielding hybrid rice, (iii) study gene action and heritability controlling floral and yield traits, (iv) determine the biochemical variations among the used parental genotypes and their crosses, and (v) study the association between grain yield and the assessed floral and yield traits.

2. Materials and methods

2.1. Plant materials and field experiments

Three CMS lines were obtained from International Rice Research Institute (IRRI) and ten diverse restorers were chosen for their genetic differences comprised of four high-yielding commercial Egyptian cultivars and six diverse lines from IRRI (Table 1). The three CMS lines were crossed with ten restorers using line × tester mating design during the summer of 2019 at the Experimental Farm of Agricultural Research Station, Sakha, Kafr El-Sheikh, Egypt (30°57′ N, 31°07′ E). The obtained thirty F_1 hybrids and their parents were assessed in a randomized complete block design (RCBD) with three replications during the summer of 2020. The experimental site is characterized as a hot and arid climate with no precipitation during the summer season and temperatures ranging from 15 to 42 °C. The soil is an old Nile valley clay throughout the profile (19 % sand, 21 % silt, and 60 % clay). Besides, it has high moisture content at field capacity (40%), available water (21%), and lower bulk density (1.2 g cm^{-3}). Thirty day old seedlings of each genotype (parents and F_1 hybrids) were individually transplanted in three rows per replicate. Each row was 5.0 m long with a spacing of 20 x 20 cm between rows and hills. All the recommended agricultural practices were applied through the growth stages. During soil preparation, 37 kg P_2O_5/ha as superphosphate (15% P_2O_5), and potassium at a rate of 50 kg K_2O kg/ha as potassium sulfate (48% K2O) were applied. Nitrogen fertilization

Table 1
Name, code, origin, and type of the parental genotypes used in the study.

Code	Genotype	Origin	Type
CMS lines			
L1	G46A/B	IRRI	Indica, short-grain
L2	K17A/B	IRRI (Kalinga cytoplasm	Indica, short-grain
L3	IR58025A/B	IRRI (WA cytoplasm, IR48483 A/8 pus A167-120-3-2//pusA 167-120-3-2)	Indica, long-grain
Restorers			
T1	Giza 179	Egypt (GZ1368-S-5-4/GZ6296-12-1-2)	Indica-Japonica, short grain
T2	Giza 178 R	Egypt (Giza 175/Milyang 49)	Indica-Japonica, short grain
T3	Giza 181 R	Egypt (IR24/IR22)	Indica, long grain
T4	Giza 182 R	Egypt (Giza181/IR39422-163-1-2//Giza 181)	Indica, long grain
T5	IR69137-34-1-3-1	IRRI	Indica, short-grain
T6	IR65617-52-23-3-2-3	IRRI	Indica, short-grain
T7	IR671017-124-2-4	IRRI	Indica, short-grain
T8	IR67413-71-4-2-2	IRRI	Japonica, short grain
T9	IR67418-238-6-2-3-2	IRRI	Indica, short-grain
T10	IR3894-40D-PN-S-1	IRRI	Indica, short-grain

was applied at a rate of 165 kg N ha^{-1} in three equal doses at transplanting, 15 days after transplanting, and 30 days after transplanting. The full irrigation with submerged depth (7.0 cm) was induced every 4 days to ensure that the irrigation water covered all surface areas in each irrigation event. The weeds were chemically controlled by adding a dose of 4.8 L/ha of Saturn four days after transplanting.

2.2. Data collection

The floral traits were measured according to Singh and Haque, (1999), and Sheeba et al., (2006) using a micrometer under a stereomicroscope for five random spikelets from each panicle. Duration of floret opening (min) was measured as the time taken from opening to closing of the floret, stigma exsertion (mm) was estimated as the length of stigma outside closed spikelet, opening floret angle (°) was calculated as the angle between lemma and palea and length of complete stigma (mm) and anther (mm) was also measured.

Yield traits were recorded for ten random plants within each plot. Length of main panicle (cm) was estimated from panicle base to the uppermost spikelet of the panicle, panicle weight (g) was measured by weighting the main panicle of the plant after drying moisture, the total number of panicles plant^{-1} was recorded when all panicles were at the fully ripe stage, seed set (%) was calculated as follows: number of filled grains/total grains number × 100, and grain yield plant^{-1} (g) was measured as the weight of grain yield of each plant.

2.3. Statistical analysis

The obtained data were subjected to ordinary analysis of variances according to Steel and Torrie (1980). General (GCA) and specific (SCA) combining ability effects and variances were estimated using line × tester analysis according to Kempthorne (1957). Heritability was calculated as suggested by Burton and Devane (1953). The heterotic effects of F$_1$ crosses were estimated relative to the better parent following Fonsecca and Patterson (1968).

2.4. Biochemical analysis

Biochemical analysis was performed at the Chinese Academy of Agricultural Science (CAAS), China. Two CMS lines (L1 and L3), four restorers (T1, T2, T3, and T5), and their respective F$_1$ hybrids were selected for this analysis. Samples of 200 mg of fresh leaves of

21 days old seedlings were collected. Each sample was mixed in 1.0 ml of an ice-cold 50 mM tris-HCl buffer (PH 6.8) including 20% (w/v) sucrose and 3 mM Dithiothreitol (DTT). The extracts were centrifuged at 15,000 rpm at 4 °C for five min., and supernatants were pipetted. Isozymes; esterase (EST) and peroxidase (POX) were determined using polyacrylamide gel electrophoresis (PAGE) as outlined by Davis (2006). Slabs of 7.5% acrylamide for separation gel and 2.5% acrylamide for stacking gel were prepared. The gel running took about four hrs at 25 mA constant current. Esterase bands were detected on the gel using α-naphthyl-acetate and β–naphthyl-acetate as substrate and subsequent color was developed with fast blue RR salts. Peroxidase zymogram was developed during 15 min following incubation of 0.25% benzidine dihydrochloride, four drops of glacial acetic acid, and 10 drops of 1% hydrogen peroxide.

3. Results

3.1. Analysis of variance

The analysis revealed highly substantial differences for parents, crosses, and parents vs. crosses in all investigated traits (Table 2). These findings imply the presence of significant genetic variation among the assessed genotypes for the evaluated traits. Furthermore, highly significant variance owing to lines (L), testers (T), and L × T interaction were detected for all evaluated traits.

3.2. Mean performance

The evaluated parents and F1 hybrids displayed a wide range in the floral and yield traits (Table 3). The duration of floret opening ranged between 28.06 and 161.95 min. The three CMS lines L1, L2, and L3, as well as the hybrids L1 × T5, L2 × T5, and L3 × T5, recorded the longest duration of floret opening. Stigma exsertion varied between 0.43 and 2.0 mm. The line L3, as well as the hybrids L2 × T5, L3 × T5, L3 × T4, L3 × T9, L3 × T6, and L3 × T8, exhibited the highest stigma exsertion. The opening floret angle ranged between 17.06 and 35.06. The parents L1 and T5, as well as the hybrids L2 × T5, L1 × T1, L3 × T2, L3 × T8, and L1 × T3, possessed the widest angle of floret opening. Stigma length varied between 0.63 and 2.28 mm. The CMS lines L3 and L2 and the hybrids L3 × T5, L3 × T6, L2 × T5, L3 × T10, and L3 × T8 had the longest stigma. Anther length varied between 1.68 and 2.61 mm. The parents L2, L3, and T1 as well as the crosses L1 × T1, L1 × T3, and

Table 2
Mean squares from the ordinary and line × tester analysis for all the studied traits.

SOV	df	Duration of floret opening (min)	Stigma exsertion (mm)	Opening floret angle (°)	Stigma length (mm)	Anther length (mm)
Genotypes (G)	42	2623.0**	0.48**	25.76**	0.55**	0.12**
Parents (P)	12	6196.9**	0.61**	36.82**	0.78**	0.14**
Crosses (C)	29	503.7**	0.13**	19.31**	0.10**	0.11**
P vs. C	1	21198.8**	8.93**	80.06**	10.88**	0.02**
Lines (L)	2	9.26*	0.48**	12.23**	0.02	0.001**
Testers (T)	9	1562.9**	0.13**	6.18**	0.18**	0.26**
L × T	18	28.97**	0.09**	26.67**	0.07**	0.05**
Error	84	1.90	0.001	0.41	0.008	0.0004
SOV	df	Panicle length (cm)	Panicle weight (g)	No. of panicles plant^{-1}	Seed set (%)	Grain yield plant^{-1} (g)
Genotypes (G)	42	7.84**	3.81**	70.84**	646.4**	334.9**
Parents (P)	12	5.22**	2.78**	37.18**	273.9**	142.0**
Crosses (C)	29	8.22**	4.08**	78.04**	778.9**	383.8**
P vs. C	1	28.47**	8.35**	266.07**	1274.14**	1232.9**
Lines (L)	2	39.07**	0.25**	241.9**	21.65**	330.9**
Testers (T)	9	12.62**	12.11**	79.59**	2476.2**	1107.4**
L × T	18	2.59**	0.49**	59.06**	14.37**	27.90**
Error	84	0.21	0.01	0.22	0.84	1.32

* and** indicate p-value < 0.05 and 0.01, respectively.

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L2 × T6 exhibited the longest anthers. Panicle length ranged between 21.39 and 29.3 cm, the crosses L3 × T5, L3 × T3, L3 × T1, L3 × T6, L1 × T3 and L3 × T9 possessed the highest desirable values. Panicle weight varied between 2.61 and 7.21 g. The hybrids L1 × T1, L2 × T3, L3 × T3, L3 × T9, and L1 × T2 recorded the heaviest weights. The number of panicles plant^{-1} ranged between 11.18 and 29.63. The hybrids L3 × T9, L3 × T6, L3 × T1, L3 × T2, L3 × T4, and L2 × T3 produced the uppermost number. The seed set varied between 38.06 and 95.77%. The parents T1, T2, T3, T4, and T10 as well as the hybrids L1 × T6, L3 × T9, L1 × T9, and L2 × T6 exhibited the highest percentage. Grain yield plant^{-1} varied between 17.84 and 64.2 g. The hybrids L3 × T1, L1 × T1, L3 × T2, L3 × T3, L3 × T6, and L1 × T2 recorded the highest grain yield.

3.3. Genotypic classification according to floral and yield traits

Floral and yield traits were employed to classify the evaluated genotypes and their crosses based on their performance. Employing hierarchical clustering the parental genotypes and their crosses were categorized into four groups based on floral traits (Fig. 1A). Group A included three CMS lines that had the highest values of floral traits; group B comprised of 9 hybrids as well as group C with 3 restorers and 21 hybrids which had intermediate values of floral traits, while group D contained 7 restorers that had the lowest values of floral traits. Likewise, the genotypes were clustered into four groups based on yield traits (Fig. 1B). Group A contained 10 hybrids that recorded the highest yield traits; group B included 11 hybrids and 6 restorers, as well as group C comprised of 4 hybrids, 3 CMS lines, and 4 restorers, had intermediate yield values, while group D contained 4 hybrids and restorer displayed the lowest yield traits.

3.4. General combining ability effects (GCA)

GCA effects for the estimated parents (CMS lines and restorers) are shown in Table 4. The results revealed that the line L1 exhibited substantial and positive GCA effects for the duration of floret opening and grain yield plant^{-1}. Moreover, the line L2 expressed highly significant and positive GCA effects for stigma exsertion, opining floret angle, and seed set percentage. Besides, line L3 posed posi-

Table 3
Mean performance for floral and yield traits of the parents and their crosses.

Genotype	Duration of floret opening (min)	Stigma exsertion (mm)	Opening floret angle (0)	Stigma length (mm)	Anther length (mm)	Panicle length (cm)	Panicle weight (g)	No. of panicles plant^{-1}	Seed set (%)	Grain yield plant^{-1} (g)
L1	154.41	1.43	31.39	1.71	2.32	23.42	4.02	16.60	92.17	35.42
L2	161.95	1.50	24.06	2.04	2.61	24.75	2.61	18.55	91.13	31.27
L3	116.29	1.78	17.06	2.14	2.40	24.86	3.44	14.69	89.10	39.32
T1	47.23	0.48	28.39	1.70	2.51	24.08	4.43	23.46	94.79	47.01
T2	49.39	0.43	26.39	0.73	1.82	24.64	4.81	22.50	95.77	42.45
T3	33.06	1.19	27.39	1.58	2.29	26.30	5.50	16.18	93.70	35.67
T4	47.06	1.36	27.39	1.58	2.09	24.39	5.04	20.18	94.85	31.35
T5	58.06	0.61	29.39	0.97	2.21	25.86	5.01	11.18	91.57	28.99
T6	29.06	0.66	23.39	0.80	2.33	24.39	3.68	17.52	77.58	32.18
T7	35.39	0.79	25.06	0.99	1.94	23.39	3.98	18.18	82.92	29.85
T8	40.39	0.61	25.06	1.01	2.30	21.39	2.98	16.85	61.51	29.51
T9	51.06	0.57	26.39	0.63	2.27	25.39	5.48	18.18	90.83	49.51
T10	70.06	0.80	23.73	1.04	2.10	22.73	3.16	12.18	93.18	31.00
L1 × T1	34.66	1.42	31.73	1.82	2.50	26.42	7.21	21.98	92.37	60.36
L1 × T2	40.39	1.38	27.39	1.78	1.68	25.32	5.68	24.03	87.10	52.45
L1 × T3	48.12	1.44	30.39	1.82	2.47	27.22	5.15	19.72	85.43	49.91
L1 × T4	35.06	1.39	28.39	1.99	2.22	25.73	5.34	21.52	91.51	39.85
L1 × T5	83.12	1.52	21.73	1.93	1.91	24.28	2.62	19.36	40.05	22.05
L1 × T6	28.06	1.24	25.39	1.94	2.35	24.39	4.98	19.63	93.01	49.18
L1 × T7	39.06	1.40	26.39	1.97	2.09	23.95	4.01	21.18	81.27	40.74
L1 × T8	36.73	1.33	27.39	2.00	2.35	22.50	3.16	17.52	60.89	37.29
L1 × T9	33.06	1.21	27.73	1.81	2.34	26.45	5.58	20.18	92.71	44.23
L1 × T10	35.06	1.33	25.39	1.99	2.25	24.39	3.28	13.18	89.18	32.00
L2 × T1	37.06	1.55	28.39	1.90	2.01	25.25	5.65	16.51	90.73	50.03
L2 × T2	42.73	1.70	27.39	2.08	1.98	23.22	5.41	21.06	90.46	45.36
L2 × T3	46.39	1.13	25.39	1.49	2.22	26.12	5.98	25.91	88.77	49.45
L2 × T4	39.39	1.53	29.06	2.00	2.30	25.06	5.43	21.52	89.51	38.18
L2 × T5	74.90	2.00	35.06	2.12	2.15	24.02	3.55	17.38	48.57	18.92
L2 × T6	28.06	1.56	26.73	2.00	2.41	24.50	4.98	12.85	92.62	43.29
L2 × T7	39.06	1.53	27.73	2.03	2.11	23.73	3.66	16.85	81.00	33.40
L2 × T8	36.06	1.58	26.06	1.98	2.38	22.00	3.05	16.52	60.49	32.62
L2 × T9	28.06	1.67	28.73	1.97	2.33	24.67	5.35	17.29	92.11	41.18
L2 × T10	37.06	1.64	27.73	2.04	2.29	24.39	3.28	15.18	90.51	36.33
L3 × T1	35.06	1.11	24.06	1.49	2.07	27.42	5.65	28.91	89.68	64.20
L3 × T2	38.06	1.30	30.73	1.61	1.81	26.70	5.41	28.48	88.67	59.23
L3 × T3	44.39	1.42	24.06	1.79	2.36	28.08	5.98	13.37	87.94	54.45
L3 × T4	40.39	1.74	25.06	2.00	2.36	25.06	5.53	26.18	86.18	40.18
L3 × T5	70.06	1.77	28.39	2.28	2.11	29.30	3.55	14.92	38.06	17.84
L3 × T6	34.06	1.71	26.39	2.19	2.31	27.39	5.38	29.29	91.01	53.91
L3 × T7	38.06	1.69	27.39	1.75	2.33	24.67	4.05	25.41	81.22	41.85
L3 × T8	33.06	1.71	29.39	2.11	2.27	24.39	3.33	25.52	63.32	39.96
L3 × T9	34.06	1.73	28.39	2.03	2.33	27.00	5.75	29.63	92.86	47.51
L3 × T10	35.06	1.62	26.73	2.12	2.36	25.39	3.53	14.85	89.18	35.67
Mean	49.25	1.34	26.97	1.74	2.23	24.99	4.55	19.59	81.73	40.35
LSD $_{0.05}$	2.24	0.05	1.04	0.15	0.03	0.75	0.17	0.76	1.49	1.86
LSD $_{0.01}$	2.97	0.07	1.38	0.20	0.04	0.99	0.22	1.01	1.98	2.47

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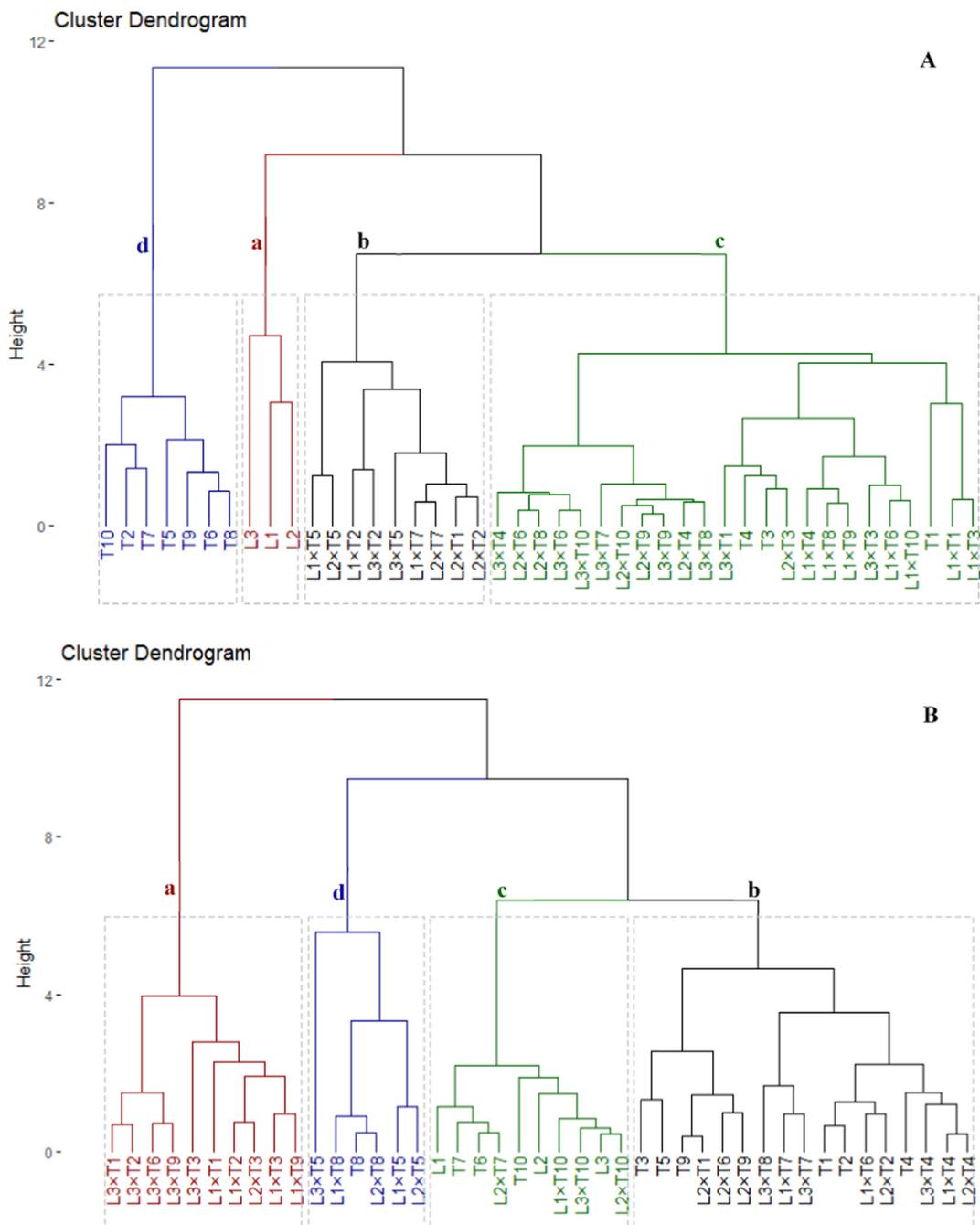
Fig. 1. Dendrogram of the phenotypic distances among ten rice genotypes and their thirteen F1s based on floral characters (A) and yield traits (B).

tive and significant GCA effects for stigma exsertion, anther length, panicle length, panicle weight, number of panicles/plant, and grain yield/plant.

Regarding the restorers, T1 and T2 displayed highly significant and positive GCA effects for opening floret angle, number of panicles/plant, panicle weight, seed set percentage, and grain yield/-plant. Moreover, T3 and T4 were good general combiners for anther length, panicle weight, and seed set %. The T5 appeared to be a good combiner for the duration of floret opening, stigma exsertion, opining floret angle, stigma length, and panicle length. The restorer T6 and T9 exhibited significant and positive effects for panicle weight, seed set %, and grain yield/plant. The restorer T7 had desirable GCA effects for stigma exsertion and number of panicles plant^{-1}. Additionally, T9 showed significant and positive

GCA effects for stigma exsertion, anther length, and seed set percentage, and accordingly, could be considered as a good combiner for improving these traits. The parents that displayed high GCA effects for grain yield also had desirable GCA effects for one or more of its contributing traits.

3.5. Specific combining ability effects (SCA)

The SCA effects in Table 5 indicated that five cross combinations L1 × T5, L2 × T2, L3 × T4, L3 × T6, and L3 × T9 possessed significant and positive SCA effects for the duration of floret opening. Besides, ten hybrid combinations had positive and significant SCA effects for stigma exsertion. The maximum desirable SCA effects for stigma exsertion were assigned for L1 × T1, L1 × T3,

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Table 4
Estimates of general combining ability (GCA) effects of the parents for all the studied traits.

Parents	Duration of floret opening (min)	Stigma exsertion (mm)	Opening floret angle (0)	Stigma length (mm)	Anther length (mm)	Panicle length (cm)	Panicle weight (gm)	No. of panicles plant^{-1}	Seed set (%)	Grain yield plant^{-1} (g)
	CMS lines									
L1	0.52*	−0.15**	−0.30*	−0.03*	−0.01**	−0.24**	−0.02	−0.70**	−0.19	0.42*
L2	0.07	0.08**	0.73**	0.03*	0.001	−1.01**	−0.08**	−2.42**	0.93**	−3.51**
L3	−0.59*	0.07**	−0.43**	0.001	0.01**	1.24**	0.10**	3.12**	−0.74**	3.09**
L.SD (gi) $_{0.05}$	0.50	0.01	0.23	0.03	0.01	0.17	0.04	0.17	0.33	0.42
L.SD (gi) $_{0.01}$	0.66	0.02	0.31	0.04	0.01	0.22	0.05	0.23	0.44	0.55
	Testers									
T1	−5.22**	−0.15**	0.57**	−0.20**	−0.03**	1.06**	1.45**	1.94**	9.38**	15.81**
T2	−0.42	−0.05**	1.01**	−0.11**	−0.40**	−0.22	0.78**	3.99**	7.20**	9.96**
T3	5.49**	−0.18**	−0.88**	−0.24**	0.13**	1.84**	0.99**	−0.87**	5.83**	8.88**
T4	−2.53**	0.04**	0.01	0.06**	0.07**	−0.02	0.72**	2.54**	7.52**	−2.98**
T5	35.21**	0.26**	0.90**	0.18**	−0.17**	0.57**	−1.48**	−3.31**	−39.32**	−22.78**
T6	−10.75**	−0.01	−1.32**	0.11**	0.13**	0.13	0.40**	0.06	10.67**	6.41**
T7	−2.09**	0.03**	−0.32	−0.02	−0.04**	−1.19**	−0.81**	0.62**	−0.38	−3.72**
T8	−5.53**	0.03**	0.12	0.09**	0.11**	−2.33**	−1.54**	−0.68**	−19.98**	−5.76**
T9	−9.09**	0.02**	0.79**	0.001	0.11**	0.74**	0.84**	1.84**	11.01**	1.92**
T10	−5.09**	0.02*	−0.88**	0.12**	0.08**	−0.57**	−1.35**	−6.12**	8.08**	−7.72**
L.SD (gi) $_{0.05}$	0.91	0.02	0.42	0.06	0.01	0.30	0.07	0.31	0.61	0.76
L.SD (gi) $_{0.01}$	1.21	0.03	0.56	0.08	0.02	0.40	0.09	0.41	0.81	1.01

* and ** indicate *p*-value < 0.05 and 0.01, respectively.

Table 5
Estimates of specific combining ability (SCA) effects of the F$_1$ hybrids for all the studied traits.

Hybrid	Duration of floret opening	Stigma exsertion	Opening floret angle	Stigma length	Anther length	Panicle length	Panicle weight	No. of panicles plant^{-1}	Seed set %	Grain yield plant^{-1}
L1 × T1	−1.45	0.20**	3.97**	0.11*	0.31**	0.29	1.06**	0.22	1.63**	1.75**
L1 × T2	−0.52	0.07**	−0.81*	−0.02	−0.14**	0.48	0.19**	0.21	−1.45**	−0.32
L1 × T3	1.30	0.26**	4.08**	0.15**	0.13**	0.31	−0.54**	0.75**	−1.75**	−1.78**
L1 × T4	−3.74**	−0.02	1.19**	0.02	−0.06**	0.68*	−0.08	−0.85**	2.64**	0.03
L1 × T5	6.57**	−0.10**	−6.37**	−0.15**	−0.14**	−1.35**	−0.60**	2.84**	−1.98**	2.03**
L1 × T6	−2.52**	−0.12**	−0.48	−0.07	0.001	−0.80**	−0.12*	−0.26	0.99	−0.03
L1 × T7	−0.19	0.01	−0.48	0.09	−0.08**	0.07	0.12*	0.74**	0.30	1.66*
L1 × T8	0.92	−0.06**	0.08	0.001	0.03*	−0.23	0.001	−1.63**	−0.48	0.25
L1 × T9	0.81	−0.18**	−0.26	−0.10*	0.01	0.64*	0.04	−1.48**	0.35	−0.50
L1 × T10	−1.19	−0.06**	−0.92*	−0.03	−0.05**	−0.10	−0.07	−0.52	−0.25	−3.09**
L2 × T1	1.40	0.11**	−0.40	0.13*	−0.18**	−0.11	−0.44**	−3.53**	−1.12*	−4.66**
L2 × T2	2.27**	0.16**	−1.84**	0.23**	0.16**	−0.86**	−0.01	−1.04**	0.79	−3.47**
L2 × T3	0.02	−0.28**	−1.96**	−0.24**	−0.13**	−0.02	0.36**	8.67**	0.46	1.69*
L2 × T4	1.05	−0.10**	0.82*	−0.02	0.01	0.78**	0.08	0.87**	−0.49	2.29**
L2 × T5	−1.19	0.16**	5.93**	−0.02	0.09**	−0.85**	0.39**	2.59**	5.41**	2.83**
L2 × T6	−2.07*	−0.02	−0.18	−0.07	0.06**	0.08	−0.05	−5.32**	−0.52	−1.99**
L2 × T7	0.27	−0.09**	−0.18	0.09	−0.06**	0.62*	−0.16**	−1.87**	−1.09*	−1.75**
L2 × T8	0.71	−0.04*	−2.29**	−0.08	0.05**	0.04	−0.05	−0.91**	−2.01**	−0.49
L2 × T9	−3.73**	0.06**	−0.29	0.01	0.001	−0.37	−0.12*	−2.65**	−1.38*	0.38
L2 × T10	1.27	0.04*	0.38	−0.04	0.001	0.67*	0.002	3.20**	−0.04	5.18**
L3 × T1	0.05	−0.32**	−3.57**	−0.25**	−0.13**	−0.19	−0.62**	3.32**	−0.51	2.91**
L3 × T2	−1.75*	−0.23**	2.66**	−0.22**	−0.03*	0.38	−0.19**	0.83**	0.66	3.79**
L3 × T3	−1.32	0.02	−2.12**	0.08	0.002	−0.30	0.18**	−9.42**	1.30*	0.09
L3 × T4	2.70**	0.12**	−2.01**	0.001	0.05**	−1.46**	0.002	−0.01	−2.15**	−2.31**
L3 × T5	−5.38**	−0.06**	0.43	0.17**	0.05**	2.19**	0.21**	−5.43**	−3.43**	−4.86**
L3 × T6	4.59**	0.14**	0.66	0.14**	−0.06**	0.72**	0.17**	5.58**	−0.47	2.03**
L3 × T7	−0.08	0.08**	0.66	−0.17**	0.14**	−0.69*	0.04	1.14**	0.79	0.09
L3 × T8	−1.64*	0.10**	2.21**	0.08	−0.08**	0.19	0.05	2.54**	2.49**	0.24
L3 × T9	2.92**	0.13**	0.54	0.09	−0.01	−0.28	0.09	4.13**	1.03	0.11
L3 × T10	−0.08	0.02	0.54	0.06	0.05**	−0.57*	0.07	−2.68**	0.29	−2.09**
LSD (Sij) $_{0.05}$	1.58	0.04	0.73	0.10	0.02	0.53	0.12	0.54	1.05	1.32
LSD (Sij) $_{0.01}$	2.10	0.05	0.97	0.14	0.03	0.70	0.16	0.72	1.39	1.75

* and ** indicate *p*-value < 0.05 and 0.01, respectively.

and L3 × T6. The best specific combiners for opining floret angle were the crosses L1 × T1, L1 × T3, L2 × T4, L2 × T5, and L3 × T8. Six hybrid combinations L1 × T1, L1 × T3, L2 × T1, L2 × T2, L3 × T5, and L3 × T6 manifested significant and positive SCA effects for stigma length. Nine hybrid combinations had significant and positive effects for anther length, the best hybrids were L1 × T1 and L2 × T1. The greatest significant and positive SCA

effects for panicle length were obtained by the crosses L1 × T4, L1 × T9, L2 × T4, L2 × T7, L2 × T10, and L3 × T5. Likewise, the hybrids L1 × T1, L1 × T2, L1 × T7, L2 × T3, L2 × T5, L3 × T3, L3 × T3, L3 × T5, and L3 × T6 had high positive SCA estimates for panicle weight. Out of the 30 hybrids, thirteen crosses were determined to have significant and positive SCA effects for the number of panicles plant^{-1}. The best three hybrids identified with

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high SCA effects for the number of panicles plant^{-1}were L1 × T6, L2 × T3, and L3 × T6. For seed set percentage, the highest positive SCA effects were assigned for the hybrids L1 × T1, L1 × T4, L2 × T5, L3 × T3, and L3 × T8. Regarding grain yield plant^{-1}, ten hybrids exhibited significant and positive SCA effects. The hybrids L1 × T1, L1 × T5, L3 × T1, L3 × T2, L3 × T3, and L2 × T10 had the highest desirable SCA effects for grain yield. Notably, these hybrids that demonstrated high SCA effects for grain yield also displayed high SCA effects for one or more traits of its components. For example, the cross L1 × T1 had advantageous SCA effects for grain yield, also revealed high SCA effects for panicle weight and seed set percentage.

3.6. Estimates of heterosis effects

The deviation of F$_1$ means from the better parent for all tested characteristics are shown in Table 6. None of the hybrid combinations demonstrated positive (desirable) heterosis over the better parent for the duration of floret opening. While, the hybrids L1 × T5, L2 × T2, L2 × T5, L2 × T6, L2 × T8, L2 × T9, and L2 × T10 showed significant and positive heterotic effects for stigma exsertion. Moreover, twelve hybrids showed significant and positive heterosis for opening floret angle with superiority of L2 × T5, L3 × T8, L3 × T2, L2 × T10, L3 × T6, and L3 × T10. The desirable heterotic effects for stigma length were detected by the hybrids L1 × T4, L1 × T5, L1 × T6, L1 × T7, L1 × T8, and L1 × T10. Only the cross combination L1 × T3 exhibited a desirable and highly significant heterotic effect for the anther length. Eleven hybrids displayed significant and positive values for panicle length; the best hybrids were L1 × T1, L1 × T4, L3 × T1, L3 × T5, and L3 × T6. Sixteen out of 30 crosses possessed significant and positive heterosis effects for panicle weight relative to the better parent. The hybrids L1 × T1, L2 × T6, L3 × T9, L3 × T6, and L3 × T5 recorded the most favorable values for panicle weight.

Furthermore, significant and positive heterotic effects for the number of panicles plant^{-1} were observed in sixteen hybrids. For the seed set percentage, only the two hybrids L2 × T6 and L3 × T6 proved significant and positive heterotic effects. Regarding grain yield/plant, data showed that twenty-two hybrids exhibited significant and positive heterotic effects. The best hybrids were L1 × T1, L1 × T2, L1 × T3, L1 × T6, L1 × T6, L2 × T3, L3 × T1, L3 × T2, L3 × T3, and L3 × T6.

3.7. Estimation of the genetic components

The genetic variance components are given in Table 7. The obtained results revealed that the dominance genetic variance (σ^2D) was superior to the additive genetic variance (σ^2A) in the inheritance of all the assessed traits, except duration of floret opening, seed set %, and grain yield/plant. which was controlled by the additive genetic variance. Heritability in a broad sense (h_b^2 %) was high for all the evaluated traits. On the other hand, heritability in the narrow sense (h_n^2 %) was relatively high for the duration of floret opening, panicle weight, seed set %, and grain yield, whereas it was low for the remaining traits.

3.8. Interrelationship among studied traits

The principal component analysis (PCA) was performed to visualize the association among evaluated traits. The first two PCAs reflected most of the variance, about 64.45% (52.17% and 12.28% by PCA1 and PCA2, respectively). Consequently, the two PCAs were used to perform the PC-biplot (Fig. 2). The evaluated traits could be classified into three groups. The first group comprises 6 traits; grain yield per plant, seed set percentage, panicle length, panicle weight, number of panicles per plant, and opening floret angle. The second group consisted of 2 traits; stigma exsertion and stigma length. The third group contained 2 traits; duration of floret open-

Table 6
Estimates of heterosis relative to the better parent for all the studied traits.

Hybrid	Duration of floret opening	Stigma exsertion	Opening floret angle	Stigma length	Anther length	Panicle length	Panicle weight	No. of panicles plant^{-1}	Seed set %	Grain yield plant^{-1}
L1 × T1	−77.55**	−0.70	1.06	6.42	−0.66	9.69**	62.63**	−6.28**	−2.56**	28.41**
L1 × T2	−73.84**	−3.27	−12.74**	3.89	−27.69**	2.76	18.17**	6.80**	−9.05**	23.54**
L1 × T3	−68.83**	1.17	−3.19	6.23	6.31**	3.47*	−6.42**	18.80**	−8.82**	39.92**
L1 × T4	−77.29**	−2.80	−9.56**	15.95**	−4.30**	5.47**	6.02**	6.61**	−3.51**	12.51**
L1 × T5	−46.17**	6.78**	−30.79**	12.65**	−17.93**	−6.11**	−47.60**	16.63**	−56.54**	−37.73**
L1 × T6	−81.83**	−13.08**	−19.11**	13.23**	0.57	0.001	23.98**	12.05**	0.92	38.86**
L1 × T7	−74.70**	−1.64	−15.93**	14.98**	−9.90**	2.28	−0.17	16.50**	−11.82**	15.02**
L1 × T8	−76.21**	−6.78**	−12.74**	16.54**	1.29	−3.90*	−21.24**	3.96	−33.93**	5.29*
L1 × T9	−78.59**	−15.42**	−11.68**	5.45	0.57	4.16**	1.82	11.00**	0.59	−10.67**
L1 × T10	−77.29**	−7.01**	−19.11**	16.34**	−3.30**	4.17*	−18.34**	−20.57**	−4.29**	−9.65**
L2 × T1	−77.12**	3.10	0.002	−6.71	22.96**	2.02	27.44**	−29.61**	−4.28**	6.42**
L2 × T2	−73.62**	12.86**	3.79	2.29	−24.23**	−6.20**	12.62**	−6.40**	−5.54**	6.85**
L2 × T3	−71.35**	−25.06**	−7.30**	−26.84**	−15.18**	−0.71	8.79**	39.68**	−5.27**	38.61**
L2 × T4	−75.86**	2.00	6.08**	−1.80	−11.99**	1.25	7.74**	6.61**	−5.62**	21.80**
L2 × T5	−53.75**	33.26**	19.28**	4.09	−17.86**	−7.14**	−29.09**	−6.29**	−46.96**	−39.49**
L2 × T6	−82.67**	3.99*	11.08**	−1.96	−7.78**	−1.00	35.33**	−30.73**	1.64*	34.52**
L2 × T7	−75.88**	1.55	10.64**	−0.49	−19.13**	−4.13**	−7.96**	−9.16**	−11.11**	6.83*
L2 × T8	−77.73**	4.88**	3.99	−2.95	−8.93**	−11.10**	2.24	−10.96**	−33.62**	4.34
L2 × T9	−82.67**	11.09**	8.84**	−3.27	−10.71**	−2.85	−2.31	−6.77**	1.08	−16.83**
L2 × T10	−77.12**	9.31**	15.24**	0.33	−12.24**	−1.44	3.80	−18.15**	−2.86**	16.20**
L3 × T1	−69.85**	−37.52**	−15.26**	−30.11**	−17.64**	10.27**	27.44**	23.25**	−5.39**	36.57**
L3 × T2	−67.27**	−26.64**	16.42**	−24.49**	−24.83**	7.37**	12.62**	26.60**	−7.41**	39.51**
L3 × T3	−61.82**	−20.08**	−12.17**	−16.38**	−1.66*	6.77**	8.79**	−17.37**	−6.15**	38.49**
L3 × T4	−65.26**	−2.25	−8.52**	−6.24	−1.94*	0.79	9.72**	29.73**	−9.14**	2.19
L3 × T5	−39.75**	−0.19	−3.40	6.86	−12.07**	13.30**	−29.09**	1.54	−58.43**	−54.63**
L3 × T6	−70.71**	−3.75*	12.82**	2.34	−3.88**	10.18**	46.20**	67.23**	2.15*	37.11**
L3 × T7	−67.27**	−4.88**	9.31**	−18.25**	−2.91**	−0.78	1.76	39.73**	−8.84**	6.43**
L3 × T8	−71.57**	−3.75*	17.29**	−1.25	−5.69**	−1.89	−3.20	51.43**	−28.93**	1.62
L3 × T9	−70.71**	−2.63	7.58**	−4.99	−2.91**	9.69**	62.63**	−6.28**	−2.56**	28.41**
L3 × T10	−69.85**	−8.82**	12.64**	−0.78	−1.80**	2.76	18.17**	6.80**	−9.05**	23.54**

* and ** indicate *p*-value < 0.05 and 0.01, respectively.

Table 7
Estimates of genetic parameters for all the studied traits.

Parameter	DFO	SE	OFA	SL	AL	PL	PW	NPP	SS	GYP
Additive variance (σ^2 A)	17.75	0.001	0.28	0.001	0.002	0.211	0.13	0.71	28.59	13.31
Dominant variance (σ^2 D)	9.02	0.03	8.75	0.02	0.02	0.79	0.16	19.61	4.51	8.86
Environmental variance (σ_2 E)	1.90	0.001	0.41	0.008	0.0004	0.211	0.011	0.22	0.84	1.32
Broad sense heritability h_b^2%	93.36	96.60	95.42	73.34	98.10	82.62	96.50	98.92	97.53	94.40
Narrow sense heritability h_n^2%	61.90	4.14	3.09	3.36	11.50	17.36	44.28	3.46	84.24	56.67

DFO: Duration of floret opening, SE: Stigma exsertion, OFA: Opening floret angle, SL: Stigma length, AL: Anther length, PL: Panicle length, PW: Panicle weight, NPP: No. of panicles plant^{-1}, GYP: Grain yield plant^{-1}, SS: Seed set %.
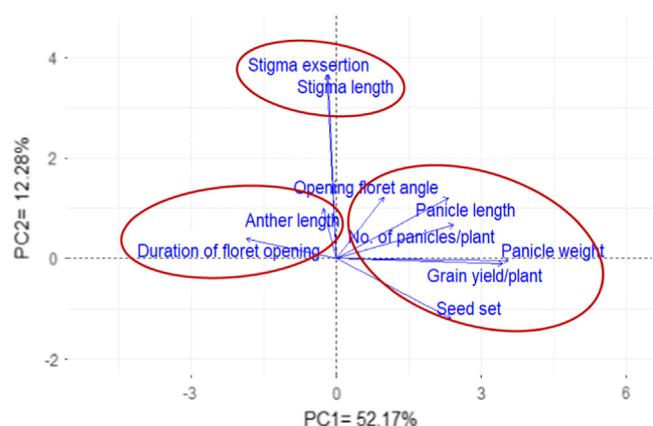
Fig. 2. Biplot of PCA presenting the interrelationship among the evaluated traits.

ing and anther length. A strong positive relationship was determined among characteristics included in each group. In contrast, a negative relationship was detected between the first group, on one side, and the second and third groups on the other side. These results suggest that panicle weight, panicle length, number of panicle/plant, and seed set % are important traits for increasing rice grain yield.

3.9. Biochemical analysis

3.9.1. Esterase isozymes polymorphism

In the current study; an attempt was performed to determine biochemical variations at the protein level in certain CMS lines

and restorers as well as their crosses. The lines L1 and L3 were investigated independently with four restorers; T1, T2, T3, and T5 and their corresponding F1 crosses. The results of line L1 displayed two monomorphic bands (No. 3 and 4) in all parents and their crosses (Table 8 and Fig. 3A). The intensity of these bands was strong in the first three crosses while was weak in the fourth cross (L1 × T5). The line L1 exhibited seven bands (No. 1, 3, 4, 5, 6, 7, and 8), four of them showed very strong intensity and the remaining were intermediate and weak. All crosses exhibited bands No. 3, 4, 5, and 6 like their parents with different intensities, except the fourth cross L1 × T5. The bands No. 5 and 6 were completely absent in this cross (L1 × T5) while were completely presented strongly in its parents. Moreover, This cross exhibited the lowest number of isozyme esterase bands (4 bands). The esterase isozyme band No. 9 was detected with intermediate to weak intensity in all genotypes except L1, T5, and L1 × T3 was absent.

The esterase isozyme patterns and their intensity for CMS line L3, four restorers, and their F$_1$ crosses are shown in Fig. 3B and Table 8. The findings indicated that only one isozyme band (No. 8) was monomorphic and presented in all parents and crosses, while the others were polymorphic. The highest number of bands was exhibited by restorer T2 with nine bands, while five were detected for CMS line L3, seven for T1, six for T3, and eight for T5 with different activities. The cross L3 × T1 as an example exhibited additional two weak bands (No. 1 and 2) which were completely absent in its parents, while one band (No. 3) was absent in the cross compared to its parents.

3.9.2. Peroxidase isozymes polymorphism

Peroxidase isozyme patterns of CMS line L1, four restorers, and their F$_1$ hybrid combinations were shown in Fig. 4A and Table 9.

Table 8
Description of esterase patterns of two CMS lines, four restorers, and their F$_1$ hybrids.

Band No.	L1	L1 × T1	T1	L1 × T2	T2	L1 × T3	T3	L1 × T5	T5
1	+++	–	–	–	+	–	–	–	+
2	–	–	–	–	+	–	–	–	+
3	+++++	++++	++	++++	++	++++	+	++	++++
4	+++++	++++	+++	++++	++	++++	++	++	+++++
5	+++++	++++	+++	++++	+++	+++++	++	–	+++++
6	+++++	++++	++	++++	+++	+++++	–	–	+++++
7	+	++	++++	+	++++	+	+	+	+++++
8	+++	++	+++	++	+++	–	+++	–	+++
9	–	+++	++++	++	+++	–	+++	++	–
Total	7	7	7	7	9	5	6	4	8
	L3	L3 × T1	T1	L3 × T2	T2	L3 × T3	T3	L3 × T5	T5
1	–	+	–	–	+	+	–	–	+
2	–	+	–	–	+	–	–	–	+
3	+++	–	++	–	++	++++	+	–	++++
4	+++++	++	+++	++	++	+++++	++	–	+++++
5	+++++	++	+++	++	+++	+++++	++	–	+++++
6	+++	++	++	++	+++	++++	–	–	+++++
7	–	+++	++++	+++	++++	+	+	–	+++++
8	+++	+++	+++	+++	+++	+++	+++	+++	+++
9	–	++++	++++	–	+++	–	+++	+++	–
Total	5	8	7	5	9	7	6	2	8

+++++: Very strong, ++++: strong, +++: intermediate, ++: weak, +: very weak, and –: absent.
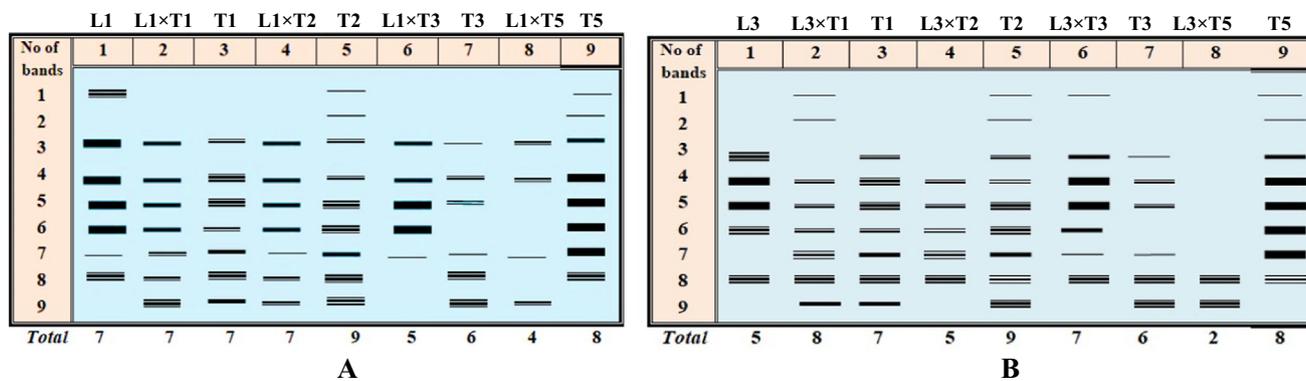
Fig. 3. The esterase isozyme electrophoretic patterns for the CMS line L1 (A) and L3 (B) and their four F$_1$ hybrids. The intensities of detected bands varied from very weak to very strong.
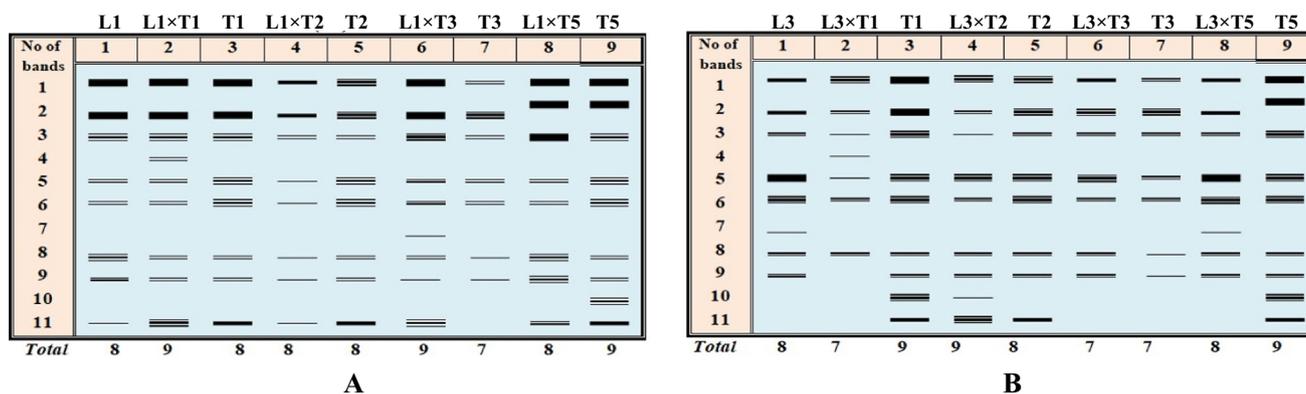
Fig. 4. The peroxidase isozyme electrophoretic patterns L1 (A) and L3 (B) with four restorers, and their four F$_1$ hybrids. The intensities of detected bands varied from very weak to very strong.

Table 9
Description of peroxidase patterns of two CMS lines, four restorers and their F$_1$ hybrids.

Band No.	L1	L1 × T1	T1	L1 × T2	T2	L1 × T3	T3	L1 × T5	T5
1	+++++	+++++	+++++	++++	+++	+++++	++	+++++	+++++
2	+++++	+++++	+++++	++++	+++	+++++	+++	+++++	+++++
3	+++	+++	+++	++	++	+++	++	+++++	+++
4	–	++	–	–	–	–	–	–	–
5	++	++	+++	+	+++	++	++	++	+++
6	++	++	+++	+	+++	++	++	++	+++
7	–	–	–	–	–	+	–	–	–
8	+++	++	++	+	++	++	+	+++	++
9	++	++	++	+	++	+	+	+++	++
10	–	–	–	–	–	–	–	–	+++
11	+	+++	++++	+	++++	+++	–	++	++++
Total	8	9	8	8	8	9	7	8	9
	L3	L3 × T1	T1	L3 × T2	T2	L3 × T3	T3	L3 × T5	T5
1	++++	+++	+++++	+++	+++	++++	++	++++	+++++
2	++++	++	+++++	++	+++	+++	+++	++++	+++++
3	++	+	+++	+	++	++	++	++	+++
4	–	+	–	–	–	–	–	–	–
5	+++++	+	+++	+++	+++	+++	++	+++++	+++
6	+++	++	+++	++	+++	++	++	+++	+++
7	+	–	–	–	–	–	–	+	–
8	++	++	++	++	++	++	+	++	++
9	++	–	++	++	++	++	+	++	++
10	–	–	+++	+	+	–	–	–	+++
11	–	–	++++	+++	–	–	–	–	++++
Total	8	7	9	9	8	7	7	8	9

+++++: Very strong, ++++: strong, +++: intermediate, ++: weak, +: very weak and -: absent.

The results revealed that the line L1 exhibited eight bands with different activities ranged from very weak (band No. 11) to very strong (bands No. 1 and 2). The restorers showed variations in the number and activity of detected bands. The restorer T1 and T2 had eight bands, T3 had seven and T5 had nine bands. All F_1 hybrids and their parents possessed common bands No. 1, 2, 3, 5, 6, 8, and 9, while with different activities. Only the cross L1 × T1 exhibited an additional peroxidase band (band No. 4) that was absent in its parent. The intensity of band No. 3 in hybrid L1 × T5 changed from intermediate to strong as well as band No. 9 from weak to intermediate compared to its parent. The line L1 and its crosses L1 × T2 and L1 × T5 showed eight bands, while L1 × T1 and L1 × T3 exhibited nine bands.

The peroxidase isozyme electrophoretic patterns of CMS line L3, four restorers, and their F_1 hybrids are presented in Fig. 4B and Table 9. All tested genotypes had common six monomorphic bands (No. 1, 2, 3, 5, 6, and 8) while the remaining bands were polymorphic. The line L3 exhibited eight bands with different intensities. The two restorers T1 and T5 exhibited nine bands as a maximum number of peroxidase isozymes while T3 had seven bands with different intensities. The cross L3 × T1 had an additional weak band (No. 4) which was absent in its parents, while band No. 9 was completely absent which was presented in the parents. The band No. 10 was absent in restorers T2 and T3 while presented in T5.

4. Discussions

Heterosis commercialization enhances rice production to cope with continuing global population growth and future food demand (Huang et al., 2017). CMS system is a highly efficient approach to facilitate hybrid seed production and attain high outcrossing potential (Abbas et al., 2021). In the current study, three CMS lines and ten restorers were crossed using lines × tester's model. The selected parents are genetically diverse and from different origins. Furthermore, the esterase and peroxidase isozyme activity was investigated to explore the genetic diversity at the protein level among the CMS lines, restorers, and their crosses (Singh et al., 2009a). All the applied isozymes displayed considerable polymorphism for the parents and the obtained crosses. The differences in the banding pattern and intensity provided accurate results on the reliable variability among the tested genotypes and their crosses. These findings verified genetic diversity among used parents and their cross combinations which could be used for developing promising hybrid rice. Likewise, El Shamey et al. (2016) and Kuwer et al. (2018) employed the isozymes to elucidate the differentiation in maintainers and restorers of CMS system in rice. The used parents and the obtained hybrids were evaluated for floral and yield traits. Five floral traits were assessed due to their influence on the efficiency of cross-pollination and hybrid seed production. Consequently, floral traits are vital to identify good parents that could be exploited in the program of hybrid seed production. The evaluated genotypes and their crosses displayed significant variation for duration of floret opening, stigma exsertion, stigma length, opening floret angle, and anther length. The detected significant differences indicated that the CMS lines behaved differently according to the crossed restorers. Furthermore, the wide range of variations revealed substantial genetic variability among the used parents and their crosses which could be employed in hybrid rice breeding. In this context, Anis et al. (2019), Hashim et al. (2021), Hasan et al. (2014), Maavimani and Saraswathi (2014) demonstrated considerable genetic variability for floral traits among evaluated genotypes.

Duration of floret opening is decisive for increasing the receptive of foreign pollen and consequently ameliorating the outcrossing rate of the CMS lines. Thereby, stigma should remain exerted out of floret and remain receptive for long period (El-Namaky, 2018). The longer duration of floret opening was assigned for the CMS lines L1, L2, and L3 as well as their crosses with T5 (L1 × T5, L2 × T5, and L3 × T5). Moreover, high stigma exsertion is required for effective interception of airborne pollen to ascertain high outcrossing rates during hybrid seed production (Hashim et al.). The line L3, as well as its crosses L3 × T5, L3 × T4, L3 × T9, L3 × T6, and L3 × T8, exhibited the highest values of stigma exsertion. Besides, The lines L3 and L2, as well as the hybrids L3 × T5, L3 × T6, L2 × T5, L3 × T10, and L3 × T8, had the highest values of stigma length. Furthermore, the wide-angle of opening floret improves exposing stigma to airborne pollen (Sato et al., 1994; Meena et al., 2021). The parental genotypes L1 and T5, as well as the hybrids L2 × T5, L1 × T1, L3 × T2, L3 × T8, and L1 × T3, possessed the highest desirable opening floret angle. Anther length is a valuable floral trait for pollen parents in hybrid rice seed production. The parents L2, L3, and T1, as well as the hybrids L1 × T1, L1 × T3, and L2 × T6 exhibited longer anther. Moreover, most of the abovementioned parents displayed significant GCA effects and the crosses demonstrated substantial and positive SCA and heterotic effects. The CMS line L2 and the restorer T5 were determined as good combiners for stigma exsertion, stigma length, opining floret angle, and duration of floret opening. Moreover, the hybrids L1 × T1, L1 × T3, L2 × T2, L2 × T5, L3 × T4, L3 × T5, and L3 × T9 had positive SCA effects for most floral traits. Therefore, these genotypes could be employed as parents in the hybrid rice breeding programs for improving floral traits.

The restorers T1, T2, and T9 as well as the hybrids L1 × T1, L1 × T2, L1 × T3, L1 × T6, L1 × T9, L2 × T1, L2 × T2 L2 × T3, L2 × T6, L3 × T1, L3 × T2, L3 × T3, L3 × T6, and L3 × T9 displayed the highest grain yield and its attributes. Furthermore, the CMS lines L1 and L3, as well as the restorers T1, T2, T3, T6, and T9, proved to be the greatest general combiners for grain yield and certain its attributes. The hybrids L1 × T1, L1 × T5, L1 × T7, L2 × T3, L2 × T4, L2 × T5, L2 × T10, L3 × T1, L3 × T2, and L3 × T6 displayed positive SCA effects for grain yield and one or more its attributes. Consequently, these genotypes could be considered promising for increasing grain yield and its attributes in rice breeding programs. Notably, the parents that showed high GCA effects for yield traits also possessed high mean performance of these traits. This displays a favored agreement between the mean performance and GCA effects. Thereby, the parental performance gave a good index for their general combining ability. Similar results were proved by Singh et al. (2020), Rasheed et al. (2021), Suvi et al. (2020), El-Mowafi et al. (2021), Rosamma and Vijayakumar (2005).

Both additive and non-additive gene effects are implicated in the governing inheritance of all assessed characteristics. This denotes that crossing can be employed to utilize both additive and non-additive gene action to increase grain yield in rice. Similarly, Zewdu (2020) and AnandaLekshmi et al. (2020) proved the importance of both gene actions with the predominance of non-additive gene effects for panicle length, productive tillers/plant, number of grains/panicle, and 1000-grain weight, test weight, and plant yield. Moreover, Anis et al. (2019) reported the significance of non-additive gene action in controlling the inheritance of most evaluated characteristics in their respective studies. This indicates that selection might be performed effectively in advanced generations for these traits. These findings agree with Bhutta et al. (2018), Gowayed et al. (2020), Sakran et al. (2020), Saleh et al. (2020).

The relationship among the studied traits elucidated that yield potentiality was principally attributed to panicle weight, panicle length, number of grains/panicle, and seed set %. Increasing these attributes is associated with high-yielding ability. These characteristics are easier in selection compared with grain yield, which is

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advantageous in breeding programs. These results are in consonance with the findings of Sheehy et al. (2001), Li et al. (2019), Oladosu et al. (2018), Zhao et al. (2020), and Gaballah et al. (2021).

5. Conclusion

Cytoplasmic male sterility (CMS) system is a beneficial approach for commercial exploitation of heterosis and producing high-yielding hybrid rice. The evaluated parental genotypes and their hybrid combinations displayed significant variation for floral and yield traits indicating substantial genetic variation that could be employed in hybrid rice breeding programs. The determined CMS lines and restorers as good combiners as well as, the hybrids with positive SCA effects forfloral and yield traits could be exploited in the hybrid rice breeding programs. A positive relationship was detected between grain yield and panicle length, panicle weight, number of grains/panicle, and seed set percentage. Consequently, these traits could be considered in the selection for improving rice grain yield due to their ease of measuring compared with grain yield.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' contributions

E.A.Z.E, R.M.S., M.A.A.E., M.E.S., S.A., B.A., M.A, designed the experiment; E.A.Z.E, S.A., B.A., M.A, R.M.S., M.A.A.E., M.E.S. performed the experiment; M.O.G., E.M., D.A.E.,S.A., B.A., M.A, analyzed the data; E.A.Z.E, M.O.G., E.M., D.A.E., S.A., B.A., M.A., write the original draft. All authors approved the final manuscript.

Availability of data and materials

Declaration of Competing Interest: The authors declare no conflict of interest

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