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The Genetic Variability of Floral and Agronomic Characteristics of Newly-Bred Cytoplasmic Male Sterile Rice

Raafat El-Namaky [1,2]

[1] AfricaRice, Senegal Regional Center, B.P. 96 Saint Louis, Senegal; relnamaky@gmail.com; Tel.: +20-11-1257-6417

[2] Rice Research & Training Center (RRTC), 33717 Sakha Kafr Sheikh, Egypt

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Abstract: Male sterility enabled commercialization of heterosis in rice but low seed set remains a constraint on hybrid dissemination. We evaluated 216 F_6 maintainer lines for agronomic and floral characteristics in augmented design and selected 15 maintainer lines, which were testcrossed with IR58025A. Five backcrosses were conducted to transfer cytoplasmic male sterility (CMS) to select maintainer lines. Newly-bred $BC_{5:6}$ CMS lines were evaluated for outcrossing rates and agronomic characteristics. There were highly significant differences among 216 F_6 maintainer lines for characteristics whose genotypic variance was higher than environmental variance. The phenotypic coefficient of variation was almost the same as the genotypic coefficient of variation, indicating that most phenotypic variation was due to genetics. There were highly significant differences among CMS lines for number of days to 50% flowering and maturity; stigma exertion; panicle exertion, length and weight; spikelet fertility; tillers per plant; plant height; grains per panicle; grain yield per plant; and 1000-grain weight, but not for pollen and panicle sterility during dry and wet seasons. Three CMS lines (CMS3, CMS12, and CMS14), exhibited high outcrossing rates (56.17%, 51.42% and 48.44%, respectively), which had a highly significant, positive correlation with stigma exertion (0.97), spikelet opening angle (0.82), and panicle exertion (0.95).

Keywords: *Oryza sativa*; CMS lines; genetic variability; hybrid seed; maintainer lines; outcrossing rate; rice

1. Introduction

Rice (*Oryza sativa*) is a staple food in West Africa, where its demand keeps increasing due to population growth. Hence, there is an urgent need to identify high-yield rice cultivars that fulfill this demand locally [1]. Hybrid rice can contribute to food security by increasing the availability of staple food for consumption by farm households and increasing on-farm income through higher grain yield [2]. Success in breeding and growing hybrid rice commercially in China has caused great interest among rice breeders throughout the world. However, the natural outcrossing rate in rice cultivars is extremely low and hybrid seed production, using male sterility or gametocides, presents some difficulties. Increasing the potential outcrossing rate would facilitate the use of hybrids in other areas of the world.

The availability of both stable cytoplasmic male sterility (CMS) and fertile restoration system is very important for commercial exploitation of heterosis in any crop. The advantage of cytoplasmic male sterility (CMS) is that the pollen in the male sterile line is aborted, while the female organ is normal. This allows stigma cross-pollination from other plants to generate heterozygous seeds [3]. Stigma exertion, panicle exertion, and angle of opened lemma and palea of male sterile lines are the key determinants of outcrossing in hybrid rice seed production [4,5]. The exerted stigma can bypass the physical barrier of glumes between the pollen of restorer lines and the stigma of CMS lines in rice. Exerted stigma of rice seems to improve the outcrossing rate of the sterile lines [6,7]. Agronomic traits should also be taken into account for achieving heterosis for grain yield. Hence, developing CMS lines with good floral and agronomic traits is necessary for hybrid rice breeding.

The floret of *O. sativa* is a terminal panicle of perfect flowers. Each spikelet has a branched stigma, six stamens, and two lodicules. At blooming, the flowers open rapidly. The flowers remain open for 1–3 h and close after anthesis, but never reopen. Some floral characteristics, such as stigma size, stigma exertion, and flower opening duration, affect the rate of outcrossing in rice. Usually the stigma remains inside the glumes during and after anthesis. In some cultivars, some stigmas remain outside the glumes after anthesis and fertilization. The rate of exerted stigma, which ranges from 0 to 90%, is correlated with stigma length. Stigma length ranges between 0.4 and 1.6 mm [8–12]. The duration of receptivity is variable and may exceed five days.

Achieving improved floral and agronomic traits in the maintainer line is the first step in developing new CMS lines with desirable traits through the backcross breeding method. Evaluation of newly developed CMS lines of rice for their morphological and floral traits to determine variation among genotypes is essential so that phenotypically excellent lines can be exploited for the development of rice hybrids to identify genotypes with desirable morphological and floral characteristics [5]. The genetics of stigma exertion is quantitative and influenced by dominance effects, followed by additive effects [13]. The genetic advance for stigma length and spikelet length appears to be greater than that of other floral characteristics, thus stigma exertion could be improved by selection in early generations.

In 2010, AfricaRice began breeding hybrid rice using both cytoplasmic male sterile and environmental genetic male sterile systems [14,15]. In this study, 216 maintainer lines were evaluated for their floral and agronomic characteristics. Five successful backcrosses with 15 selected maintainer lines led to developing 15 new CMS lines. The 15 new CMS were evaluated for outcrossing rates and agronomic characteristics along with popular CMS lines as checks. The main objective of this research was to study the genetic variability of some floral and agronomic traits of newly bred CMS lines in rice.

2. Materials and Methods

This research was carried out at AfricaRice Regional Center, Saint Louis, Senegal from 2013 to 2017, as described briefly in Figure 1. The study included 216 maintainer lines (F_6) derived from four breeding populations, namely V20B/IR72793B (46 lines), IR80561B/IR72793B (54 lines), IR93561B/IR58025B (65), and IR93561B/IR68897B (51 lines), which were evaluated for agronomic and floral characteristics using an augmented design with 12 blocks. Each block contains 18 lines and two popular maintainers IR68897B (early maturity) and IR58028B (late to medium maturity) as checks. Each line was transplanted in four rows 3 m long and with a spacing of 20 × 20 cm. The experiment used a recommended package of crop husbandry practices during plant growth. Data were recorded on five random plants within each plot for number of days to 50% flowering (DAF), number of days to maturity (DAM), stigma exertion (SE, %), panicle length (PnL), panicle weight (PnW), spikelet fertility (SF, %), panicle exertion (PnE, %), tillers per plant (NPT), plant height (PH) grains per panicle (NGP), grain yield per plant (GYP), and 1000-grain weight (GW).

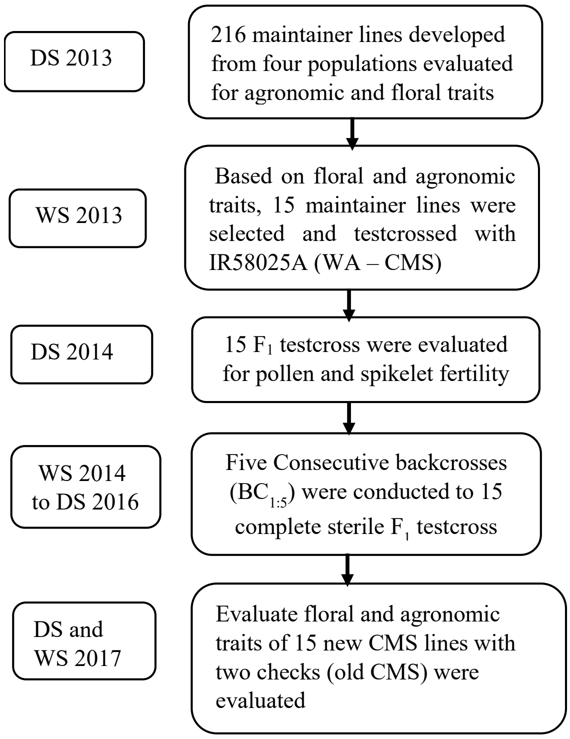

Figure 1. Scheme of evaluation of maintainer lines and developing new cytoplasmic male sterility (CMS) lines in dry (DS) and wet (WS) seasons at Saint Louis, Senegal.

2.1. Backcrossing and Derived Offspring Assessment

Out of 216 maintainer lines evaluated for floral and agronomic traits, 15 lines were selected and testcrossed with IR58025A (a popular WA CMS used for commercial production in many countries). Five consecutive backcrosses ($BC_{1:5}$) were conducted to complete the transfer of cytoplasmic male sterile (CMS) to selected maintainer lines. The backcross offspring and its parent were transplanted side by side on two or three rows based on the seeds available. Pollen fertility (POF), DAF, and phenotypic acceptability (PAC) were evaluated for all offspring and their parents. The new CMS lines derived from $BC_{5:6}$ were evaluated for agronomic and floral characteristics.

2.2. Outcrossing Experiment

The 15 newly-bred CMS lines derived from $BC_{5:6}$, plus two old CMS lines and their respective maintainers, were evaluated for outcrossing rates and agronomic characteristics during the dry and wet seasons in 2017. The maintainer of each line was sown four days after the male sterile lines to optimize the synchronization of flowering. The CMS and their maintainer were evaluated in a randomized complete block design with three replications. The CMS lines were transplanted in six rows, while their respective maintainer used two rows of 3 m long; i.e., 6:2 row ratio. Flag leaf cutting was done for all CMS before heading to facilitate outcrossing. Correlation coefficients between floral characteristics and outcrossing rates were estimated using SPSS version 16 for Windows [16].

Data were recorded on five plants per plot for DAF, DAM, SE, PnE (%), PnL, PnW, SF, NPT, PH, NGP, GYP, and GW. About 15 spikelets from the freshly emerged panicles of at least 10 plants were collected and examined under a microscope with 1% iodine potassium iodide (IKI) solution for pollen sterility assessment [17]. For this purpose, panicles emerging from the sheath were bagged with paper bags prior to anthesis to prevent cross-pollination. Data recording was done according to the standard evaluation system for rice [18]. For glume angle, at the time of flowering, three well-opened florets of the primary panicle were collected. The angle of the opened floret was measured using a protractor. The stigma exertion was calculated by the ratio of spikelets with exerted stigma to the total number of spikelets and expressed as a percentage. Bagged panicles were also harvested to assess spikelet percentage. Digital cameras were used to take photos every 4 min to estimate the flowering time of each CMS lines.

Estimation of phenotypic and genotypic variances:

The phenotypic and genotypic coefficients of variation were computed as per the methods suggested by [19,20]:

$$\text{Genotypic variance } ({\sigma^2}_g) = (Ms_g - Ms_e)/r$$

$$\text{Environmental variance } ({\sigma^2}_e) = Ms_e$$

where Ms_g and Ms_e are the mean sum of squares for the genotypes and error in the analysis of variance, respectively, and r is the number of replications.

The phenotypic variance was estimated as the sum of the genotypic and environmental variances:

$$\text{Phenotypic variance } ({\sigma^2}_{ph}) = {\sigma^2}_g + {\sigma^2}_e$$

Estimation of genotypic and phenotypic coefficient of variability:

The genotypic and phenotypic coefficients of variability were calculated according to the following formulae:

$$\text{Genotypic Coefficient of Variation (GCV)} = (\sigma_g\ /\text{grand mean}) \times 100$$

$$\text{Genotypic Coefficient of Variation (PCV)} = (\sigma_{ph}\ /\text{grand mean}) \times 100$$

where σ_g and σ_{ph} are the genotypic and phenotypic standard deviations, respectively.

Heritability and genetic advance:

Heritability in a broad sense (${h^2}_b$) for all characters was computed as per the following formula:

$${h^2}_b = {\sigma^2}_g\ /{\sigma^2}_{ph} \times 100.$$

The genetic advance for selection intensity (k) at 5% was estimated by the following formula [21,22]:

$$EGA = {h^2}_b\ /100 \times \sigma_{ph} \times k$$

where

EGA = the expected genetic advance under selection;
σ_{ph} = the phenotypic standard deviation;
${h^2}_b$ = heritability in a broad sense;
k = selection intensity.

$$\text{Genetic advance per population mean} = (EGA/\text{grand mean}) \times 100$$

3. Results

3.1. Maintainer Lines

There were highly significant differences among the 216 F_6 maintainer lines for all characteristics except angle of floret opening (°) and number of tillers per plant (Table 1), thereby indicating that these lines differed significantly for these characteristics.

Table 1. Analysis of variance of maintainer lines for agronomic and floral traits.

Agronomic Characteristics								
Source of Variation	**Degrees of Freedom**	**DAF**	**DAM**	**NPT**	**PH**	**GW**	**GYP**	
Block	11	63.05	1898.9	8.05	255.8	0.316	277	
Lines	217	34.35 **	46.5 **	2.33 NS	40.73 **	0.11 **	59.16 **	
Error	11	0.678	1.041	1.5	6.06	0.01	12.56	
Panicle and Floral Characteristics								
Source of Variation	**Degrees of Freedom**	**SE**	**SOA**	**PnE**	**PnL**	**PnW**	**NS/Pn**	**SF%**
Block	11	465.1	15.62	20.5	8.58	0.758	1045.7	6.72
Lines	217	278.6 **	12.18 NS	29.19 **	2.47 **	0.323 *	269.4 **	25.21 *
Error	11	10.1	6.74	0.5	0.4	0.12	26.79	10.21

* and ** indicate significance at $p \leq 0.05$ and 0.01, respectively. DAF: number of days to 50% flowering, DAM: number of days to maturity, NPT: number of tillers per plant, PH: plant height, GW: 1000-grain weight, GYP: grain yield per plant. SE: stigma exertion (%), SOA: angle of floret opening (°), PnE: panicle exertion (%), PnL: panicle length, PnW: panicle weight, SF: spikelet fertility (%); NS/Pn: number of spikelet per panicle.

The significant variation for most characteristics revealed the availability of maintainer lines with good agronomic and floral features (Table 2). Days to 50% flowering ranged between 69 and 108 (average: 97 days), while with the two checks (IR68897B and IR58025B) it was 86 and 104 days, respectively. The days to maturity of the 216 maintainer lines ranged between 83 and 140 (average: 126 days), while it was 118 and 134 for IR68897B and IR58025B, respectively. This large variation of days to 50% flowering and maturity in the maintainer lines calls for breeding new CMS lines with early, medium, and late maturity. Large variation was also observed for number of tillers per plant (average: 15) and plant height (86 cm). On average, 1000-grain weight and grain yield per plant were 25 g and 32 g, respectively. A promising maintainer line had 76% stigma exertion whereas it was 25% and 30% for the two checks. The spikelet opening angle ranged between 15.30° and 37.17°. Both checks and maintainer lines exhibited good panicle exertion, ranging between 78% and 100%. The average panicle length and panicle weight were 26 cm and 23 g, respectively. The highest spikelet per panicle was 198 and the lowest was 92. Spikelet fertility of both maintainer lines and checks was above 73%.

Table 2. Range and average for agronomic and floral characteristics of 216 maintainer lines and two checks.

Characteristics	**Range**	**Average**	**IR68897B**	**IR58025B**	**$LSD_{0.05}$**	**CV †**
Days to 50% flowering	69.46–108.46	96.95	85.67	104.25	1.89	0.01
Days to maturity	83.04–140.54	126.30	117.58	133.50	2.34	0.01
Tillers per plant (#)	10.87–20.57	15.44	17.00	18.33	2.80	0.08
Plant height (cm)	56.92–104.37	86.44	87.25	91.09	5.65	0.03
1000-grain weight (g)	18.8–33.7	25.3	23.70	23.00	2.20	0.40
Grain yield per plant (g)	10.30–63.11	31.49	25.56	30.60	8.10	0.11
Stigma exertion (%)	1.13–76.16	30.14	24.86	29.84	7.27	0.11
Spikelet opening angle (°)	15.30–36.17	25.64	24.84	26.67	5.95	0.10
Panicle exertion (%)	78.0–100.0	96.84	100.00	100.00	0.00	0.00
Panicle length (cm)	20.46–29.76	26.06	24.45	25.64	1.47	0.02
Panicle weight (g)	1.79–5.88	3.25	3.23	3.24	0.79	0.11
Spikelets per panicle	91.82–197.67	126.10	115.92	125.30	12.47	0.04
Spikelet fertility (%)	73.76–97.74	84.78	87.54	88.09	7.28	0.04

† Coefficient of variation (%).

Variance and Heritability

The phenotypic variance was partitioned into genotypic and environmental variances for a clear understanding of the pattern of variation (Table 3). Number of days to maturity exhibited higher environmental variance than genetic variance, while the phenotypic variance of spikelet fertility was affected by both environmental and genotypic variances. Overall, the genotypic variance was higher than environmental variance for most characteristics. The phenotypic coefficient of variation (PCV) was close to the genotypic coefficient of variation (GCV) for all characteristics. Stigma exertion and grain yield per plant exhibited the highest estimates of GCV and PCV, followed by panicle weight, spikelet per panicle, 1000-grain weight and spikelet opening angle. The lowest estimates of PCV were for number of days to maturity.

Table 3. Variance components and genetic parameters of agronomic and floral traits of 216 maintainers and two checks.

Characteristic	σ^2_G	σ^2_E	σ^2_P	GCV	PCV	H^2	GA
Days to 50% flowering	27.53	0.63	28.15	5.41	5.42	75.0	11.14
Days to maturity	6.61	35.65	42.26	2.04	2.45	69.0	3.48
Tillers per plant (#)	0.99	0.91	1.90	6.43	6.68	93.0	12.77
Plant height (cm)	30.32	11.45	41.77	6.37	6.47	97.0	12.92
1000 grain weight (g)	1.10	0.10	1.20	12.90	12.93	99.0	26.50
Grain yield per plant (g)	49.58	11.60	61.18	22.36	22.57	98.0	45.62
Stigma exertion (%)	295.52	5.99	301.51	57.03	57.08	97.0	117.39
Spikelet opening angle (°)	7.24	5.37	12.61	10.50	10.82	94.0	20.98
Panicle exertion (%)	26.72	0.12	26.84	5.24	5.34	98.0	11.00
Panicle length (cm)	2.07	0.35	2.41	5.52	5.56	99.0	11.29
Panicle weight (g)	0.23	0.11	0.34	14.75	15.04	96.0	29.81
Spikelets per panicle (#)	265.18	28.90	294.08	12.91	12.97	99.0	26.48
Spikelet fertility (%)	12.57	11.37	23.95	4.18	4.34	93.0	8.31

σ^2_G: genotypic variance, σ^2_E: environmental variance, σ^2_P: phenotypic variance, GCV: genotypic coefficient of variation (%), PCV: phenotypic coefficient of variation, H^2: broad-sense heritability (%), GA: genetic advance.

Broad-sense heritability estimates were relatively high for all characteristics, thereby suggesting a great genetic advance associated with breeding for agronomic and floral characteristics such as stigma exertion, grain yield per plant, panicle weight, spikelet per panicle, 1000-grain weight and spikelet opening angle. High heritability, associated with high genetic advance, was also noted for duration of floret opening, thus indicating that this characteristic was simply inherited, involving few major genes and likely having additive gene effects.

3.2. Newly-Bred CMS Lines

There were highly significant differences among the 17 CMS lines for most characteristics except pollen sterility and panicle sterility during both dry and wet seasons (Table 4). The non-significant differences observed for pollen and spikelet sterility were due to the fact that CMS lines were almost sterile. Only days to 50% flowering and plant height showed significant differences between the dry and wet seasons. Non-significant interactions were noted among all characteristics except pollen and spikelet fertility.

Data in Table 5 shows the agronomic and floral traits of newly developed CMS lines with two checks. A wide variation range was for noticed for number of days to 50% flowering; being CMS 13 the earliest (79 days) and IR58028A the latest (98 days). The highest number of tillers per plant was observed for CMS 11 and CMS 12 (18 tillers). Most CMS lines were semi-dwarf for plant height, which ranged between 69 and 91 cm. The semi-dwarf height is a very desirable characteristic in hybrid rice seed production because it facilitates outcrossing to the CMS. Pollen and spikelet sterility were almost 100% in the 17 CMS lines, thus indicating the stability of this male sterility type. Most of the newly-bred CMS (particularly CMS 3, CMS 12 and CMS 14) had better stigma exertion than the two checks. The spikelet opening angle ranged between 23.33° and 39.83°, while it was 28.67° and 29.67° for the two checks. CMS 2 and CMS 5 were the earliest for spikelet opening time (9:32 a.m.), while CMS 13 was the latest (11:03 a.m.). Except for CMS 13 and CMS 15, the other newly-bred CMS lines exhibited better panicle exertion than the old CMS lines, CMS 3 recorded highest panicle exertion

(73%). Outcrossing rates ranged between 22.87% and 56.17%. CMS 3, CMS 12, and CMS 14 had the highest outcrossing rates: 56.17%, 51.42%, and 48.44%, respectively. We also observed three CMS lines (CMS 3, CMS12, and CMS 14) exhibiting the highest percentages for both stigma and panicle exertion. Broad-sense heritability was relatively high (>0.85) for most agronomic and floral characteristics.

Table 4. Analysis of variance for agronomic and floral traits in 17 newly-bred cytoplasmic male sterility (CMS) lines.

Source of Variation	DF †	DAF	NPT	PH	PS	SpS
Replication	2	1.24	1.92	3.42	0.0022	0.00005
Line	16	278.12 **	12.01 **	269.90 **	0.0004	0.00024
Season	1	10,360.60 **	0.09	23.83 *	0.0004	1.6×10^{-5}
Season × Line	16	17.79 **	1.30	4.33	0.0014 **	0.00182 **
Residual	66	4.12	1.82	4.62	0.0055	0.00050
Source of Variation	**DF**	**SE**	**SOA**	**SOT**	**PnE**	**OCR**
Replication	2	11.92	3.21	0.004	18.30	3.31
Line	16	1631.90 **	106.60 **	1.800 **	382.90 **	520.90 **
Season	1	4.96	12.50	0.004	5.99	5.52
Season × Line	16	16.91	5.83	0.009	17.12	5.19
Residual	66	25.93	8.80	0.240	15.81	6.84

* and ** indicate significance at $p \leq 0.05$ and 0.01, respectively. † DF: degrees of freedom, DAF: days to 50% heading, NPT: number of tiller per plant, PH: plant height, PS: pollen sterility, SpS: spikelet sterility, SE: stigma exertion, SOA: spikelet opening angle (°), SOT: spikelet opening time, PnE: panicle exertion, OCR: outcrossing rate.

Table 5. Agronomic and floral traits of 17 newly-bred CMS lines and two checks.

CMS Line	DAF	NPT	PH	PS%	SpS	SE	SOA	SOT	PnE	OCR
CMS 1	93.83	16.67	68.60	99.96	99.95	31.16	26.89	10.69	62.69	36.09
CMS 2	81.83	17.00	78.17	99.96	99.95	23.17	29.67	9.32	55.00	31.67
CMS 3	84.50	14.50	84.50	99.96	99.95	64.67	39.83	10.16	72.67	56.17
CMS 4	82.00	15.67	79.67	99.95	99.96	24.83	32.67	10.18	58.00	36.17
CMS 5	82.83	14.83	80.50	99.95	99.95	30.83	31.33	9.32	65.67	38.33
CMS 6	84.00	15.50	85.50	99.94	99.95	33.67	34.50	9.48	65.83	40.83
CMS 7	85.00	15.17	89.17	99.94	99.94	40.67	35.00	9.68	70.33	44.00
CMS 8	87.33	14.17	89.00	99.95	99.95	42.33	34.50	10.25	67.00	43.33
CMS 9	86.83	14.33	85.67	99.96	99.96	51.28	34.17	10.20	70.50	45.67
CMS 10	94.00	16.67	80.27	99.96	99.95	19.15	26.30	10.79	58.25	32.28
CMS 11	96.50	18.00	88.47	99.95	99.94	19.01	26.93	10.58	55.27	30.31
CMS12	88.67	18.00	74.13	99.95	99.95	58.98	31.50	10.43	69.10	51.42
CMS 13	78.50	13.83	78.37	99.94	99.95	19.69	25.47	11.03	50.87	26.87
CMS14	97.33	17.50	90.55	99.94	99.94	53.61	29.83	10.43	69.54	48.44
CMS 15	86.33	17.67	69.50	99.95	99.95	9.13	23.33	10.87	47.96	22.87
IR58025A	97.67	17.17	75.60	99.96	99.96	19.79	28.67	9.92	51.72	27.55
IR68897A	79.50	15.50	78.17	99.97	99.96	16.00	29.67	9.38	54.33	31.83
Standard Error	1.15	1.11	1,71	0.02	0.14	4.13	2.41	0.17	3.17	2.06
$LSD_{0.05}$	1.82	1.86	2.45	0.32	0.26	7.18	4.32	0.24	6.43	3.99

DAF: days to 50% heading, NPT: number of tillers per plant, PH: plant height, PS: pollen sterility (%), SpS: spikelet sterility (%), SE: stigma exertion (%), SOA: spikelet opening angle (°), SOT: spikelet opening time, PnE = panicle exertion (%), OCR = outcrossing rate (%).

Highly significant positive correlations were observed between DAF and NTP (r = 0.70), pollen and spikelet sterility (r = 0.62), Table 6. Stigma exertion and plant height (r = 0.42), and SOA with both SE (r = 0.75) and PnE (0.79). Likewise, outcrossing rates had a highly significant positive correlation with SE (0.97), SOA (r = 0.82), and PnE (r = 0.95). Moreover, there was a significantly positive correlation between PnE and PH (r = 0.54). We also observed a highly significant positive correlation between pollen and spikelet sterility (r = 0.62).

Table 6. Correlation coefficients between agronomic and floral characteristics in 17 CMS lines.

	NPT	PH	PS	SpS	SE	SOA	SOT	PnE	OCR
DAF	0.70 **	−0.026	−0.089	−0.058	0.22	−0.23	0.39	0.14	0.15
NPT		−0.33	−0.067	−0.033	−0.21	−0.52	0.2	−0.31	−0.24
PH			−0.29	−0.52	0.42 **	0.56	−0.22	0.54 *	0.48
PS				0.62 **	−0.16	0.031	−0.19	−0.16	−0.14
SpS					−0.21	−0.1	−0.16	−0.31	−0.26
SE						0.75 **	−0.051	0.92 **	0.97 **
SOA							−0.49	0.79 **	0.82 **
SOT								−0.21	−0.16
PnE									0.95 **

* and ** indicate significance at $p \leq 0.05$ and 0.01, respectively. DAF: days to 50% heading, NPT: number of tiller per plant, PH: plant height, PS: pollen sterility, SpS: spikelet sterility, SE: stigma exertion, SOA: spikelet opening angle, SOT: spikelet opening time, PnE: panicle exertion, OCR: outcrossing rate.

4. Discussion

Low seed set remains a major challenge in hybrid seed production for rice. Improvement of floral characteristics, especially stigma and panicle exertion, of CMS lines is very important to improving outcrossing rates and increasing seed set for producing hybrid seed. This requires us to develop maintainer lines with desired floral and agronomic characteristics and transfer CMS through backcrossing. We were able to breed new CMS lines with outcrossing rates above 50%. Out of 216 maintainer lines evaluated for floral and agronomic characteristics, 16 were selected for further use in breeding.

There were highly significant differences among the 216 F_6 maintainer lines for all characteristics except angle of floret opening (°) and number of tillers per plant, thus indicating that these lines differed significantly for most desired traits. Similar results were obtained by the authors of [23], who found highly significant differences for various traits among 11 genotypes. Significant variation was observed for all characteristics, which provides a source for further breeding of maintainer lines combining the desired agronomic and floral characteristics. The large variation in days to 50% flowering and maturity of maintainers and CMS is very important to optimize the synchronization of flowering with restorer lines of different duration in hybrid seed production. The ratio of GCV to PCV was high, thus indicating that most of the phenotypic expression of the agronomic and floral characteristics was due to genetics. This finding suggests the potential for genetic gains due to selection for these traits in such CMS lines of rice. The results also indicated significant genetic variability among the tested maintainer lines for most characteristics, which agreed with previous research [24,25]. Broad-sense heritability estimates were relatively high for all characteristics, thereby indicating that selection of superior genotypes based on phenotypic performance should be very effective. [26]. The high heritability estimates along with high genetic advance should allow for selecting the best offspring among CMS lines, which also agreed with previous results elsewhere [27,28]. Low heritability estimates along with low genetic advance reveal a non-additive type of gene action and significant genotype × environment interaction affecting the expression of characteristics [25,28,29].

The newly-bred CMS lines exhibited highly significant differences for most characteristics. Most of them exhibited desirable traits vis-à-vis the two checks. The high outcrossing rate associated with high stigma and panicle exertion indicated that selection for such floral characteristics along with spikelet angle opening could be efficient for improving outcrossing rates. These results agreed with previous research [12,30], in which natural outcrossing rates ranged from 14.8 to 51%, while 14 of our newly developed CMS lines had an outcrossing rate above 35%. Our results suggest that additive genes play a major effect on floral and agronomic characteristics, thus being selected in earlier generations was very effective, as noted previously by Tian et al. [29].

Highly significant positive correlations were observed between different traits with outcrossing rates, as also found elsewhere [31–33]. This finding suggests that indirect selection to increase outcrossing rates may be effective in our breeding population for hybrid rice. A high panicle exertion in CMS lines was essential to attain high outcrossing rates, as noted previously [34]. Plant height, panicles per plant, and panicle length should also be regarded as very important for enhancing outcrossing rates in rice [35].

5. Conclusions

Hybrid seed production is the key to success in the breeding and cultivation of hybrid rice. The adoption of hybrid rice is severely limited, primarily because of its higher seed cost due to low hybrid seed set. Understanding the genetic variability of floral and agronomic characteristics is very important for improving hybrid seed set in rice. Evaluation and selection of maintainer lines with good floral and agronomic characteristics was the first step in developing new CMS lines with high outcrossing rates. High genotypic variance and high heritability estimates along with high genetic advance suggest the potential for selecting the best offspring as CMS lines. In this study three newly-bred CMS lines (namely, CMS 3, CMS 12, and CMS 14) were the best and had the highest outcrossing rates. Their high panicle and stigma exertion (Figure 2) are very important floral characteristics to achieve a suitable outcrossing rate for hybrid seed production in rice, while their outstanding agronomic traits will be necessary to obtain both high seed set and grain yield.

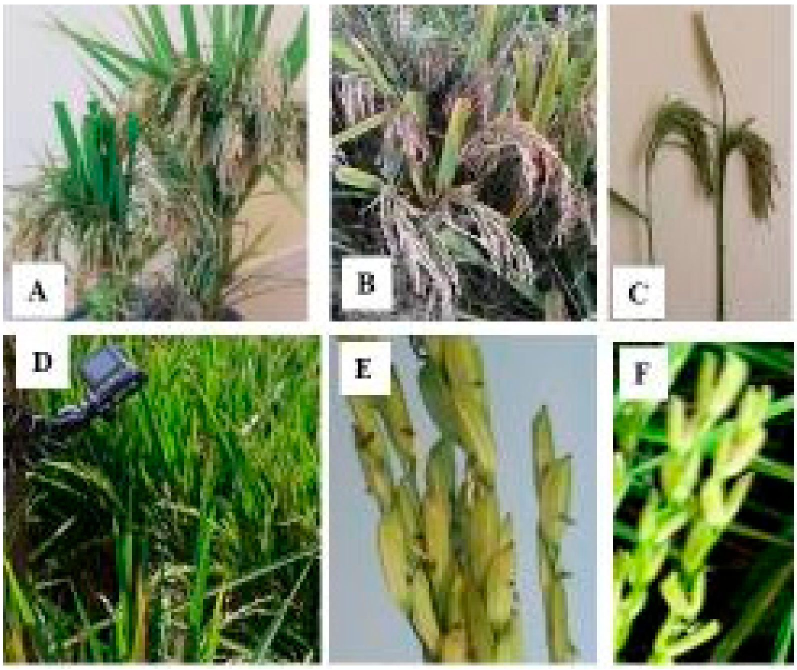

Figure 2. Improved floral and agronomic characteristics of newly-bred CMS lines. (**A**) Good agronomic traits of CMS line; (**B**) high outcrossing rate of improved CMS line as a result of desired floral characteristics; (**C**) good panicle exertion for CMS (left side) and maintainer lines (right side); (**D**) the use of a digital camera to record spikelet characteristics; (**E**) good stigma exertion for newly-bred CMS lines; (**F**) large glume opening of newly-bred CMS line.

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