IMPROVEMENT OF GENETIC PURITY FOR THE CYTOPLASMIC MALE STERILE LINE IR58025A WITH ITS MAINTAINER OF HYBRID RICE UNDER EGYPTIAN CONDITIONS

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Abstract

The present investigation aims at improving the genetic purity of Cytoplasmic Male Sterile line (CMS) IR58025A and its maintainer line IR58025B during three growing seasons of 2014, 2015 and 2016. Seeds of parental line were planted in 2014 season in isolated plots to obtain the parent seeds. This study was performed at the experiment farm of Rice Research Dept., Sakha, Kafr-Elsheikh, and Plant Pathology and Biotechnology Lap., Faculty of Agriculture, Kafr-Elsheikh univ. A total of 50 single crosses were made and harvested separately for the CMS line with its maintainer line to be evaluated during 2015 season. Some seeds of each of the crosses were used for identification, and the rest were kept to be grown with their maintainer seeds for multiplication. The same methods were repeated during, 2015 and 2016 seasons. Five replicates were used in randomized complete block design. Genetic markers were used to develop a reliable Polymerase Chain Reaction (PCR) assay to distinguish IR58025A and its maintainer. The primers used were; RMT6, cms and RM239. The maximum impurity value for IR58025A line was 4% after three paired-cross evaluations. The present investigation showed highly significant variance among the studied characters and the values of heritability in broad sense for IR58025A line were 89.08 and 97.84 for flag leaf area and stigma breadth, respectively. The recorded data for the primer RMT6 showed two DNA bands with molecular size of 200 and 210 bp for line IR 58025A and 200 bp for its maintainer. The cms primer showed only one DNA band with molecular size 400 bp for IR58025A line and it was absent in its maintainer. RM239 showed one DNA band with molecular size of 160 bp in IR58025A and it was absent in its maintainer, indicating that these primers can be used as marker aided selection (MAS) for pure parental line to develop new hybrid rice variety.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important food crops in Egypt, contributing over 20% of the cereal consumption. Rice provides 23% of global human per capita energy and 16% per capita protein. The rapidly increasing demand for rice and the continuous decrease in rice growing areas have resulted to search for improving rice production. In addition to producing improved varieties and new plant type, the use of hybrid rice may provide the target requirement of 15t/ha (Virmani *et al,* 1997) and (Yuan, 2010).

Hybrid rice is the commercial rice crop grown from F_1 seeds of a cross between two genetically dissimilar parents. Commercial exploitation of hybrid vigor is one of the most important applications of genetics in agriculture. It has not only contributed to food security, but has also benefited the environment (Xu, 2003). Hybrids have the potential of yielding 15–20% more than the best inbred variety grown under similar conditions.

The purification of the parental lines is very important to produce F_1 hybrid seeds with high genetic purity; this is because the decrease of 1% in the genetic purity reduces the yield about 100 kg ha^{-1}. Maximum yield potential was obtained from using 100% pure seed of the A line (Cytoplasmic male sterile line) and nucleus seed produced through paired – cross cycles of the respective parental lines every year (Ingale and Waghmod, 2005). The Indian Seed Acr prescribes that, for hybrid rice, the purity should be 98% (Verma, 1996), while in the People's Republic of China it is mandated that the purity of hybrid rice should be at least 96% Yan (2000). To ensure the required levels of purity in hybrid seed, the parental lines that are utilized in hybrid seed production should be 99% genetic purity.

Floral traits are of most importance in increasing the out crossing rate in hybrid rice seed production, such as stigma exsertion, duration of floret opening and angle of floret opening (Ingale *et al,* 2004) and (Abo-youssef *et al,* 2013). Among them, stigma exsertion is especially emphasized as a major component in increasing pollination and seed set (Yadav *et al*, 2008).

The present study had been undertaken to validate some known SSR markers for discriminating CMS lines with their isogenic counterparts, maintainer lines. All the markers used during the study are PCR-based and are of different nature.

MATERIALS AND METHODS

This study was carried out at the Experimental Farm of Rice Research Department, Sakha, Kafrelsheikh, Egypt, Plant Pathology and Biotechnology Lab, Faculty of Agriculture, Kafrelsheikh University during the three successive seasons; 2014, 2015 and 2016. The cytoplasmic male sterile line (IR58025A) WA type; (Virmani *et al*, 1996) was tested with its respective maintainer (B line) to determine their genetic purity and related traits.

In 2015 season, the 50 populations of the CMS line under isolated plots were sown in the nursery during the first week of May for identification, and after 21 and 24 days for multiplication to the CMS line and its maintainer, respectively. Five replicates were sown in randomized complete block design, each replication consisted of one row for the maintainer (1-50) and one row of $F_{1\ (1\text{-}50)}$ crosses between the

cytoplasmic male sterile line and maintainer line (A/B). Each row was 5m long and contained 25 individual plants. Seedlings were carefully pulled out from the nursery 30 days after sowing seeding and transferred to the permanent field. Seedlings were manually transplanted in one seedling /hill. The same method was applied in 2016 season.

All the observations on floral characters were taken during flowering peak and at the time of anthesis. Three plants were randomly marked in each row from each replicate and three spikelets from each plant were labeled for recording observations on the studied floral characters.

The measurements for each morphological, floral, yield and its component characters were conducted by using five replicates for all 50 populations of the CMS line with its maintainer. The data were recorded according to SES of IRRI (1998). These characters were; pollen test, spikelet pagged as a method to measure spikelet fertility test, days to heading (day), flag leaf area (cm^2), flag leaf angle(ô), plant height (cm), duration of floret opening (min.), angle of floret opening (ô), stigma exsertion %, stigma breadth (mm), panicles $plant^{-1}$, panicle exsertion %, seed set % and yield $plant^{-1}$(g). Fifty populations were produced from the line used in the present study. Data were subjected to analysis of variance for randomized complete block design as suggested by Panse and Sukhatme(1954).

Biochemical and molecular techniques

Total protein banding patterns were determined electrophoretically using SDS discontinuous gel as slabs (4% and 7.5% for stacking and separating gels, respectively) according to Stegemann *et al*. (1985).

DNA isolation and purification was carried out using CTAB (Cetyl-tetramethyl ammonium bromide) method, Murray and Thompson (1988)

Eight SSR primers were used to study the genetic purity for the parental lines.

primers	Sequences	
	Forward	**Reverse**
RMT6	GATGGTTTGGAAGGCTG	**GGGTTTAGAGTCGCCAC**
CMS	ACTTTTTGTTTTTGTGTAGG	**TGCCATATGTCGCTTAGATTTAC**
RM9	GGTGCCATTGTCGTCCTC	**ACGGCCCTCATCACCTTC**
RM5	TGCAACTTCTAGCTGCTCGA	**GCATCCGATCTTGATGGG**
RM284	ATCTCTGATACTCCATCC	**CCTGTACGTTGATCCGAAGC**
RM44	ACGGGCAATCCGAACAACC	**TCGGGAAAACCTACCCTACC**
RM178	TCGCGTGAAAGATAAGCGGCGC	**GATCACCGTTCCCTCCGCCTGC**
RM239	**TACAAAATGCTGGGTACCCC**	**ACATATGGGACCCACCTGTC**

RESULTS AND DISCUSSION

Results in Table 1 showed that the pollen and spikelet pagged tests are important for traits under identification in order to remove the undesirable population before heading for multiplication. From 50 hand crosses of IR58025A were planted in 2015 season in the identification and multiplication experiments, five populations exhibited partial fertility to pollen grains, as well as, seed set in spikelet pagged. The results indicated that the percentage of purity was 90%, but the genetic purity percentage was increased to 96% in 2016 season. There were only two populations showing partial fertility to pollen grains and seed set in pagged spikelet. Therefore, the recurrent selection for the CMS line with the hand crossing method is the best method to maintain and increase the genetic purity for IR58025A line.

Table 1. Pollen fertility test for the CMS line IR58025A/B in 2015 and 2016 seasons.

SEASON	POLLEN TEST		SPIKLETE PAGGED	
2015	**Population No.**	**Fertility %**	**Population No.**	**Fertility %**
	6	23	6	29
	17	18	17	28
	24	12	24	14
	40	16	40	18
	45	13	45	27
2016	15	20	15	19
	48	18	48	21

The selection was done for the populations showing complete sterility on the bases of pollen test and spikelet pagged. These results agree with those of Sawant *et al.* (2003) who mentioned that the CMS lines IR68886A, IR68897A, IR 68901A, IR 68885A, G 46A, IR58025A, IR69625A, and PMS 2A had complete pollen and spikelet sterility. Also, the results were in agreement with those of Abo-youssef *et al.* (2013).

Results in Table 2 showed that the general mean values of the IR58025A/B_{1-50} for days to heading trait were 107.22 and 106.46 days in 2015 and 2016 seasons, respectively. The average for days to heading ranged from 104.70 to 111.10 days in 2015 season and in 2016 season ranged from 103.56 to 110.6 days.

Moreover, the desirable recorded mean values for IR58025A were resulted from populations No. 2, 9 and 23 in 2015 season but in 2016 season were resulted from populations No. 7, 22 and 47. Thus, the produced populations showed duration of 106.84 days to heading. Similar results were described before by Gireesh *et al* (2009) who reported that generally A lines took more number of days to 50 percent flowering than that of the corresponding B lines.

Regarding to the mean performances for flag leaf area, the general mean value of the IR58025A/B_{1-50} was 43.66 and 42.59 cm^2 in 2015 and 2016 season, respectively. As well as, the average for flag leaf area ranged from 33.14 to 56.85 cm^2 in 2015 season and in 2016 season ranged from 32.77 to 56.14 cm^2. The desirable recorded mean values for IR58025A were resulted from populations No. 9, 23 and 42 in 2015 season and populations No.7, 14 and 47 in 2016 season. Meanwhile, the average of flag leaf area for the obtained populations reached to 43.13 cm^2.

The results showed a wide range of differences among the IR58025A/B_{1-50} line with its populations for flag leaf angle trait. The general mean values of the IR58025A/B_{1-50} for this trait were 45.67 and 47.44° in seasons 2015 and 2016, respectively. The data for flag leaf angle ranged from 36 to 62° in 2015 season and in 2016 season was ranged from 33.20 to 62°. Also, the desirable mean values were obtained from IR58025A populations No.2, 36 and 42 in 2015 season and in 2016 season from IR58025A populations No. 22, 47 and 50. Thus, the average of flag leaf angle for the produced populations was 46.56°. The results were in agreement with what was found before by Pandey *et al.* (2010).

For plant height, the general mean value of the IR 58025A/B_{1-50} was 87.54 and 85.58 cm in 2015 and in 2016, respectively. As well as, the data for plant height ranged from 81.23 to 95.81, in 2015 and in 2016 ranged from 92.80 to 79.00. The desirable recorded mean values for IR58025A were resulted from populations No. 9, 23 and 42 in 2015 and No.14, 47 and 50 in 2016. Therefore, the average of plant height for the produced populations in CMS line reached to (86.56 cm). These results were in full agreement with the results obtained by Ingale *et al*. (2004).

For duration of floret opening, the general mean values of the IR580A/B_{1-50} were 182.12 and 185.35 minute in 2015 and 2016 seasons, respectively. The average for duration of floret opening ranged from 176.60 to 194.80 min. in 2015 season and in 2016 season ranged from 177.60 to 198.80 min. Moreover, the desirable recorded mean values for IR58025A were attained by populations no. 2, 36 and 44 in 2015 season and in 2016 season were attained by populations no. 7, 22 and 47. Thus, the produced populations showed duration of to (183.74 min.) floret opening. These results were in agreement with those obtained by Patil (1983) who observed longer duration of flowering in KMS1 compared to other lines.

Table 2. Mean performance values of the CMS line IR58025A/B for some morphological, floral, yield and its component characters in 2015 and 2016 seasons.

Season	Entries	Population No.	Days to Heading (day)	Population No.	Flag leaf area (cm^2)	Population No.	Flag leaf angle (o)	Population No.	Plant height (cm)	Population No.	Duration of floret opening (min)	Population No.	angle of floret opening (o)
2015	IR58025A-H	49	111.1	50	56.85	43	62.00	6	95.81	42	194.80	9	26.00
	IR58025A-L	40	104.7	40	33.14	17	36.00	10	81.23	17	176.60	45	18.05
	IR58025A-S	2	107.51	9	44.34	2	48.87	9	87.77	2	188.12	23	21.81
	IR58025A-S	9	108.78	23	45.55	36	49.35	23	88.14	36	187.33	36	21.63
	IR58025A-S	23	108.11	42	45.91	42	48.11	42	88.62	44	186.87	42	22.00
	X- (1-50)	-	107.22	-	43.66	-	45.67	-	87.54	-	182.12	-	21.95
2016	IR58025A-H	33	110.6	38	56.14	47	62.00	15	92.80	50	198.80	47	27.60
	IR58025A-L	15	103.56	50	32.77	48	33.20	7	79.00	12	177.60	15	18.40
	IR58025A-S	7	107.11	7	43.55	22	48.95	14	88.55	7	188.34	9	22.00
	IR58025A-S	22	107.48	14	44.01	47	49.47	47	88.00	22	189.00	47	22.84
	IR58025A-S	47	108.02	47	44.80	50	49.83	50	87.29	47	189.71	34	23.00
	X- (1-50)	-	106.46	-	42.59	-	47.44	-	85.58	-	185.35	-	22.87
Season	**Entries**	**Population No.**	**Stigma exsertion %**	**Population No.**	**Stigma breadth (mm)**	**Population No.**	**Panicles $plant^{-1}$**	**Population No.**	**Panicle exsertion %**	**Population No.**	**Seed Set %**	**Population No.**	**Yield $plant^{-1}$ (g)**
2015	IR58025A-H	2	69.79	37	0.41	40	18.80	6	85.40	6	58.20	6	29.00
	IR58025A-L	17	61.20	45	0.39	11	9.40	13	70.00	19	20.60	26	13.40
	IR58025A-S	23	71.64	23	0.40	2	15.00	9	75.00	23	44.00	23	21.30
	IR58025A-S	42	72.39	9	0.41	23	15.82	23	76.20	36	45.63	36	22.00
	IR58025A-S	45	73.00	44	0.41	36	16.32	42	76.41	9	46.00	42	21.50
	X- (1-50)	-	65.37	-	0.40	-	13.45	-	77.85	-	38.81	-	21.42
2016	IR58025A-H	22	72.02	29	0.42	10	19.40	15	90.20	15	56.62	15	28.60
	IR58025A-L	48	63.06	48	0.39	19	10.56	17	69.80	3	23.70	9	14.20
	IR58025A-S	34	73.55	7	0.41	22	16.00	34	78.74	7	45.12	14	21.40
	IR58025A-S	47	74.00	22	0.41	47	16.70	22	79.00	22	46.60	47	22.00
	IR58025A-S	50	75.36	38	0.41	50	17.00	50	79.22	50	47.45	22	22.50
	X- (1-50)	-	67.26	-	0.40	-	14.51	-	78.27	-	35.53	-	22.00

H : The highest value ***L : The lowest value*** ***S : selected value***s

For angle of floret opening, the general mean values of the IR58025A/B_{1-50} were 21.95° in 2015 season and 22.87° in 2016 season. The average of angle of floret opening ranged from 18.05 to 26.00° in 2015 season and in 2016 season ranged from 18.40 to 27.60°, and the desirable mean values were resulted from IR58025A populations No. 23, 36 and 42 in 2015 season and No. 9, 47 and 34 in 2016 season. Thus, the produced populations had angle of floret opening with an average of 22.41°. Similar results were described by Gireesh *et al* (2009) who showed that the genotype KCMS 11A and IR 68888A were promising for panicle exsertion, the CMS lines IR 70369A, CRMS 32A, KCMS 17A, KCMS 12A, KCMS 22A and KCMS 25A had long stigma. The style length of RTN 10A and CRMS 32A was high, while KCMS 25A and CRMS 32A exhibited greater angle between stigma lobes.

Regarding to stigma exsertion%, the general mean values of the IR58025A/B_{1-50} were 65.37 and 67.26 % in seasons 2015 and 2016, respectively. The average of stigma exsertion % ranged from 61.20 to 69.79 % in 2015 season and ranged from 63.06 to 72.02 % in 2016 season. The best mean values were recorded by IR58025A populations No. 23, 42 and 45 in 2015 season and in 2016 were recorded by IR58025A No. 34, 47 and 50. Thus, the average of stigma exsertion % for the produced populations was 66.32 %. These results were in agreement with those obtained by Huang (2006) who reported that M98A is a new and early-maturing medium indica CMS line, derived from the cross Nonghuayou 808 × (M98A × MR0208). Its main characteristics include desirable floral traits, stable sterility, stigma exsertion rate of 80% and outcrossed seed setting rate of above 60%.

When stigma breadth was taken into consideration, the general mean values of the IR58025A/B_{1-50} were 0.40 mm in both seasons of 2015 and 2016. The average for stigma breadth ranged from 0.39 to 0.41 in 2015 season and from 0.39 to 0.42 in 2016 season. The desirable mean values were resulted IR58025A populations No. 32, 9 and 44 in 2014 and from populations No. 7, 22 and 38 in 2016 season. Also, the average of stigma breadth for the produced populations reached 0.40 mm.

For panicles plant^{-1}, the general mean value of the IR 58025A/B_{1-50} was 13.45 in 2015, it was 14.51 in 2016. The obtained data for this trait ranged from 9.40 to 18.80 in 2015 and in 2016 ranged from 10.56 to 19.40. The desirable mean values for IR58025A were recorded by populations No. 2, 23 and 36 in 2015 and populations No. 22, 47 and 50 in 2016 season. The mean value for panicles plant^{-1} in the obtained populations reached 13.98 panicles.

Concerning panicle exsertion %, the general mean values of line IR58025A/B_{1-50} were 77.85 and 78.27 % in seasons 2015 and 2016, respectively. The average of panicle exsertion ranged from 70.00 to 85.40 % in 2015 season and from 69.80 to

90.20 % in 2016 season. The selectsd mean values were recorded by IR58025A populations No. 9, 23 and 42 in 2015 season and in 2016 season were recorded by IR58025A populations No. 34, 22 and 50. Thus, the average of panicle exsertion % for the produced populations was 78.06 %. Similar results were obtained by Pardhe *et al.*, (2011) who observed that the CMS line KJT 5A had high panicle exsertion.

For seed set %, the general mean values of line IR58025A/B_{1-50} were 38.81 and 35.53 % in 2015 and 2016 seasons, respectively. The average for seed set % ranged from 20.60 to 58.20 % in 2015 season and from 23.70 to 56.62 % in 2016 season. The desirable mean values were resulted from IR58025A populations No. 23, 36 and 9 in 2015 and from populations No. 7, 22 and 50 in 2016 season. Also, the average of seed set for the produced populations reached 37.17 %.

For the latest trait, the mean values of yield plant $^{-1}$ for line IR58025A/B_{1-50} were 21.42 and 22.00g in 2015 and 2016 seasons, respectively. Thus, the average for yield plant $^{-1}$ ranged from 13.40 to 21.30g in 2015 and in 2016 ranged from 14.20 to 21.40g. Moreover, the desirable recorded mean values were attained by IR58025A populations No. 23, 36 and 42 in 2015 and in 2016 were attained by IR58025A populations No. 14, 47 and 22. The mean value of yield plant $^{-1}$ trait for the selected populations was 21.71g. These results were in general agreement with those reported by Sawant *et al.,* (2003) who stated that, IA2 and IB2 with strong disease resistance, good flowering characteristics, high out-crossing rate, good yields and stable fertility status were selected.

It is very important mentioning that, the selection was done to the desirable values obtained in 2016 season for all above studied traits in the CMS line IR58025A. On the other hand, the results showed also that the produced seeds from all selected populations could be used as nucleus seeds for all traits.

Results in Table 3 showed that, the selected populations values of all traits for line IR58025B_{1-50} for were selected around of general mean. The desirable recorded mean values of all traits for IR58025A were attained by populations no. 16, 17, 23, 32, 40, 43 and by population 46 in 2015 season. Also, the desirable mean values of all traits were obtained from IR58025B populations no.1, 4, 12, 27, 30 and from population 42 in 2016 season.

The general mean values of days to heading for IR58025 B_{1-50} were 105.31 and 105.64 in 2015 and 2016, respectively. The general mean values of flag leaf area for line IR58025A/B_{1-50} were 40.08 and 38.89 in 2015 and 2016, respectively. The general mean values of flag leaf angle for line IR58025B_{1-50} were 36.33 and 36.86 in 2015 and 2016 respectively. The general mean values of line IR58025 B_{1-50} for plant height were 100.84 and 102.38 in 2015 and 2016, respectively.

Table 3. Mean performance values of the maintainer line IR58025B for some morphological, floral, yield and its component characters during 2015 and 2016 seasons.

Season	Entries	Population No.	Days to heading	Population No.	Flag leaf Area	Population No.	Flag leaf angle	Population No.	Plant height	Population No.	Duration of floret opening	Population No.	angle of floret opening
2015	IR58025 B-H	16	107.60	5	47.08	49	45.00	12	107.20	17	67.00	33	21.00
	IR58025 B-L	4	102.80	46	33.67	1	29.00	29	95.80	5	52.20	20	14.60
	IR58025 B-S	23	105.40	32	40.46	16	37.20	23	102.00	16	63.00	17	19.00
	IR58025 B-S	32	106.00	16	42.00	32	37.00	40	103.20	32	64.20	23	19.50
	IR58025 B-S	40	106.35	40	43.12	46	38.00	43	104.00	46	64.82	40	20.00
	$\bar{X}$ (1-50)	-	105.31	-	40.08	-	36.33	-	100.84	-	60.62	-	18.19
2016	IR58025 B-H	31	107.80	18	46.81	18	45.00	50	108.20	22	69.20	11	22.80
	IR58025 B-L	32	103.00	10	30.26	19	30.00	34	97.30	41	54.60	37	15.60
	IR58025 B-S	4	106.80	1	39.20	4	36.80	1	104.00	4	65.00	1	20.00
	IR58025 B-S	12	106.30	30	39.84	27	37.00	27	105.00	30	66.00	27	20.25
	IR58025 B-S	30	107.00	42	40.00	30	37.50	42	105.57	42	65.80	30	21.00
	$\bar{X}$ (1-50)	-	105.64	-	38.89	-	36.86	-	102.38	-	62.96	-	19.54
Season	**Entries**	**Population No.**	**Stigma exsertion %**	**Population No.**	**Stigma breadth**	**Population No.**	**Panicles plant^{-1}**	**Population No.**	**Panicle length**	**Population No.**	**Seed Set %**	**Population No.**	**yield plant^{-1}**
2015	IR58025 B-H	14	64.21	16	0.34	20	20.00	40	26.40	33	97.42	21	48.60
	IR58025 B-L	5	55.12	10	0.31	18	10.00	9	21.00	47	80.32	9	27.60
	IR58025 B-S	16	59.70	23	0.33	16	14.00	23	24.56	16	95.00	16	44.20
	IR58025 B-S	40	61.00	32	0.33	40	13.80	32	25.00	32	93.70	23	45.00
	IR58025 B-S	46	63.43	40	0.33	46	15.20	46	24.90	40	94.84	46	42.30
	$\bar{X}$ (1-50)	-	59.25	-	0.32	-	13.66	-	24.05	-	91.62	-	41.78
2016	IR58025 B-H	43	65.61	4	0.34	39	21.00	27	26.80	12	98.02	39	49.00
	IR58025 B-L	36	55.71	9	0.32	17	10.80	11	20.75	3	81.88	16	30.24
	IR58025 B-S	1	61.44	12	0.33	1	15.50	4	25.40	1	94.93	1	44.60
	IR58025 B-S	27	63.00	27	0.33	27	16.22	30	25.00	27	95.47	4	47.20
	IR58025 B-S	30	63.27	42	0.33	30	15.00	42	26.08	42	96.00	42	46.80
	$\bar{X}$ (1-50)	-	60.33	-	0.33	-	14.67	-	24.92	-	93.03	-	42.66

H : The highest value ***L : The lowest value*** ***S : selected values***

The general mean values of duration of floret opening for line IR58025A/B_{1-50} were 60.62 and 62.96 in 2015 and 2016 seasons, respectively. The general mean values of angle of floret opening for line IR58025B_{1-50} were 18.19 and 19.54 in 2015 and 2016, respectively. The general mean values of stigma exsertion % for line IR58025B_{1-50} were 59.25 and 6.33 in 2015 and 2016, respectively. The general mean values of stigma breadth for IR58025B_{1-50} were 0.32 and 0.33 in 2015 and 2016 seasons, respectively. The general mean values of panicles plant^{-1} for line IR58025B_{1-50} were 13.66 and 14.67 in 2015 and 2016 seasons, respectively. The general mean values of panicle length for line IR58025B_{1-50} were 24.05 and 24.92 in 2015 and 2016 seasons, respectively. These results are in general agreement with those reported by Ingale *et al*, (2004). The general mean values of seed set % for line IR58025 B_{1-50} were 91.62 and 93.03 in 2015 and 2016, respectively. The general mean values of IR58025B_{1-50} for yield plant $^{-1}$ were 41.78 and 42.66 in 2015 and 2016 seasons, respectively.

The results revaled that, the selection was done for the desirable values during 2016 season in all the above studied traits. Also, these results showed that, the produced seeds from all selected populations could be used as nucleus seeds with desirable mean values for all traits.

Results in Table 4 showed that, the analysis of variance revealed highly significant differences among the IR58025A CMS line with its 50 populations for all the studied characters. This indicates that the average improvement was significant in all populations for all studied characters and it has not been affected by the environmental conditions. The results in 2015 season summarized the heritability in broad sense of IR58025A which was 90.48 for days to heading, 89.08 for flag leaf area, 90.09 for flag leaf angel, 92.23 for plant height, 91.12 for duration of floret opening, 91.24 for angle of floret opening, 90.73 for stigma exsertion %, 96.36 for stigma breadth, 91.80 for panicle plant^{-1}, 90.32 for panicle exsertion %, 92.42 for seed set % and 92.36 for yield plant^{-1}. In the same trend, while in 2016 season, the heritability in broad sense for line IR58025A recorded 92.08 for days to heading, 91.35 for flag leaf area, 92.93 for flag leaf angel, 93.66 for plant height, 92.60 for duration of floret opening, 92.05 for angle of floret opening, 94.23 for stigma exsertion %, 97.84 for stigma breadth, 93.43 for panicle plant^{-1}, 91.31 for panicle exsertion %, 93.27 for seed set % and 93.07 for yield plant^{-1} indicating that the environmental effects were very low and these traits were controlled by genetic variances and could be used for selection in early generations.

Overall mean values, error variances and coefficients of variability (CV) of the genotypes for morphological, floral and yield components are presented in Table 4.

Table 4. Mean square values of the CMS line IR58025A/B for some morphological, floral, yield and its component characters in 2015 and 2016 seasons.

S.O.V	d.f	Days to heading		Flag leaf area		Flag leaf angel		plant height		Duration of floret opining		Angle of floret opining	
		2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Reps.	4	0.706 ns	0.674 ns	25.521 ns	15.512 ns	31.456 ns	12.726 ns	2.190 ns	1.946 ns	25.93 ns	15.246 ns	0.546 ns	0.607 ns
Population	49	18.735**	15.733**	279.443**	198.250**	365.433**	265.090**	83.912**	81.694**	352.587**	290.421**	26.643**	29.259**
Error	196	0.949	0.937	8.740	3.239	8.701	7.466	1.733	1.501	8.438	7.956	2.013	1.806
Hbs	-	90.48	92.08	89.08	91.35	90.09	92.92	92.23	93.66	91.12	92.60	91.24	92.05
S	-	4.966	3.328	16.716	14.080	19.116	16.281	9.160	9.338	18.777	17.0417	5.161	5.208
CV	-	0.040	0.037	0.383	0.3305	0.418	0.3431	0.1075	0.1044	0.1031	0.09194	0.2352	0.2405
S.O.V	d.f	Stigma exseration %		Stigma breadth		Panicle plant^{-1}		Panicle exseration %		Seed set %		Yield plant^{-1}	
		2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Reps.	4	1.681 ns	0.669 ns	0.0009 ns	0.0006 ns	3.636 ns	1.190 ns	0.0009 ns	0.0005 ns	0.003 ns	0.0018 ns	13.414 ns	12.466 ns
Population	49	25.448**	24.060**	0.0003**	0.0003**	61.626**	37.259**	0.014**	0.009**	0.046**	0.032**	278.380**	248.781**
Error	196	0.919	0.803	0.0002	0.0001	1.868	1.431	0.0005	0.0004	0.0005	0.0005	9.616	6.0742
Hbs	-	90.73	94.23	96.36	97.84	91.80	93.43	90.32	91.31	92.42	93.27	92.36	93.07
S	-	5.045	4.905	0.018	0.019	7.850	6.104	0.117	0.099	0.215	0.178	16.684	15.779
CV	-	0.0772	0.0729	0.0449	0.0471	0.5820	0.4632	0.1509	0.1269	0.554	0.5012	0.6831	0.6191

For the statistical parameters, wide differences were detected among the genotypes in the most studied characters, the CV indicates the degree of precision with which the treatments are compared and is a good index of the reliability of the experiment. It expresses the experimental error as percentage of the mean; thus, the higher value of CV doesn't mean reliability of experiment, but the lower value of CV mean the reliability of the experiment. All studied traits showed lower CV values for the studied 50 populations. These results are in agreement with those obtained by Bansal *et al* (2000) who reported that the heterosis was significant for grain yield and its component characters in most hybrids.

Results in Table 5 showed that, the analysis of variance revealed highly significant differences among the IR58025B line and its 50 populations. The parental lines and the selected populations showed highly significant differences for some characters, indicating that, the average improvement was significant for all studied characters in all populations.

In 2015 season the results summarized the heritability in broad sense of IR58025B that recorded 92.51 for days to heading, 91.40 for flag leaf area, 86.58 for flag leaf angel, 90.66 for plant height, 92.25 for duration of floret opening, 86.97 for angle of floret opening, 92.05 for stigma exsertion %, 95.39 for stigma breadth, 92.12 for panicle plant^{-1}, 88.19 for panicle length, 91.46 for seed set and 91.97 for yield plant^{-1}.

The results obtained in 2016 season, showed that, heritability values in broad sense of IR58025B were: 93.23 for days to heading, 92.94 for flag leaf area, 88.76 for flag leaf angel, 92.23 for plant height, 94.39 for duration of floret opening, 89.76 for angle of floret opening, 93.91 for stigma exsertion %, 97.27 for stigma breadth, 93.73 for panicle plant $^{-1}$, 89.75 for panicle length, 92.11 for seed set and 93.44 for yield plant $^{-1}$. These characters gave the highest values, indicating very low environmental effect and these traits were controlled by additive and non-additive gene action and could be selected in early generations. The high recorded values of heritability in broad sense for these traits in both seasons indicated very low environmental effect which make them easier to be selected in early generations. These results are in agreement with the results obtained by Bansal *et al*., (2000) who studied the heterosis for yield and its component characters through 11 parents and their crosses. Data revealed that the heterosis was significant for grain yield and its component characters in most of the hybrids.

Table 5. Mean square values of the maintainer line, IR58025B for some morphological, floral, yield and its component characters during 2015 and 2016 seasons.

S.O.V	d.f	Days to heading		Flag leaf area		Flag leaf angel		plant height		Duration of floret opining		Angle of floret opining	
		2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Reps.	4	0.91 ^{ns}	0.506 ^{ns}	4.729 ^{ns}	3.844 ^{ns}	9.106 ^{ns}	8.254 ^{ns}	2.024 ^{ns}	1.140 ^{ns}	3.950 ^{ns}	2.034 ^{ns}	0.086 ^{ns}	0.030 ^{ns}
Population	49	17.904**	11.379**	80.601**	81.547**	122.562**	110.642**	44.155**	39.259**	83.504**	79.466**	10.86**	13.259**
Error	196	0.751	0.453	2.860	2.358	8.049	7.265	2.527	2.166	3.068	2.687	1.48	1.480
Hbs	-	92.51	93.23	91.40	92.94	86.58	88.76	90.66	92.23	92.25	94.39	86.97	89.76
S	-	4.232	3.373	8.978	9.030	10.519	10.071	6.644	6.266	9.138	8.914	3.241	3.641
CV	-	0.0401	0.0320	0.2239	0.2321	0.2895	0.2846	0.0658	0.0612	0.1507	0.1415	0.1781	0.1863
S.O.V	d.f	Stigma exseration %		Stigma breadth		Panicle plant^{-1}		Panicle length		Seed set %		Yield plant^{-1}	
		2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Reps.	4	1.308 ^{ns}	0.972 ^{ns}	0.0009 ^{ns}	0.0007 ^{ns}	1.046 ^{ns}	0.19 ^{ns}	0.206 ^{ns}	0.001 ^{ns}	0.0007 ^{ns}	0.0006 ^{ns}	21.646 ^{ns}	14.036 ^{ns}
Population	49	25.031**	26.460**	0.0003**	0.0003**	33.900**	27.896**	8.692**	7.651**	0.0110**	0.0102**	423.606**	366.365**
Error	196	0.8518	0.743	0.029	0.018	2.196	1.224	0.957	0.873	0.0003	0.0002	8.083	7.638
Hbs	-	92.05	93.91	95.39	97.27	92.12	93.73	88.19	89.75	91.46	92.11	91.97	93.44
S	-	5.003	5.044	0.0177	0.183	5.822	5.282	2.948	2.766	0.105	0.101	20.575	19.14
CV	-	0.0844	0.0852	0.0541	0.0556	0.3968	0.3867	0.1226	0.1136	0.115	0.1098	0.4822	0.4580

Analysis of variance for traits studied (Table 5) showed wide range of difference in most of them between the populations of line IR58025B in both seasons. It was observed that CV values for all studied traits were low indicating high level of processes and give good index of reliability of the results of the experiment.

Total Soluble Protein Analysis:

Biochemical study was carried out for the CMS line IR58025A and its maintainer line IR58025B. Fig. (1) showed three bands of the maintainer line, with molecular weights of (123, 93 and 28KD), but these bands were absent in the CMS line and its selected populations indicating that these bands may be related to maintaining ability for the maintainer line under study.

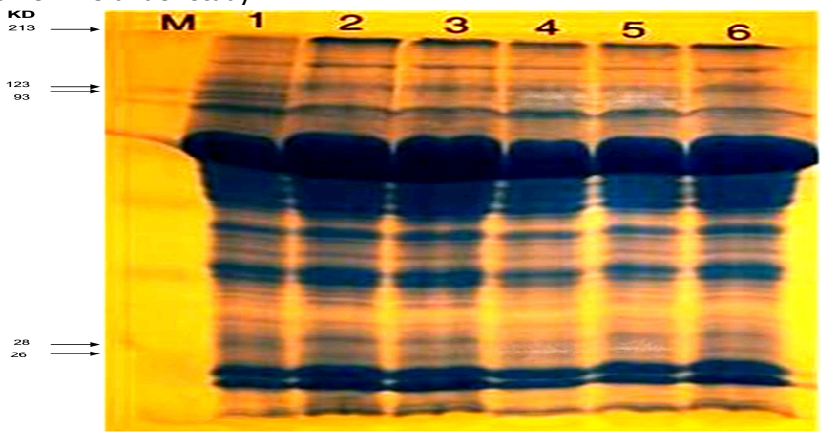

Fig. 1. Total soluble protein for maintainer line, CMS line and its selected populations

A band with molecular weight of 26 KD, was present in all CMS lines, but it was absent in its maintainer lines, indicating that it may be related to complete sterility gene(s) for the CMS line, the remain soluble proteins banding patterns, showed monomorphic in maintainer and CMS line.

From the above results, this analysis could be used to determine the genetic purity for CMS line and its maintainer line, especially that there are in confirmation with the obtained results under filed condition.

Molecular Studies:

Detection of genetic purity for CMS line by using molecular markers was used in this investigation to detect the sterility gene (s) in the CMS line IR58025A with its maintainer IR58025B as well as selected populations of IR58025A line.

To study the differences between the CMS line and its maintainer on the molecular level, eight SSR primers were used. Five primers out of them showed only monomorphic DNA bands with different sizes, and these bands were appeared in CMS and maintainer line. On the other hand, only three primers (i.e. RMT6, cms and RM239) proved to be able to amplify DNA fragments and showed polymorphic

patterns in both CMS and maintainer line. Fig (2A) showed the amplified DNA bands as a result of using the SSR primer, RMT6. The results for the CMS line and its selected populations, which produced from two pairing cross cycle method, showed two DNA bands with molecular sizes of 210 and 200 bp and only one band with molecular size of 210 bp was demonstrated in its maintainer line. Results in Fig (2B) for cms primer, showed that only one DNA band with molecular size of 400 bp was appeared in the CMS line and selected populations, but it was absent in its maintainer line. Results in Fig (2C) for RM239 primer, showed that only one DNA band with molecular size of 160 bp was appeared in the CMS line and selected populations, but it was absent in its maintainer line.

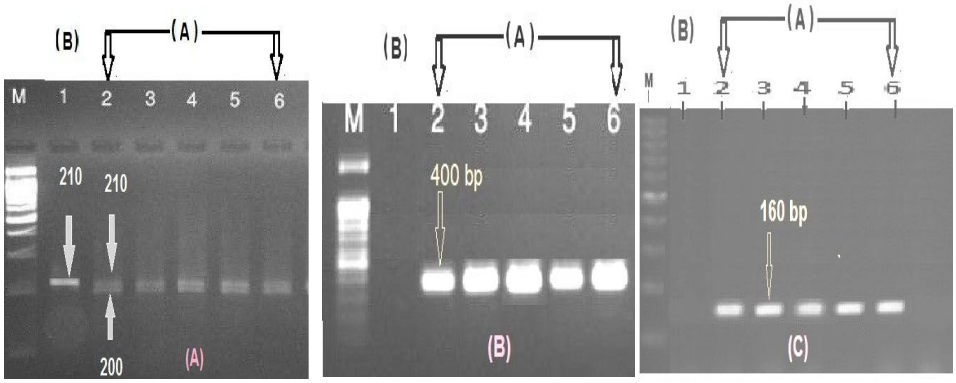

Fig. 2. PCR zymogram for maintainer line, CMS line and its selected populations

From the obtained data, it could be concluded that, the genetic purity for the selected populations showed complete sterility in the CMS line. The DNA fragments with size of 200 bp which produced by the RMT6 primer, it was found in the CMS line with its selected populations may indicate that it carries a gene(s), responsible for the sterility process because it was absent in the maintainer line.

Moreover, the obtained results were confirmed with the results obtained by the cms primer which produced only one DNA band (400 bp) that appeared only in the CMS line but was absent in its maintainer. These results were also confirmed in the morphological characters, especially pollen test and spikelet fertility.

Finally, the results suggested that the primers RMT6, cms and RM239 could be used to identify the genetic purity for the CMS lines used in multiplication experiment instead of identification experiment. Similar results were obtained by Sunil *et al*, (2015) and Devanand *et al*, (2000) who mentioned that, the level of sterility should be tested in crosses between CMS lines, maintainers and restorers before the formers are used for backcrossing to create new CMS lines.

CONCLUSION

The maximum impurity which was (4 %) in line IR58025A was dropped from 2014 to 2016 season after three paired – cross evaluation. The 96 % purity of IR58025A plants was used for nucleus seed multiplication through paired cross cycles every year to produce breeder seed. This procedure was successful in improving the purity of the parental lines to achieve the maximum yield potential 99 %, and pure seeds of the A line should be used. For PCR analysis, the results suggested that the primers RMT6, cms and RM239 could be used to identify the genetic purity for CMS line under multiplication experiment in hybrid rice seed production of Egypt.

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تحسين النقاوة الوراثية فى الأرز للسلالة ذات العقم الذكرى السيتوبلازمي IR58025A والأب المحافظ علي خصوبتها تحت الظروف المصرية

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أقيمت هذه التجربة بمزرعة قسم بحوث الارز بسخا – كفرالشيخ وذلك بهدف تحسين النقاوة الوراثية للسلالة ذات العقم الذكرى السيتوبلازمي IR58025A والأب المحافظ علي خصوبتها تحت الظروف المصرية خلال ثلاث مواسم زراعة ٢٠١٤، ٢٠١٥، ٢٠١٦. تم زراعة حبوب السلالة الأبوية خلال موسم زراعة ٢٠١٥م. تم استخدام بعض من هذه الهجن فى التجربة الإستكشافية ، والجزء المتبقى تم إستخدامه فى تجربة الإكثار وتم تكرار ما سبق خلال موسمي زراعة ٢٠١٥،و٢٠١٦. أستخدم خمس مكررات بنظام قطاعات كاملة العشوائية. المعلمات الجزيئية المستخدمة والمعتمدة علي تكنيك الـــPCR إستخدمت لتوضيح مدي الإختلاف ما بين السلالة ذات العقم الذكري السيتوبلازمي والسلالة المحافظة علي خصوبتها. وتم إستخدام ثلاث من المعلمات الجزيئية وهي علي التوالي RMT6 و cms و RM239 وكانت أقصي نسبة لعدم النقاوة الوراثية ٤٪ للسلالة IR58025A بعد تقييمها خلال ثلاثة مواسم زراعة متتالية. وقد أوضحت نتائج هذه الدراسة قيم عالية المعنوية لتحليل تباين المتوسطات بين الصفات المدروسة. تراوحت قيم المكافئ الوراثي بالمعني الواسع للسلالة IR58025A ٨٩,٠٨ و ٩٧,٨٤ لصفة مساحة سورقة العلم وعرض الريشة علي التوالي. بإستخدام المعلم الجزئيئ RMT6 أوضحت وجود إثنين من حزم الـــ DNA بحجم ٢٠٠ و ٢١٠ زوج من القواعد للسلالة IR58025A وبحجم ٢٠٠ زوج من القواعد للسلالة IR58025B . بالنسبة للمعلم الجزيئي cms أوضحت وجود حزمة من الـــــــــDNA بحجم ٤٠٠ زوج من القواعد للسلالة ذات العقم الذكري السيتوبلازمي IR58025A وغيابها في السلالة المحافظة عليها IR58025B. بالنسبة للمعلم الجزيئي RM239 اوضحت وجود حزمة من الـــــــ DNA بحجم ١٦٠ زوج من القواعد للسلالة ذات العقم الذكري السيتوبلازمي IR58025A وغيابها في السلالة المحافظة عليها IR58025B .