CHARACTERIZATION OF SOME RICE GENOTYPES FOR FERTILITY RESTORING GENES USING RAPD AND SSR MARKERS

A. N. EL-BANNA, I. A. KATTAB AND MONA A. FARID

Genetics Department, Faculty of Agriculture, Kafrelsheikh University, Kafr El-Sheikh, 33516, Egypt

To meet the demand of increasing population and maintain self-sufficiency the present rice production needs to be increased by 30% by the year 2020 (Singh *et al*., 2012). The task is quite challenging and the options available are very limited in view of plateauing trend of yield in high productivity areas, decreasing and degrading land and scarcity of water and labour (Santhanalakshmi *et al*., 2010). Rice (*Oryza sativa* L.) is one of the major crop species in which hybrids are used commercially (Selvaraj *et al*., 2011). It is a major staple food that feeds more than one-half of the human population. During the past decade, hybrid rice technology has emerged as one of the most practical and acceptable approaches to achieve this target (Tiwari *et al*., 2011). It has not only contributed to food security but also benefited the environment (Sreedhar *et al*., 2011). The current level of rice production will not meet future demand either in national or international level. Egypt has to be increase the average national rice yield by about 25-30% to meet the demand of increasing population. Yield of traditional rice cultivar is now considered to have reached the plateau. So, achieving further yield improvement is now unsatisfactory. Exploitation of heterosis through hybrid rice technology is an important strategy to increase grain yield beyond the present ceiling. Identification of effective maintainers and restorers are of great importance in any hybrid rice breeding program based on cytoplasmic male sterility (CMS) (Bijral *et al*., 1991). It is a common phenomenon in higher plants characterized by sterile, non functional pollen grains due to the alterations in mitochondrial genome. The sterility can be restored by crossing CMS lines with the restorer lines containing fertility restorer genes (*Rf*). In rice, wild abortive CMS (WA-cms) source has been utilized extensively in commercial hybrid rice seed production duo to its stability, excellent out crossing potential and the availability of broader genetic base for the restorer lines (Virmani *et al*., 1994). Restorer lines play an important role in successful hybrid rice development. They are detected conventionally through test cross procedure by crossing rice germplasm lines (male parents) with the sterile CMS lines (female parents) and the F_1's are evaluated for the pollen and spikelet fertility.

Molecular marker assisted identification with high power of genetic resolutions has emerged as a robust technique for cultivar fingerprinting, identity profil-

Egypt. J. Genet. Cytol., 44:253-264, July, 2015
Web Site (*www.esg.net.eg*)

ing, estimating and comparing genetic similarity, and variety protection. Several types of molecular marker such as RAPD (Wang and Lu, 2006; Ichii *et al*., 2003), SSR (Yashitola *et al.*, 2002; Nandakumar *et al.*, 2004) have been used in this term. These markers are highly polymorphic and easy to detect. The high polymorphism means that these markers can be used in germplasms that is closely related (Ni *et al.*, 2002). Recently, a fairly dense SSR map of rice has been published (McCouch *et al*., 2002). Agronomical important genes can provide useful information for plant breeders. In this line, our objective was to study the maintenance and restoring ability of the pollen parents over different cytoplasmic sources, and to detect an important *Rf* locus in rice using RAPD and SSR markers which could be used in hybrid rice breeding program depending on molecular assisted selection (MAS).

MATERIALS AND METHODS

Four CMS lines namely, G46A (Gambica cytoplasmic source), D297A (Dissi), Yimi15A (Dian type) and V20A (Wild abortive cytoplasmic source) and ten elite cultivated rice genotypes namely, Sakha 106, Giza 182, Giza 178, Giza 181, Sakha 101, Wita 4, Wita 12, Gz.1368-5-4, IET 1444 and Sakha 105 rice cultivar were comprised the materials for present study. The pollen parents were planted in plot with a spacing of 20x20 cm. Before crossed, all CMS plants were tested separately by pollen sterility to ensure 100% pollen sterility of CMS plants. The Line x Testers design was used according to Kempthorne (1957). The generated 40 crosses along with their parents were grown in randomized complete block design with three replications. Thirty day old seedlings were transplanted in a single seedling per hill; each experimental unit consisted of 5 row of 3 m length with the spacing of 20 cm. x 20 cm. All the recommended agronomic package of practices was followed. In each entry, five plants were randomly selected from each replication and the observations were recorded for spikelets fertility percentage, No. of filled grains/ panicle, No. of panicles/ plant, 1000-grain weight (g) and grain yield (kg/m^2) following the Standard Evaluation System for Rice (IRRI, 1996).

Classification of pollen parents

The pollen parents were classified into four categories; Maintainers (M), Partial Maintainers (PM), Partial Restorer (PR) and Restorer (R) according to the scale of Manuel and Rangaswamy (1993) (Table 1).

Plant materials and DNA isolation

Seven rice genotypes, including one cytoplasmic male-sterile line (GA46A), three restorer lines (Giza 181, Giza 182 and Giza 178) and their F_1 hybrids combinations were used in this study. Genomic DNA was isolated from young leaves according to Doyle and Doyle (1990) procedure. The quantity and quality of DNA was assessed with Nano Drope.

RAPD and SSR analyses

Twelve randomly decamer primers were tested. PCR was carried out in 25 µl reaction mixture containing 30 ng of template DNA, 200 µM of each dNTPs, 10x *Taq* buffer PCR (10 mM Tric-HCl, 50 mM KCl (pH 8.8), 2.5 mM of $MgCl_2$, 1.2 U of *Taq* polymerase, and 1 µM each of the RAPD primers using a MJ research thermal cycler. Amplification reaction was started by initial denaturation of template DNA at 94°C for 4 min and followed by 40 cycles at 92°C for 1 min, 35°C for 1 min, and 72°C for 2 min. Final extension was at 72°C for 5 min. PCR amplified products were separated by electrophoresis in 1.5% agarose gels at 70 V in 0.5x TBE buffer. Gels were stained with ethidium bromide and imaged using Biometra (UV-solo model) gel documentation system. Each reaction was repeated twice and only reproducible bands were considered for analysis.

Ten microsatellite markers (SSRs) were used in this study. The sequences of the polymorphic primer pairs are presented in Table (2). Polymerase chain reaction (PCR) was performed in a volume of 20 µl reaction mixture containing 30 ng of template DNA, 200 µM of each dNTPs, 10x *Taq* buffer PCR (10 mM Tric-HCl, 50 mM KCl (pH 8.8), 2.5 mM $MgCl_2$, 1 U of *Taq* polymerase and 0.2 µM each of the SSR primer pair (Metabion International AG, Germany) using a MJ research thermal cycler. Amplification reactions were initiated by 5 min pre-denatutation at 95°C and followed by 35 cycles each at 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min. A final extension step at 72°C for 7 min was performed. PCR amplified products were separated by electrophoresis in 2% agarose gels at 70 V in 0.5x TBE buffer. Gels stained with ethidium bromide and then were imaged using Biometra (UV-solo model) gel documentation system.

RESULTS AND DISCUSSION

Means of yield and its component traits

Means of the forty line x tester hybrids (Table 3) indicated worth of genetic variability for the fertility (%), No. filled grains/panicle, No. of panicles/plant, 1000-grain weight (g) and grain yield (kg/m^2), which are important in hybrid rice yield. The hybrids V20A x Giza 178 was the most desirable hybrid for high fertility percentage (95.4%) followed by V20A x Wita 12 (94%), D297A x Giza 182 (93.4%) and G46A x Giza 178 (93%). Wide variation among rice genotypes were detected for No. of filled grains/ panicle. G46A x Giza 181 recorded the highest mean value of this trait (221) compared with the other hybrids followed by G46A x Wita 12 (192). V20A x Sakha 101 rice hybrid showed the best combination for No. of panicles/ plant compared with other tested genotypes, the F_1 hybrid rice combination between such parents and G46A recorded the most favorable value for 1000-grain weight. D297A x Giza 182 recorded the best mean value for grain yield (1.04 kg/m^2) followed by V20A x Giza 178 (0.98 kg/m^2), G46A x

Giza 178 (0.95 kg/m^2) and D297A x Wita 4 (0.93 kg/m^2). The highest performance values of F_1 hybrids concerning grain yield may be attributed to increasing No. of filled grains/ panicle, No. of panicles/plant and 1000-grain weight.

Maintenance and restoring ability

The fertility percentage and category of restorer and maintainer genotypes are shown in Table (3). Maintainers and restorers genotypes were identified according to the scale of Manuel and Rangaswamy (1993) as presented in Table (1). It is clear that Giza 182, Giza 178 and Wita 12 rice parents were identified as restorers for the CMS lines G46A, D297A and V20A (spikelet fertility more than 80%). Contrary, Sakha 106 and Sakha 101 rice cultivars were identified as maintainers for the same aforementioned CMS lines. Giza 181 was identified as restorers for the CMS line G46A and D297A and partial restorer for Yimi 15A and V20A. While, Sakha 105 rice cultivar was identified as maintainers for G46A and D297A. However, the other combinations were identified either partial maintainers or partial restorers which have less of interest in hybrid rice breeding programs.

Results also showed that four testers (Giza 182, Giza 178, Giza 181 and Wita 12) restored the CMS lines G46A and D297A. Also, three testers (Giza 182, Giza 178 and Wita 12) restored V20A while, none of the tested parents restored the CMS line Yimi 15A, indicating that G46A and D297A were easy to restore while, Yimi 15A was hardly to restore. The difficultly of restoring ability of Yimi 15A may be due to this CMS line belongs to Japonica type and most of restorer lines frequency were belongs to Indica type, suggesting high sterility percentage in F_1 hybrids. These results were, in general, agree with those reported by Murayama and Sarker (2002) who found that F_1 hybrids from crosses between japonica and Indica rice show variable degrees of sterility.

Previous studies showed that among 239 rice genotypes tested for their status in hybrid rice gene pool, 12 restorers and 16 maintainers were identified. Most of genotypes were found to be partial restorers and partial maintainers (Akhter *et al*., 2008). There are considerable variations in frequency of maintainers for different CMS lines. The average frequency was 5% over the tested crosses. Considerable variability was found in frequency of restorers over different CMS lines and with lines from different sources (Eusebio *et al.*, 2002).

RAPD profiling analysis

Ten informative out of twelve RAPD primers were chosen for DNA profiling (Table 2). A total of 88 reproducible amplifications products were observed and 57 polymorphic bands were scored (64.7%). The lowest and highest numbers of polymorphic products obtained with primers OPK09 and OPK03 with 4 and 13 bands, respectively. The selected CMS line, restorer cultivars and their F_1 hyprids could be uniquely identified by RAPD multilocus amplified profile at the used

ten informative RAPD markers. Concerning the restoring linked markers, two primers OPK08 and OPK10 produced fertility restoration linked markers. The markers with the molecular size of 200 and 690 bp for the primers OPK08 and OPK10, respectively (Table 4), were absent in the CMS line whereas found in the selected restorer cultivars and their F_1 hybrids (Fig. 1).

Microsatellite profiling analysis

Six out of the ten SSR loci amplified polymorphic bands in the seven genotypes under study (Table 2). Four markers, RM315, RM288, RM171 and RM179 were monomorphic. The average numbers of bands per primer were 1.9. The number of genotypes possessing a particular locus ranged from 1 (RM315, RM288 and RM179) to 3 for RM302 and RM510 markers. Microsatellite RM302 marker was the most informative locus for genotypic DNA profiling and differentiation. This marker produced two extra bands only in the hybrid genotypes with band size of 495 and 220 bp. The markers RM510 and RM493 could differentiate the CMS line, restorer and their hybrids. RM510 and RM493 produced bands with size of 186 and 128 bp in the restorer cultivar Giza 182 and the hybrid G46A x Giza 182, respectively (Table 4), whereas they produced bands with the size of 165 and 123 bp in the restorer cultivar Giza 181 and the hybrid G46A x Giza 181, respectively, while it amplified bands with the size of 162 and 126 bp in the restorer cultivar Giza 178 and the hybrid G46A x Giza 178, respectively. Non-parental bands were also observed in addition to parental bands in the hybrid between CMS line G46A and the restorer cultivars using primer RM320. In addition to the monomorphic band with the size of 200 bp, this marker produced two extra specific bands to the hybrids with the size of 495 and 220 bp (Fig. 2**)**.

A common feature of the minority of SSR markers is the presence of a non-parental extra band in hybrid individuals, these bands aside from the parent-specific markers indicating hybrid vigour. Xiao *et al*. (1996) also showed that hybrid vigour has a positive relation with extra banding of DNA. The result from our study suggests that heterosis is positively correlated with the extra DNA banding that appeared in the DNA profile of F_1 and the accumulation of all the parental genes in the F_1 reveals hybrid vigor. In addition the heterosis observed in the F_1 hybrid could be combined alleles associated with hybrid vigor (Young *et al*., 2004).

Ten microsatellite and twelve RAPD markers were used for DNA profiling of hybrids and their parental lines in this investigation. The results revealed that the SSR loci RM493, RM302 and RM510 and RAPD multiloci OPK08 and OPK10 have the highest efficiency for DNA profiling and discrimination the CMS, restorer lines and their corresponding hybrids. Garg *et al.* (2006) suggested that some the RM primers were linked to a single *Rf* gene that possesses co-dominant

status can provide a precise and quick alternative to grow out test. F_1 hybrid genotypes were characterized by non-parental band(s) produced by some primers. Similar features were also reported in other markers and plants viz: RAPD codominant markers in soybean (Zheng *et al.*, 2003), rice (Wu *et al.*, 2002), *Chrysanthemum* hybrids (Huang *et al.*, 2000) and SSR markers in maize heterozygous DH lines (Heckenberger *et al*., 2002). In addition to the restoring fertility associated marker(s), a single polymorphic marker should suffice to ascertain hybrid seed purity in rice. Also, Nandakumar *et al.* (2004) successfully employed a single restorer gene linked marker assessment for testing genetic purity of hybrid seeds that substantially reduced the time, space and labor. Concisely, concordant with results of the present study, a non-parental extra band is not only useful for fingerprinting the rice hybrids and unambiguous hybrid identity, but could also be used as specific feature for characterization and genetic purity test and detection of off-type seeds in the hybrids based on monomorphic markers. The present study showed that SSR and RAPD markers are quick, effective and results are generally consistent with agronomical traits in the field. Markers identified in the study could be utilized for fertility restoration testing. These markers could be of immense help for hybrid rice seed industry to select appropriate restorer(s) and to detect an important *Rf* loci in rice.

SUMMARY

With the objective of identifying restorers and maintainers, to identify fertility restoration linked markers that can distinguish cytoplasmic sources and restorer lines, four male sterile and fertile counterparts of Cytoplasmic Male Sterile (CMS) lines and 10 Restorers (R) lines were characterized. Based on spikelet fertility percentages, ten suggested restorer were identified. Giza 182, Giza 178 and Giza 181 were identified as restorers for the CMS lines G46A and D297A. Ten SSR and 12 RAPD markers were used for the identification of the fertility restoration gene in restorer lines. Among ten suggested restorer lines, only three Egyptian rice cultivars, Giza 182, Giza 178 and Giza 181 used in molecular analysis. Two out of ten SSR markers (RM493 and RM510) could differentiate the CMS line, restorer and their hybrids. RM493 produced bands with size of 123, 128 and 126 bp in the restorer cultivars Giza 181, Giza 182 and Giza 178, respectively. Whereas, the SSR marker RM510 produced bands with size of 165, 186 and 162 bp in the restorer cultivars Giza 181, Giza 182 and Giza 178, respectively. These specific markers were found in their corresponding hybrids with CMS line G46A. In addition, two RAPD primers OPK08 and OPK10 produced fertility restoration linked markers with the molecular size of 200 and 690 bp, respectively. These markers were found only in the restorer cultivars and their respective hybrids with the CMS line G46A. The results suggested effective utilization of SSR and RAPD markers in hybrid test-

ing and marker aided heterosis breeding in rice.

ACKNOWLEDGMENT

The authors thank Dr. Ibrahim El Degwy, Dept. of Agronomy, Faculty of Agriculture, Kafrelsheikh University for providing plant material and agronomical characteristics analysis.

REFERENCES

Akhter, M., M. A. Zahid, M. Sabar and M. Ahmed (2008). Identification of restorers and maintainers of the development of rice hybrids. The Journal of Animal and Plant Sciences, 18: 39-41.

Bijral, J. S., T. R. Sharma, B. B. Gupta, K. Singh and C. L. Raina (1991). Restorers and maintainers for to cytoplasmic male sterile lines. IRRN 16: 7.

Doyle, J. J., and J. L. Doyle (1990) Isolation of plant DNA from fresh tissue. Focus, 12: 13-15.

Eusebio, W., C. Casal, B. Parado, V. Bartolome, V. Virmani and G. Mclaren (2002). Variations in frequency of maintainers and restorers in test crosses of the hybrid rice breeding project. Philippine Journal of Crop Science, 27 (supplement 1) pp. 80.

Garg, A., A. K. Singh, K. V. Prabhu, T. Mohapatra, N. K., Tyagi, N. Nandakumar, R. Singh and F. U. Zaman (2006). Utility of a fertility restorer gene linked marker for testing genetic purity of hybrid seeds in rice (*Oryza sativa* L.). Seed Sci. Tech., 34: 9-18.

Heckenberger, M., M. Bohn, J. S. Ziegle, L. K. Joe, J. D. Hauser, M. Hutton and A. E. Melchinger (2002). Variation of DNA fingerprints among accessions within maize inbred lines and implications for identification of essentially derived varieties. Mol. Breed., 10: 181-191.

Huang, S. C., C. C. Tsai and C. S. Sheu (2000). Genetic analysis of *Chrysanthemum* hybrids based on RAPD molecular markers. Bot. Bull. Acad. Sin., 41: 257-262.

Ichii, M., D. L. Hong, Y. Ohara, C. M. Zhao and S. Taketa (2003). Characterization of CMS and maintainer lines in indica rice (*Oryza sativa* L.) based on RAPD marker analysis. Euphytica, 129: 249-252.

International Rice Research Institute (IRRI). (1996). Standard Evaluation System for Rice. 4th ed Manila (Philippines): International Rice Research Institute, 39: 323-334.

Kempthorne, O. (1957). An Introduction to Genetic Statistics. XVII, John Wiley and Sons Inc., New Yourk, pp. 545.

Manuel, W. W. and M. Rangaswamy (1993). Restorers and maintainers for cytoplasmic male sterile line (CMS) lines in rice. IRRN, 18: 6.

McCouch, S. R., L. Teytelman, Y. Xu, K. B. Lobos, K. Clare, M. Walton, B. Fu, R. Maghirang, Z. Li, Y. Zing, Q. Zhang, I. Kono, M. Yano, R. Fjellstrom, G. DeClerck, D. Schneider, S. Cartinhour, D. Ware and L. Stein (2002). Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Res., 9: 199-207.

Murayama, S. and M. A. Sarker (2002). Agronomic performance of F_1 hybrids of rice (*Oryza sativa* L.) in japonica-indica crosses: Heterosis for and relationship between grain yield and yield related characters. Plant Production Science, 5: 203-210.

Nandakumar, N., A. Singh, K. Sharma, R. K. Mohapara, T. K. V. Prabhu and F. U. Zaman (2004). Molecular fingerprinting of hybrids and assessment of genetic purity of hybrid seeds in rice using microsatellite markers. Euphytica, 136: 257-264.

Ni, J., P. M. Colowit and D. J. Mackill (2002). Evaluation of genetic diversity in rice subspecies using microsatellite markers. Crop Sci., 42: 601-607.

Santhanalakshmi, S., S. Saikumar, S. Rao, A. SaiHarini, P. Khera, H. E. Shashidhar and P. Kadirvel, (2010). Mapping genetic locus linked to brown planthopper resistance in rice *Oryza sativa* L. Int. J. Plant Breed. Gene., 4: 13-22.

Selvaraj, C. I., P. Nagarajan, K. Thiyagarajan, M. Bharathi and R. Rabindran (2011). Studies on heterosis and combining ability of well known blast resistant rice genotypes with high yielding varieties of rice (*Oryza sativa* L.). Int. J. Plant Breed. Genet., 5: 111-129.

Singh, A. K., P. Khera, R. Priyadarshi, V. Patil, M. Dhasmana and V. Shenoy (2012). Occurance of tifid stigma morphotype in maintainer line of rice (*Oryza sativa* L.). International Journal of Plant Breeding and Genetics, 6: 252-255.

Sreedhar, S., T. D. Reddy and M. S. Ramesha (2011). Genotype x Environment interaction and stability for yield and its components in hybrid rice cultivars (*Oryza sativa* L.). Int. J. Plant Breed. Genet., 5: 194-208.

Tiwari, D. K., P. Pandey, S. P. Giri and J. L. Dwivedi (2011). Heterosis studies for yield and its components in rice hybrids using CMS system. Asian J. Plant Sci., 10: 29-42.

Virmani, S. S., M. N. Prasad and J. Kumar (1994). Breaking the yield barrier

of rice through exploitation of heterosis. In: Murlidharan K., Siddiq E. A., editors. New frontiers in rice research. Hyderabad (India): Directorate of Rice Research, p. 76-85.

Wang, S. and Z. Lu (2006). Genetic diversity among parental lines of Indica hybrid rice (*Oryza sativa* L.) in China based on coefficient of parentage. Plant Breed., 125: 606-612.

Wu, J. Y., H. K. Wu and M. C. Chung (2002). Co-dominant RAPD markers closely linked with two morphological genes in rice (*Oryza sativa* L.). Bot. Bull Acad. Sin., 43: 171-180.

Xiao, J., J. Li, L. Yuan, S. R. McCough and S. Tanks (1996) Genetic diversity and its relationship to hybrid performance and heterosis as revealed by PCR based markers. Theor. Appl. Genet., 92: 637-643.

Yashitola, J., T. Thirumurgan, R. M. Sundaram, M. K. Naseerullah, M. S. Ramesha, N. P. Sarma and R. V. Sonti (2002). Assessment of purity of rice hybrid using microsatellite and STS markers. Crop Sci., 42: 1369-1373.

Young, C., C. W. Park, S. W. Kwon, J. H. Chin, H. S. Ji, K.J. Park, S. McCouch and H. J. Koh (2004). Key DNA markers for predicting heterosis in F_1 hybrids of *japonica* rice. Breed. Sci., 54: 389-394.

Zheng, C., R. Chang, L. Qiu, P. Chen, X. Wu and S. Chen (2003). Identification and characterization of a RAPD/SCAR marker linked to a resistance gene for soybean mosaic virus in soybean. Euphytica, 132: 199-210.

Table (1)**:** Classification of elite lines into maintainers and restorers**.**

Spikletes fertility%	Catogary
Restorers (R)	80%
Partial restorers (PR)	20 – 80%
Maintainers (M)	5%
Partial maintainers (PM)	5 – 20%

Table (2): Details of selected primers used in RAPD and microsatellite DNA (SSR) profiling analysis.

RAPD primers		TNB	NPB	PPB	UB
OPK-01	CATTCGAGCC	8	8	100.0	-
OPK-02	GTCTCCGCAA	9	2	22.2	-
OPK-03	CCAGCTTAGG	13	11	84.6	-
OPK-04	CCGCCCAAAC	7	2	28.5	-
OPK-05	TCTGTCGAGG	9	7	77.7	-
OPK-06	CACCTTTCCC	9	9	100.0	-
OPK-07	AGCGAGCAAG	12	1	8.3	-
OPK-08	GAACACTGGG	9	7	77.7	2
OPK-09	CCCTACCGAC	4	2	50.0	-
OPK-10	GTGCAACGTG	8	8	100.0	2
SSR primers		TNB	NPB	PPB	UB
RM231	F: CCAGATTATTTCCTGAGGTC R: CACTTGCATAGTTCTGCATTG	2	2	100.0	1
RM315	F: CGGTCAAATCATCACCTGAC R: CAAGGCTTGCAAGGGAAG	1	0	0.0	-
RM302	F: TCATGTCATCTACCATCACAC R: ATGGAGAAGATGGAATACTTGC	3	2	66.6	1
RM525	F: AGAGTTATGAGCCGGGTGTG R: GATTTGGCGATCTTAGCAGC	2	2	100.0	-
M493	F: TAGCTCCAACAGGATCGACC R: GTACGTAAACGCGGAAGGTG	2	2	100.0	1
RM510	F: AACCGGATTAGTTTCTCGCC R:TGAGGACGACGAGCAGATTC	3	3	100.0	1
RM593	F:TCCCGTATGTAACGTGCCA R:GACAAGAGAACATCGCTAGG	2	1	50.0	-
RM288	F: ATGCCGCCAGTGAATAGC R: CTGAGAATCCAATTATCTGGG	1	0	0.0	-
RM171	F: CATCCCCCTGCTGCTGCTGCTG R: CGCCGGATGTGTGGGACTAGCG	2	0	0.0	-
RM179	F: CATAGTGGAGTATGCAGCTGC R: CCTTCTCCCAGTCGTATCTG	1	0	0.0	-

TNB: Total number of bands; NPB: number of polymorphic bands;
PPB: percent of polymorphic bands; UB: Unique bands.

Table (3): Mean performance of F_1 hybrids for grain yield and yield related characters, category of restoring ability.

Crosses	Grain yield and yield related characters					Category
	Fertility (%)	No. filled grains/ panicle	No of panicles/ plant	1000-grain weight (g)	Grain yield (kg/m^2)	
G46A x Sakha 106	1.4	3.4	18.4	30.7	0.02	M
G46A x Giza 182	92.5	160.4	15.4	27.8	0.89	R
G46A x Giza 178	93.0	154.0	19.8	25.1	0.95	R
G46A x Giza 181	88.3	221.0	13.6	24.4	0.91	R
G46A x Sakha 101	0.20	0.50	14.4	28.5	0.01	M
G46A x Wita 4	30.7	73.8	16.2	17.9	0.36	PR
G46A x Wita 12	80.1	192.0	15.6	21.9	0.87	R
G46A x Gz.1368	67.0	128.4	13.2	23.1	0.60	PR
G46A x IET 1444	9.9	21.8	16.0	24.3	0.11	PM
G46A x Sakha 105	0.80	2.0	12.2	30.9	0.01	M
D297 x Sakha 106	4.3	7.9	16.2	27.0	0.03	M
D297 x Giza 182	93.4	136.0	19.6	27.5	1.04	R
D297 x Giza 178	90.2	140.0	16.2	24.8	0.84	R
D297 x Giza 181	89.7	171.2	12.6	24.9	0.77	R
D297 x Sakha 101	0.49	1.20	15.6	28.5	0.01	M
D297 x Wita 4	68.6	115.8	15.8	26.5	0.93	PR
D297 x Wita 12	80.8	160.6	14.4	22.0	0.64	R
D297 x Gz.1368	77.3	130.0	16.6	21.8	0.70	PR
D297 x IET 1444	7.7	12.8	15.4	24.4	0.11	PM
D297 x Sakha 105	1.4	1.80	18.0	23.6	0.02	M
YIMI15A x Sakha 106	11.6	20.0	13.4	28.5	0.10	PM
YIMI15A x Giza 182	39.0	64.4	17.0	27.2	0.42	PR
YIMI15A x Giza 178	39.0	70.8	17.4	26.9	0.50	PR
YIMI15A x Giza 181	35.1	57.0	14.2	27.6	0.34	PR
YIMI15A x Sakha 101	6.8	10.8	14.8	26.0	0.05	PM
YIMI15A x Wita 4	12.0	22.8	15.6	25.7	0.16	PM
YIMI15A x Wita 12	73.8	108.8	15.6	26.3	0.72	PR
YIMI15A x Gz.1368	32.1	48.4	18.0	26.4	0.50	PR
YIMI15A x IET 1444	55.8	101.2	17.8	26.6	0.70	PR
YIMI15A x Sakha 105	17.8	23.8	15.4	27.1	0.08	PM
V20A x Sakha 106	0.52	1.2	11.6	28.7	0.01	M
V20A x Giza 182	85.3	132.0	12.4	27.0	0.51	R
V20A x Giza 178	95.4	137.0	19.6	23.8	0.98	R
V20A x Giza 181	76.6	130.2	16.2	20.9	0.41	PR
V20A x Sakha 101	3.9	7.20	22.8	27.3	0.05	M
V20A x Wita 4	49.5	88.0	18.4	26.8	0.43	PR
V20A x Wita 12	94.0	145.6	14.5	26.3	0.74	R
V20A x Gz.1368	77.0	112.2	18.0	19.8	0.73	PR
V20A x IET 1444	17.5	23.6	16.6	23.7	0.18	PM
V20A x Sakha 105	7.2	13.2	14.4	23.3	0.10	PM
LSD 0.05	3.8	4.5	0.93	0.52	0.12	
0.01	5.4	6.5	1.30	0.74	0.16	

Table (4): Genotype-specific markers for different rice genotypes based on RAPD and SSR analysis.

Genotypes	Markers molecular size (bp)			
	RAPD (OPK08)	RAPD (OPK10)	SSR (RM510)	SSR (RM493)
G46A (CMS)	-	-	-	-
Giza 181 (P1)	200	690	165	123
Giza 182 (P2)	200	690	186	128
Giza 178 (P3)	200	690	162	126
CMSxP1	200	690	165	123
CMSxP2	200	690	186	128
CMSxP3	200	690	162	126

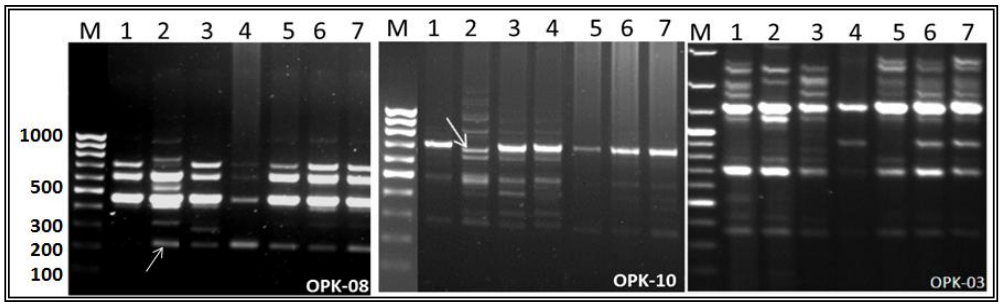

Fig. (1): Amplification pattern of representative RAPD markers. M- 100 bp ladder, 1-G46A, 2- Giza 182, 3- G181, 4- G178, 5- G64A x Giza182, 6- G64A x Giza181, 7- G64A x Giza178. Arrows indicated restorer gene(s) linked marker.

Fig. (2): Amplification pattern of representative SSR markers. M- 100 bp ladder, 1-G46A, 2- Giza 182, 3- G181, 4- G178, 5- G64A x Giza182, 6- G64A x Giza181, 7- G64A x Giza178. Arrows indicated restorer gene(s) linked marker.