# RELATIONSHIP BETWEEN DIFFERENT ALLOGAMIC ASSOCIATED TRAIT CHARACTERISTICS OF THE FIVE NEWLY DEVELOPED CYTOPLASMIC MALE STERILE (CMS) LINES IN RICE

**Nematzadeh GHORBAN ALI[1], Ali ABUBACKER JAUHAR[2]*, Sattari MAJID[2], Valizadeh AHMED[2], Alinejad EISHA[2], Nouri MOHAMMAD ZAMAN[2]**

[1]Sari College of Agricultural Sciences, Sari, IRAN,

[2]Rice Research Institute of Iran, Rasht, IRAN.
Telephone : 0098-131-6690052; Fax : 0098-131-6690051

*Corresponding author, E-mail: jauharali02@yahoo.com

Manuscript received: September 8, 2005; Reviewed: May 5, 2006; Accepted for publication: June 9, 2006

## ABSTRACT

Five suitable maintainer varieties were identified through testcrosses with IR58025A and the transfer of wild abortive cytoplasm was carried out by seven successive backcrosses. Five new CMS lines were developed by this approach in well adapted high yielding improved varietal background such as 'Nemat', 'Neda', 'Dasht', 'Amol3' and 'Champa'. Agronomical characterization and allogamy-associated traits of the five newly developed CMS lines were studied for their interrelationship. Anther length had a significant positive correlation with the duration of glume opening (0.759) and high correlation of (0.698) with the angle between lemma and palea. The results indicated that 'Nemat' A, 'Neda' A, 'Dasht' A are more suitable as parents for hybrid seed production due to their favorable and superior floral characteristics in comparison to IR58025A.

**KEY WORDS: Oryza sativa , wild abortive cytoplasm, floral traits, CMS lines,**

## INTRODUCTION

Rice is a second main staple crop after wheat in Iran, cultivated in 570,000 hectares of irrigated area and 2.4 million tonnes of milled rice is produced. Iran still largely depends on rice imports to the tune of 0.8 million tonnes each year to meet the domestic consumer's demand. Therefore, hybrid rice with 20-25% yield heterosis over conventional varieties happens to be the only viable option to enhance the production and productivity levels, since the area under rice cultivation cannot be further increased due to water shortages. Exploitation of heterosis phenomenon through commercial F1 hybrid seed plays a significant role in increasing the production and productivity of rice. Hybrid rice seed production in China had started from 1976 onwards on a regular basis and this technology had caused real changes in rice production (17, 20). Ideal cytoplasmic male sterility (CMS) is essential for developing a successful hybrid seed production primarily as they render the male anthers completely sterile especially in a perfect flower of rice (20). Encouraged by the developments achieved by China, the International Rice Research Institute (IRRI) took the initiative in 1979 to explore the potentials and problems of developing this technology for countries outside China (19,24). Hybrid rice varieties demonstrated 15-20% more yield comparing to the conventional inbred varieties (3,23,24,25). The first attempts in Iran for hybrid rice research was initiated as early as 1987 simultaneously at Sari Agricultural College and Rice Research Institute of Iran, Amol with the introduction of two CMS lines such as V20A and W32A from IRRI (9). Another two CMS lines namely IR58025A and IR62829A were introduced from IRRI in 1991 and the development of new CMS lines in well adapted and high yielding improved varietal background was initiated in 1994 (9). In order to develop superior rice hybrids it is pre-requisite to develop CMS lines suitable for the target environment and with desirable floral traits. Therefore, an experiment was carried out with the objective to develop adaptable CMS lines to Iranian conditions for breeding superior rice hybrids and to study the inter-relationship of different floral traits.

## MATERIALS AND METHODS

A total of 8 varieties with 3 traditional cultivars having superior grain quality but with tall plant stature and low yield (Tarom, Domsiah and Rashtisadri) and 5 improved semi-dwarf high yielding varieties with medium grain quality i.e.('Nemat', 'Neda', 'Dasht', 'Champa' and 'Amol3') were test crossed with IR58025 A (WA cytoplasm) in the wet cropping season of the year 1994 to identify the potential maintainers and restorers. The pollen viability of the $F_1$'s of the testcrosses between CMS line IR58025A and 8 different varieties from the source nursery were studied in 1995 under microscope by staining with 1% IK-I as shown by Virmani et al., (21). According to the pollen fertility of the $F_1$'s of the testcrosses, the pollen parents of the crosses were classified as maintainers when the fertile pollens were less than 1%, semi-maintainer(1-20% fertile pollens), semi-restorer (21-60% fertile) and restorer when more than 61% fertility (2). Further, the percent of spikelet fertility was calculated on the bagged $F_1$ panicles. The complete sterile $F_1$'s in the testcrosses confirmed through pollen and spikelet sterility were then backcrossed to their respective pollen parents, in order to transfer the sterile WA cytoplasm into new nuclear background. After every successive backcross, 10 single plants were selected from a progeny of 50 plants for each backcross that were precisely checked for 100% percent pollen grain sterility under microscope (1% IK-I staining) before being backcrossed with their respective pollen parent (recurrent parent). Finally, after 7 generations of backcrossing, in the wet cropping season of the year 2001, the newly developed CMS lines were characterized for their agronomic and allogamic associated traits.

The agronomic traits comprised of tiller number (TN) (number of panicle bearing tillers), plant height (PH) (measured as average number of centimeters from the ground to the tip of the tallest panicle), panicle length (PL) (measured as the average number of centimeters from the panicle neck to the panicle tip -excluding the awn), flag leaf length(FLL) (measured in average number of centimeters from the flag leaf auricle to the leaf tip), flag leaf width (FLW) (measured in average number of centimeters at the widest middle part of the flag leaf), flag leaf angle (FLA) (angle in degrees formed between flag leaf and panicle at the time of complete emergence of the panicle), days to 50% flowering (DFF) ( time taken in days from sowing to 50% of the plants came to flowering), number of spikelets per panicle (NSP) (calculated by counting the total number of spikelets from the 10 plants divided by the number of panicles from all 10 plants), 1000 seed weight (TSW) (measured in grams as average of three different samples of 1000 fully filled seeds for each A line seed derived from their respective A x B CMS seed multiplication plot under natural field conditions) and were all measured according to the standard evaluation system (4, 20). The allogamy associated traits i.e. ovary length (OL)(average length of the 10 ovaries in millimeter), style length (SL) (average length of 10 styles in millimeter), stigma length (STL) (average length of 10 stigmas in millimeter), filament length (FL) (average length of 10 filaments in millimeter), anther length (AL) (average length of 10 anthers in millimeter), angle between lemma and palea (ALP)(average angle in degrees formed between lemma and palea from a sample of 10 spikelets randomly selected panicles in the middle row at the time of flowering), glume opening time (GOT) (time recorded at the time of opening of lemma and palea), duration of glume opening (DGO)(as measured in minutes for the time duration from opening to closing of the lemma and palea), stigma colour (STC) of blooming spikelets between 9.00AM to 2.00 PM (stigma colour as per visual observation and given scores 1: white, 2: light green, 3:yellow, 4: light purple, 5:purple) were all measured according to the standard evaluation system (4, 20). Mean for each trait over different CMS lines and standard deviation was computed for making effective comparison. Spearman's rho correlation coefficient was calculated for different floral trait variables (N=6) with SPSS 9.0 software. Testcrosses were made in wet cropping season of the year 2001 between newly developed CMS line 'Neda' A and potential restorers to identify partial and complete restorers by studying their pollen and spikelet fertility percentage. The classification of the testcross $F_1$ results for pollen and spikelet fertility percentage into partial and complete restorers was carried out according to Govindaraj et al., (2).

## RESULTS

Preliminary results showed F1's of testcrosses with IR58025A with traditional rice varieties like Tarom, Domsiah and Rashtisadri were male sterile and possessed rfrf gene in recessive condition in the nucleus. In spite of their excellent quality, these varieties were eliminated and not used for the male sterile conversion program because of their undesirable features like tall plant stature, narrow and weak culm. However, the other five high yielding semi-dwarf improved varieties such as 'Nemat', 'Neda', 'Dasht', 'Amol3' and 'Champa' were also found to possess a pair of recessive nuclear fertility genes rfrf based on the test cross data that showed complete pollen sterility in the $F_1$ plant and suitable for male sterile conversion program. Finally, after 7 generations of successive back crosses 'Nemat' A, 'Neda' A, 'Dasht' A, 'Amol3' A and 'Champa' A CMS lines with complete male sterility were developed.

Agronomic characterization of PH, TN, FLL, FLW, FLA, DFF, PL, NSP, TSW of A line (derived from AxB CMS seed multiplication plots) for the newly developed CMS lines in comparison to IR58025A which showed considerable range of variation for each of the trait (Table 1). Likewise, the allogamy associated traits i.e. OL, SL, STL, FL, AL, ALP, DGO, GOT, STC for the different CMS lines showed considerable range of variation. Amongst the newly developed CMS lines 'Neda' A showed highest tillering ability (35.0) with wider ALP of 25.5° with 183 minutes of DGO. 'Nemat' A had the longest FLL (29.6 cm), PL (28.6 cm), highest TSW (A x B crossed seeds), widest FLW (1.9 cm) and with least FLA (14.1°A). The 'Nemat' A also possessed the longest STL (1.66 mm) and with good DGO of 203 minutes. Although 'Dasht' A showed the least number of 19 tillers /plant, it recorded the highest NSP (211) and longest DGO (230 minutes). 'Neda' A and 'Nemat' A showed promise especially with the better STL, ALP and DGO in comparison to IR58025A (Fig.1, Table 2).

A significant positive correlation was observed between OL and SL (0.771) was observed. But the STL was negatively correlated with OL (-0.371) and SL (-0.6) although not significant (Table 3). FL had a significant negative correlation with SL (-0.829). AL had a significant positive correlation with the DGO (0.759) and high correlation of (0.698) with the ALP (Table 3).

Furthermore, three promising restorer lines i.e. IR24R, IR60966 and IR56R were identified for the newly developed CMS line 'Neda' A (Table 4). The highest $F_1$ pollen grain fertility of 88.7% was observed for 'Neda'A/IR62030R cross but on spikelet fertility basis, the $F_1$ of 'Neda'A/IR24R cross recorded the highest (84.04%) (Table 4).

## DISCUSSION

Several countries that had introduced Chinese hybrids and parental materials especially in the tropical regions like India, Bangladesh, Philippines and Sri Lanka met with failure primarily due to their susceptibility to tropical insect pests and diseases. Therefore, they had no choice but to introduce the IRRI bred IR58025A and IR62829A CMS lines and simultaneously develop their own CMS lines in adaptive local backgrounds. This lead us to develop our own adaptable CMS lines by transferring WA cytoplasm from IR58025A. Most of the released hybrids are currently based on CMS lines IR58025A & IR62829A. This narrow choice of female lines is one of the factors limiting higher heterosis and improved quality of hybrids. In the initial phases of hybrid rice technology in India, the number of promising hybrids was negligible mainly because of non-adaptable CMS lines that were used in developing hybrids resulted in hybrid impurity. Therefore, several promising CMS lines like DRR2A, APMS 5A, PMS12A, CRMS31A and CR 32 A were developed in India for breeding new hybrids (7). In Bangladesh, potential elite maintainers

Table 1: Agronomic traits of the newly developed CMS lines in comparison to IR58025 A

| CMS lines | TN (nos) | PH (cm) | PL (cm) | FLL (cm) | FLW (cm) | FLA ($A^o$) | DFF (days) | NSP (nos) | TSW* (g) |
|---|---|---|---|---|---|---|---|---|---|
| Nemat A | 26.3 | 92.2 | 28.6 | 29.4 | 1.9 | 14.1 | 116 | 165.6 | 29.8 |
| Neda A | 34.9 | 83.3 | 24.3 | 26.8 | 1.4 | 31.4 | 114 | 125.5 | 28.5 |
| Dasht A | 19.3 | 108.6 | 27.7 | 22.3 | 1.5 | 23.1 | 114 | 210.7 | 22.9 |
| Amol 3 A | 20.8 | 97.2 | 26.1 | 26.2 | 1.4 | 29.3 | 118 | 154.4 | 24.5 |
| Champa A | 26.7 | 91.7 | 25.1 | 24.1 | 1.3 | 27.5 | 119 | 172 | 22.2 |
| IR58025 A | 24.3 | 71.7 | 26.3 | 29.3 | 1.3 | 14.1 | 104 | 127.6 | 26 |
| Mean | 25.4 | 90.78 | 26.35 | 26.35 | 1.47 | 23.25 | 114.17 | 159.3 0 | 25.65 |
| SD | 5.52 | 12.50 | 1.59 | 2.82 | 0.23 | 7.60 | 5.38 | 31.67 | 3.04 |

*A line seed derived from A x B line cross; TN:tiller number(nos), PH: plant height(cm), PL:panicle length (cm), FLL:flag leaf length (cm),
FLW: flag leaf wdth (cm), FLA:flag leaf angle($A^o$),DFF:days to 50% flowering, NSP: number of spikelets per panicle(nos), TSW: thousand seed weight of A line seed(g)

Table 2: Allogamy associated traits of newly developed CMS lines in comparison to IR58025 A.

| CMS lines | OL(mm) | SL (mm) | STL (mm) | FL (mm) | AL (mm) | GOT (AM) | STC (score) |
|---|---|---|---|---|---|---|---|
| Nemat A | 0.46 | 0.22 | 1.66 | 2.34 | 2.11 | 9.1 | 3 |
| Neda A | 0.48 | 0.19 | 1.56 | 2.68 | 2.11 | 9.2 | 4 |
| Dasht A | 0.43 | 0.25 | 1.5 | 2.14 | 2.24 | 9.5 | 2 |
| Amol 3 A | 0.52 | 0.26 | 1.57 | 1.8 | 2.23 | 9.15 | 2 |
| Champa A | 0.53 | 0.27 | 1.54 | 2.1 | 2.43 | 10 | 2 |
| IR58025 A | 0.6 | 0.5 | 1.46 | 2.03 | 2.11 | 9.15 | 3 |
| Mean | 0.50 | 0.28 | 1.55 | 2.18 | 2.21 | 9.35 | 2.66 |
| SD | 0.06 | 0.11 | 0.07 | 0.30 | 0.13 | 0.35 | 0.82 |

OL:ovary length(mm), Sl:style length(mm),STL:stigma length(mm),FL:filament length(mm), AL:anther length(mm), GOT:glume opening time(AM), STC: stigma colour (score 1 to 5)

Table 3: Coefficients of phenotypic correlations among different floral traits of CMS lines.

| Floral traits | SL | STL | FL | AL | ALP | DGO |
|---|---|---|---|---|---|---|
| OL | 0.771* | -0.371 | -0.600 | -0.030 | 0.371 | 0.429 |
| SL | | -0.600 | -0.829* | 0.334 | -0.257 | 0.143 |
| STL | | | 0.257 | -0.213 | -0.029 | -0.086 |
| FL | | | | -0.334 | 0.429 | -0.086 |
| AL | | | | | 0.698 | 0.759* |
| ALP | | | | | | 0.600 |

* correlation is significant at the .05 level (1-tailed) ; OL:ovary length (mm), Sl:style length (mm), STL:stigma length(mm), FL:filament length (mm), AL:anther length (mm), ALP:angle between lemma and palea ($A^o$), DGO:duration of glume opening (minutes)

have been identified in their own local germplasm such as BR29, BR5876-6-2-1, BR5690-62-23, BR5882-12-2-1, BR5892-32-5-3 (6). The two widely used CMS lines are IR58025A and IR62829A; however do not possess specific resistance to any of the major diseases and insect pests (12). The CMS lines like 'Nemat' A, 'Neda'A, 'Amol3'A developed by us are in high yielding backgrounds and at the same time possess high levels of resistance to blast and moderate tolerance to stem borer. 'Nemat' A had the longest FLL with least FLA ($14.1^{o}$) making it highly efficient in photosynthesis. The 'Nemat' A also possessed longest panicle with 28.6 cm, highest 1000 grain weight (A x B crossed seeds) and with good opening period of 203 minutes making it ideal for CMS conversion.

Selection for floral traits that increased cross-pollination such as stigma length and the proportion of extruded stigmata improved hybrid rice seed production (15, 16). In the backcross program of developing the 5 CMS lines we were careful enough to select for extruded stigma and longer stigma. Xu and Li (22) reported out crossing rates ranging from 14.6 to 53.1% in various experiments conducted at Changsha, Hunan, China. Extent of natural out crossing on CMS lines of rice depends largely on the floral trait characteristics of both the CMS and the pollen parent and prevailing the environmental factors at the time of flowering. Certain agronomic traits like the PH, FLL, FLA, panicle exertion (PE) etc., also affects natural out crossing in the CMS lines.

Besides sterility, several other factors could prolong the DGO. For instance, changes in morphology or ALP could prolong the DGO (1, 5, 8). Simultaneous action of lodicules, filaments, anther, ovary and stigma helps the glumes to open in rice (10). Short, coarse spikelets with thick anthers, for instance, had wider angles, whereas the long slender spikelets with narrow anthers had narrow angles (10). Wide and prolonged DGO in the CMS lines enables the well-exerted stigma to intercept with ease the airborne pollen. However, such an ideal type of CMS line in the present study fitted well with 'Neda' A and 'Dasht' A , 'Champa' A (Fig.1, Table 2).

In tropical conditions, indica rice cultivars bloom at 08.30 h and is terminated between 1100 h to 1200 h. Peak blooming in rice occurs between 1000 and 1030h irrespective of the spikelet morphology (10). However, a japonica CMS line WU 10A bloomed between 1000 h to 1100 h at IRRI, Philippines(18). In the present study all the A lines including IR58025A initiated blooming between 0910 h to 0920 with an exception of 'Champa' A and 'Dasht' A line that had opened at 0950 h and 1000 h respectively. The majority of rice varieties initiated blooming at 1000-1100 h in Northwest Indian conditions (10).

Parmar et al., (11) reported a distinct positive correlation between stigma surface and the frequency of varieties with exerted stigma. In their study, the length of stigma surface tended to show a negative correlation with anther size. A similar negative correlation (-0.213) was observed but not of much significance between STL and AL. However, in the present study we found a significant positive correlation (0.771) between the OL and the SL. However, showed negative correlations of STL with OL (-0.371) and SL (-0.60). Interestingly, the AL showed a significant positive correlation (0.759) with the DGO. Likewise, the AL had non-significant high correlation with ALP (0.698) which indicated clearly its importance for visual selection in developing better CMS lines. Out crossing in rice depends upon the capacity of stigmas to receive alien pollen and it is a function of the time interval from flowering to pollen emission, stigma size and extrusion of stigmas from the flower as conditioned by the style plus stigma length (18). These results indicate that improvement in floral traits of female and male parents has to be done separately to increase out crossing potential in rice in relation to hybrid rice breeding and seed production programs. The out crossing rate for the five different CMS lines varied from 14 % to 28% (data not shown) and was not stable on account of improper flowering synchronization and unfavourable weather conditions at the time of flowering. However, 'Neda' A, 'Dasht' A and 'Nemat' A showed higher out crossing rate over IR58025A (18 % out crossing rate) probably due to longer stigma length, wider angle and duration of lemma and palea opening.

We were successful in developing as many as 5 CMS lines in locally adapted background for an effective transfer of hybrid rice technology in Iran. Amongst these 5 CMS lines, 'Nemat'A and 'Neda'A are in high yielding (8 t/ha) recombinant inbred varietal backgrounds with complete resistance to blast disease and moderate tolerance to stem borer.

In this study all the eight Iranian varieties were found to be good maintainers. Later on, through our testcross program, we found lack of restorers in the local Iranian germplasm and therefore we are currently depending on IRRI bred restorers. However, this has helped us to identify combinations that are more heterotic due to involvement of diverse parents. IR24 was found to be good restorer for 'Neda'A line, based on the high pollen and spikelet fertility of 75.5 and 84.4 percent in the $F_1$ testcross of 'Neda' A/IR24 respectively.

Currently, we are conducting yield and quality evaluation trials of the promising hybrids. Simultaneously, attempts on large-scale CMS multiplication and hybrid seed

Table 4: Partial and complete restorers of CMS 'Neda' A line

| S.No. | Crosses | $F_1$ Pollen fertility (%) | $F_1$ Spikelet fertility (%) | Status of pollen parent |
|---|---|---|---|---|
| 1 | Neda A /IR28 | 81.5 | 12.1 | Partial Restorer |
| 2 | Neda A /Amol 2 | 66.0 | 12.2 | Partial Restorer |
| 3 | Neda A /IR62030 | 88.7 | 55.5 | Partial Restorer |
| 4 | Neda A / IR56 | 75.5 | 61.2 | Restorer |
| 5 | Neda A / IR36 | 61.6 | 48.9 | Partial Restorer |
| 6 | Neda A / IR60966 | 78.8 | 80.0 | Restorer |
| 7 | Neda A / IR24 | 75.5 | 84.4 | Restorer |
| 8 | Neda A / Amol 1 | 87.3 | 52.4 | Partial Restorer |

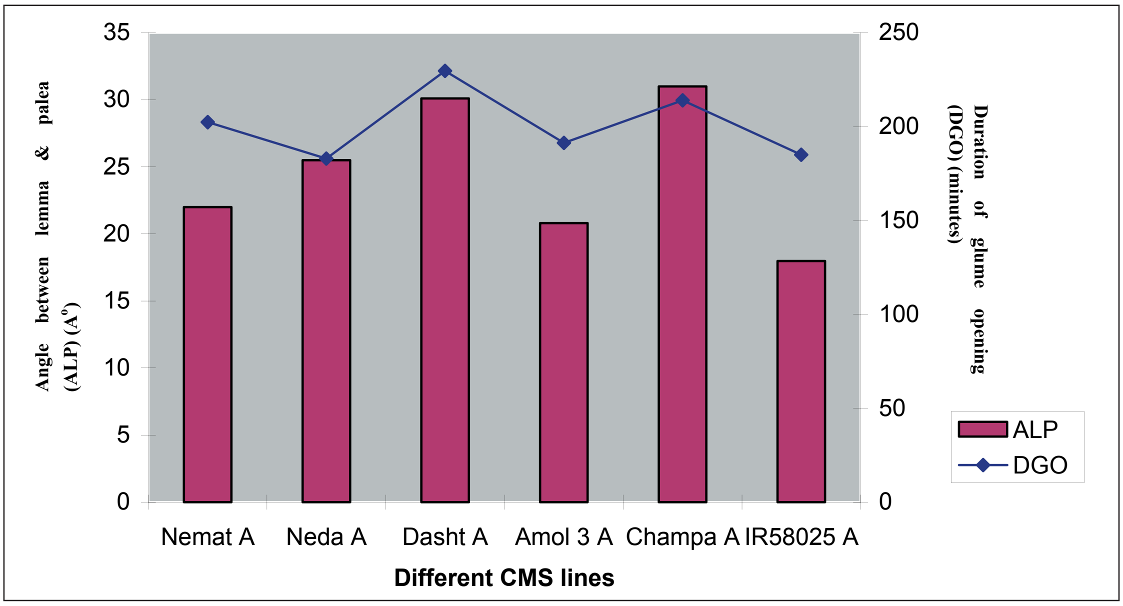


Figure 1: Floret opening angle and duration of newly developed CMS lines in comparison to IR58025A

production are being worked out.

## ACKNOWLEDGEMENTS

We are thankful to International Rice Research Institute, Philippines for kindly providing us the parental seed material. The authors also thank the Director of AREO and Deputy Minister of Agriculture, Iran. We acknowledge the support and facility provided by Mazandaran University, Sari, Rice Research Institute of Iran, Amol and Sari College of Agricultural Sciences, Sari, Iran.

## REFERENCES

[1] Gill K. S., Saini, S. S., George M. M., Kandola H. S. Open –spikelet, a radiation induced mutant character in rice. Curr Sci. (1969) 38:82-84.

[2] Govindaraj K., Virmani S. S. Causes of incomplete fertility-restoring ability of some established restorer lines of WA cytosterility system in rice. RGN. (1987) 4:104-107.

[3] Ikehashi H., Wan J. The wide compatibility system: current knowledge of its genetics and use for enhanced yield heterosis, in: Virmani S.S., Siddiq

E.A., Muralidharan K. (Eds). Advances in hybrid rice technology, International Rice Research Institute, Manila, Philippines. 1998, pp. 67-77.

[4] IRRI. Standard evaluation system. International Rice Research Institute, Manila, Philippines. 1996.

[5] Jachuck P. J., Sampath S. Palea less mutant in African cultivated rice, Oryza glaberrima S. Curr Sci. (1969) 39: 372.

[6] Julfiquar A.W., Virmani S. S., Haque M. M., Mazid M. A., Kamal M. M. Hybrid rice in Bangladesh. opportunities and challenges, in. Peng S., Hardy B. (Eds). Rice research for food security and poverty alleviation, International Rice Research Institute, Manila, Philippines. 2001, pp. 167-177.

[7] Krishnaiah K., Virakthamath B. C. Ilyas Ahmed M. Vijaykumar C. H. M., Ramesha M. S. Recent developments in hybrid rice research in India, in. Peng S., Hardy B. (Eds). Rice research for food security and poverty alleviation, International Rice Research Institute, Manila, Philippines. 2001, pp. 179-191.

[8] Narhari P., Bora K.C. Radiation induced spikelet abnormalities and mutations in rice. Indian J Genet. (1963) 23:7-18.

[9] Nematzadeh G. A., Planning and programming of national hybrid rice research project. Program Report. Rice Research Institute of Iran, Rasht, Iran. 1994. pp 1-5

[10] Parmar K. S., Siddiq E.A., Swaminathan M.S. Variation in components of flowering behaviour of rice. Indian J Genet. (1979) 39:542-550.

[11] Parmar K. S., Siddiq E.A., Swaminathan M. S. Variation in anther and stigma characteristics in rice. Indian J Genet. (1979) 39:542-550.

[12] Siddiq E. A., Ilyas Ahmed M., Viraktamath B. C., Rangaswamy M.

Vijaykumar R., Vidyachandra B., Zaman F. U., Chatterjee S. D. Hybrid rice technology in India. current status and future outlook, in: Virmani S.S., Siddiq E.A., Muralidharan K. (Eds). Advances in hybrid rice technology, International Rice Research Institute, Manila, Philippines. 1998, pp. 311-324.

[13] Singh R. J., Shrisha Y., Singh A. K., Singh A. Evaluation of CMS lines for various floral traits that influence outcrossing in rice (Oryza sativa L.). IRRN (2003) 28: 24-26.

[14] Suh H. S., Multiple pistilate male sterile rice for hybrid seed production, in. Hybrid rice, International Rice Research Institute, Manila. Philippines. 1988, pp. 181-187.

[15] Taillebois J., Guimaraes E.P. Improving outcrossing rate in rice (Oryza sativa L.). In. Hybrid rice, International Rice Research Institute, Manila. Philippines. 1988, pp. 175-180.

[16] Virmani S. S., Athwal D.S. Genetic variability for floral characters influencing outcrossing in Oryza sativa L. Crop Sci. (1973): 13.66-67.

[17] Virmani S. S., Edwards I. B. Current status and future prospects for breeding hybrid rice and wheat. Adv. Agron. (1983) 36:145-214.

[18] Virmani S. S., Heterosis and hybrid rice breeding, Monographs on Theoretical and Applied Genetics 22, Springer-Verlag, Berlin. 1994.

[19] Virmani S.S., Chaudhary R.C., Khush G.S. Current outlook on hybrid rice. Oryza (1981) 18:67-84.

[20] Virmani S. S., Viraktamath B. C., Casal C. L., Toledo R. S., Lopez M. T., Manalo G.O. Hybrid rice breeding manual, International Rice Research Institute, Manila, Philippines. 1997.

[21] Virmani S.S., Aquino R.C., Khush G. S. Heterosis breeding in

rice (Oryza sativa L.). Theor Appl Genet. (1982) 63:373-380.

[22] Xu S., Li B. Managing hybrid rice seed production, in. Hybrid rice, International Rice Research Institute, Manila. Philippines. 1988, pp. 157-163.

[23] Yuan L.P., Virmani S.S., Mao C. X. Hybrid rice-achievements and future outlook, in. Progress in Irrigated Rice Research, International Rice Research Institute, Manila, Philippines, 1989, pp 219 – 235.

[24] Yuan L. P., Increasing yields potential in rice by exploitation of heterosis, in. Virmani S.S. (Ed.) Hybrid Rice Technology. new developments and future prospects, International Rice Research Institute, Manila, Philippines. 1994, pp. 1-6.

[25] Yuan L. P., The execution and theory of developing hybrid rice. zhong gue mongye kexut (Chinese Agric). (1977) 1:27-31.