International Journal of Advanced Information Science and Technology (IJAIST) ISSN: 2319:2682
Vol.3, No.12, December 2014 DOI:10.15693/ijaist/2014.v3i12.88-93

A Study on Out Crossing influencing Mechanisms in Rice

Rajesh Singh*
Professor/Department of GPB,
Instt. of Agril. Sciences, BHU,
Varanasi-221005 (U.P.), India
* Corresponding Author

B. Singh
Student, Department of GPB
BHU, Varanasi

Lekha Ram
Student, Department of GPB
BHU, Varanasi

***Abstract*— In large scale hybrid rice seed production plots in china, natural out crossing on male sterile lines has been reported up to 75 per cent with the help of external and artificial parameters including growth hormone GA_3. The cultivated rice varieties express limited natural out crossing only up to 1-2 per cent. In the present study an attempt has been made to identify a cheaper alternative of GA_3. Further, the rice germplasm and certain male sterile lines have also been screened for certain suitable floral traits, which may also influence the out crossing. The growth hormones along with suitable floral traits can together enhance out crossing of the cyto-sterile lines significantly. In the present study the effects of 25 treatments were evaluated on Cyto-Sterile line i.e., IR 58025 A/B. Based on this experiment another experiment was planned to evaluate four selected treatments 60 ppm GA_3, 40 ppm GA_3, 60 ppm Mangiferin and 40 ppm Mangiferin on four cyto-sterile line viz., IR 58025A, PMS 2A, PMS 3A and PMS 10A. It was observed that number of treatments scored much higher values than control for the characters like duration of florets opening, angle of opened florets and some other out crossing influencing traits. The study also helped in identification of mangiferin as cheaper alternative of GA_3.**

***Index terms*–Out crossing, Rice, GA_3, Mangiferin, Hybrid seed production.**

I. INTRODUCTION

Low out crossing of CMS lines in rice is one of the major problems for low seed set in hybrid seed production plots. A cyto-sterile line with high out crossing potential will economize the cost of hybrid seed including male sterile line (parental line) seed production. Male sterility is most important trait to influence out crossing. Male fertile plant show very little or no out crossing due to self pollinating nature of rice flower. However in male sterile plants, extent of out crossing is further influenced by its floral trait. A wide range (0-44%) of natural out crossing has been observed in male sterile plants/lines (Stansel and Craigmiles, 1966; Athwal and Virmani, 1972; Carnahan *et al.*, 1972; Azzini and Ruteger, 1987). Xu and Li (1988) reported out crossing rates ranging from 14.6 to 53.1% in various experiments conducted at Changsha, Hunan, China. Variability in extent of natural out crossing in male and female lines of rice can be attributed to variations in flowering behavior, floral characteristics of male sterile and pollen parents, and variations in environmental factors. Growth hormones like GA_3 are known to influence floral traits and out crossing. Some recent studies have indicated Mangiferin as cheaper alternative of GA_3 (Singh and Sahoo, 1997 & 1998). The aim of present experiment was to establish the effectiveness of Mangiferin as new growth hormone and cheaper alternative of GA_3 by testing its performance over number of cyto-sterile lines and identifying certain floral traits which can influence out crossing.

II. MATERIALS AND METHOD

Experiments were conducted during 2011-13 to evaluate the treatments and understand the flowering behavior of male sterile lines influencing out crossing of cyto-sterile lines in rice. This experiment comprised of twenty five treatments (Table 1), which included GA_3, glycine, boric acid, urea solution and a new growth hormone Mangiferin either singly or in combination. Leaf clipping and rope pulling were common practice with all the treatments. These treatments were tested on cyto-sterile line seed production of IR 58025 A/B. Based on this study four treatments (GA3 40 ppm & 60 ppm; Mangiferin 40 ppm & 60 ppm) were selected and were tested on four cyto-sterile lines viz., IR 58025A, PMS 2A, PMS 3A and PMS 10A. Cyto-sterile lines A/B were planted into 4:2 ratio.

A spacing of 20 cm was maintained between lines and 15 cm with in lines. Each plot consisted of two set of A:B in 4:2 ratio of two meter row length. The experiment was laid out in Randomized Block Design with three replications during Kharif, 2010-11 at Experimental Farm of Institute of Agricultural Sciences, BHU, Varanasi. The Flag leaf clipping and Rope Pulling were common practice to whole experiment. At five per cent flowering stage, the flag leaves were clipped with the help of scissor. This may enhance the out crossing by allowing free flow of pollen of the B to A. Further, rope pulling was performed to increase the supplementary pollination in the absence of wing current. The treatments were applied with low volume sprayer at 5 per cent of flowering stage. The observations on following characters were recorded i) Duration of opening of florets (minutes), ii) Exserted stigma percentage, iii) Angle of florets opening, iv) Length of spikelets (mm), v) Plant height (cm), vi) Panicle exsertion percentage, and vii) grain yield per hectare (kg.). The data recorded on the different treatments were subjected to statistical analysis based on simple means of the various characters of five plants under observation in three replications. Analysis of variance was performed following Panse and Sukhatme, 1954.

Table 1: Twenty five treatments used to evaluate hybrid seed production technology

T_1	60 ppm GA_3 + Flag leaf clipping + Rope pulling.
T_2	60 ppm Mangiferin + Flag leaf clipping + Rope pulling
T_3	40 ppm Mangiferin + Flag leaf clipping + Rope pulling
T_4	40 ppm GA_3 + Leaf cutting + Rope pulling
T_5	40 ppm glycine + Flag leaf clipping + Rope pulling
T_6	80 ppm Mangierin + Flag leaf clipping + Rope pulling
T_7	80 ppm Glycine + Flag leaf clipping + Rope pulling
T_8	1.5% Boric acid + 2% Urea solution + Flag leaf clipping + Rope pulling
T_9	60 ppm GA_3 + 1.5% Boric acid + 2% Urea solution + Flag leaf clipping + Rope pulling
T_{10}	60 ppm Mangiferin + 1.5% Boric acid + 2% Urea solution + Flag leaf clipping + Rope pulling
T_{11}	40 ppm GA_3 + 1.5% Boric acid + 2% Urea solution + Flag leaf clipping + Rope pulling
T_{12}	40 ppm Mangierin + 1.5% Boric acid + 2% Urea solution + Flag leaf clipping + Rope pulling
T_{13}	40 ppm Glycine + 1.5% Boric acid + 2% Urea solution + Flag leaf clipping + Rope pulling
T_{14}	80 ppm Mangiferin + 1.5% Boric acid + 2% Urea solution + Flag leaf clipping + Rope pulling
T_{15}	80 ppm Glycine + 1.5% Boric acid + 2% Urea solution + Flag leaf clipping + Rope pulling
T_{16}	60 ppm GA_3 + 60 ppm Mangiferin + Flag leaf clipping + Rope pulling
T_{17}	40 ppm GA_3 + 40 ppm Glycine + Flag leaf clipping + Rope pulling
T_{18}	40 ppm GA_3 + 40 ppm Mangiferin + Flag leaf clipping + Rope pulling
T_{19}	40 ppm GA_3 + 40 ppm Mangiferin+1.5% Boric acid + 2% Urea solution + Flag leaf clipping + Rope pulling
T_{20}	40 ppm GA_3 + 40 ppm Glycine + 1.5% Boric acid + 2% Urea solution + Flag leaf clipping + Rope pulling
T_{21}	60 ppm GA_3 + 60 ppm Mangiferin + 1.5% Boric acid + 2% Urea solution + Flag leaf clipping+Rope pulling
T_{22}	Only leaf clipping.
T_{23}	Only rope pulling.
T_{24}	Flag leaf clipping + Rope pulling.
T_{25}	Control.

III. Results and Discussion

A. To evaluate available hybrid seed production technology along with new growth hormone ‘Mangiferin’

Rice being predominantly a self pollinated crop, doubts have often been raised about the economic viability of the hybrid seed production.

Efficient seed production package has been given as much research emphasis as the improvement of cyto-sterile lines and development and evaluation of hybrids. So, with the help of present investigation an attempt has been made to evaluate the available hybrid seed production technology along with new growth hormone 'Mangiferin'. Keeping in view, the high cost of GA_3, an attempt in the present study has been made to replace it with some other cheaper growth hormones/chemicals, like 'Mangiferin' and 'Glycine'. Response of twenty five treatments involving GA_3, boric acid, urea and new growth hormone or chemical mangiferin and glycine were valuated in different concentrations and combinations to study their effect on characters like duration of opening of florets, stigma exsertion percentage, angle of opened florets, plant height before spray and after spray, panicle exsertion at 5 per cent, 50 per cent flowering and maturity, seed yield per hectare, test weight and spikelet length. Analysis of variance showed highly significant difference for all the characters except panicle exsertion at 50 per cent flowering, which was significant at 5 per cent level of significance. In general, it was observed that treatment combination having GA_3 and mangiferin gave positive response for most of the floral and associated traits. Treatment combination having GA_3 like T1–(60 ppm GA_3), T4 – (40 ppm GA_3) and T9 – (GA_3 + 1.5% boric acid) influenced more than one floral and other characters viz., duration of opening of florets, percentage of exserted stigma, grain grain yield per plant, plant height, panicle exsertion, etc. Treatment combination T9 was one of the most effective combinations as it influenced almost all the characters presently studied. Similar results have been obtained by the GA_3 spray by Virmani *et al.* (1981), Virmani and Edwards (1983), Yuan (1985), Xu and Li (1988), Virmani (1994, 1996), Singh and Sahoo (1996) and Singh (2012, 2014).

Table 2: Ranking of Top 10 treatments affecting the grain yield of IR 58025 A/B multiplication.

Sl. No.	Treatment No.	Treatment	Yield Kg/ha.*
1	T1	60 ppm GA_3 + FLC + RP	1417 (100.14%)
2	T4	40 ppm GA_3 + FLC + RP	1230 (73.72%)
3	T9	60 ppm GA_3 + B.A. + U + FLC + RP	1081 (52.68%)
4	T21	60 ppm GA_3 + 60 ppm Mangiferin + U + FLC + RP	1043 (47.31%)
5	T2	60 ppm Mangiferin + FLC + RP	984 (38.98%)
6	T5	40 ppm Glycine + FLC + RP	984 (38.98%)
7	T15	80 ppm Glycine + BA + U + FLC + RP	890 (25.70%)
8	T17	40 ppm GA_3 + 40 ppm Glycine + FLC + RP	868 (22.60%)
9	T3	40 ppm Mangiferin + FLC + RP	859 (21.32%)
10	T25	Control	708

Values in parenthesis are % increase over control; FLC:Flag leaf clipping,RP:Rope pulling,BA:Boric acid, U: Urea.

It was interesting to note that the new growth hormone in different combinations and concentrations almost responded as well as GA_3. Some of the important treatments having Mangiferin were T2 (60 ppm mangiferin), T12 (40 ppm mangiferin + Boric acid), T6 (80 ppm mangiferin), T14 (80 ppm mangiferin + 1.5% boric acid) which influenced the traits like duration of opening of florets, percentage of exserted stigma, angle of opened florets, grain yield per hectare, plant height and panicle exsertion by recording much higher values than control. The treatment T16 which contain GA_3 as well as mangiferin in 60 ppm concentrations along with flag leaf clipping and rope pulling gave much higher values for duration of opening of florets and percentage of exserted stigma than control. Another important finding was use of Glycine as a out crossing promoting agent as established by the treatment T17 (GA_3 40 ppm + Glycine 40 ppm) by scoring 23 per cent more yield than control. It is clear from this experiment that GA_3 as well as mangiferin are influencing the important floral associated traits and seed yield equally. GA_3 has advantage of influencing grain yield per hectare more substantially than mangiferin. More experiments will be required to establish mangiferin as a growth regulator influencing hybrid seed production in rice. In general it was also observed that flag leaf clipping and rope pulling helped in more seed set by out yielding control values where these procedure were not followed. Virmani (1994) observed that flag leaves taller than the panicles are the main obstacles to cross pollination, their cutting helps to remove pollen barrier to pollen spread, Singh and Sinha (1987) also observed that removal of flag leaves gave higher seed set in V20 A/B fields than flag leaf not removed. Better seed setting after flag leaf removal were also observed by Satato (1984), Sahai *et al.* (1987), Virmani (1994, 1996). As far as use of GA_3 is concerned, better seed setting has been observed by number of workers spraying GA_3 at 5 to 10 per cent flowering stage. There are reports of Vasundhara (1987), Kim and Heu (1990), Authammar *et al.* (1993), Virmani (1994, 1996), Singh and Sahoo (1996) etc. Prasad *et al.* (1988) substituted GA_3 with boric acid and urea and found that they were as effective as GA_3 in increasing hybrid seed set. However, effect of mangiferin and glycine in higher seed setting is being reported first time.

Table 3: On the basis of effectiveness with respect to important floral and related traits some of the promising treatments have been ranked as follows.

Sl. No.	Treat. No.	Treatment	Characters influenced*
1	T1	60 ppm GA_3	Duration of opened florets, angle of opened florets, percentage of exserted stigma, plant height, panicle exsertion, grain yield per hectare, spikelet length, test weight
2	T17	40 ppm GA_3 + 40 ppm Glycine	Duration of opened florets, angle of opened florets, percentage of exserted stigma, plant height, panicle exsertion, grain yield per hectare, spikelet length, test weight
3	T3	40 ppm Mangiferin	Duration of opened florets, angle of opened florets, percentage of exserted stigma, plant height, panicle exsertion, grain yield per hectare, spikelet length
4	T16	60 ppm GA_3 + 60 ppm Mangiferin	Duration of opened florets, angle of opened florets, percentage of exserted stigma, plant height, panicle exsertion, grain yield per hectare, spikelet length
5	T9	60 ppm GA_3 + 1.5 % Boric acid	Duration of opened florets, angle of opened florets, percentage of exserted stigma, plant height, panicle exsertion, grain yield per hectare and spikelet length
6	T12	40 ppm Mangiferin + 1.5% Boric acid	Duration of opened florets, angle of opened florets, percentage of exserted stigma, plant height, panicle exsertion, grain yield per hectare
7	T14	80 ppm Mangiferin + 1.5% Boric acid	Duration of opened florets, angle of opened florets, percentage of exserted stigma, plant height, panicle exsertion
8	T4	40 ppm GA_3	Duration of opened florets, angle of opened florets, percentage of exserted stigma, plant height, panicle exsertion, grain yield per hectare, spikelet length
9	T6	80 ppm Mangiferin	Duration of opened florets, angle of opened florets, percentage of exserted stigma, plant height, panicle exsertion, grain yield per hectare, spikelet length

B. Evaluating Comparative effect of Comparative effect GA_3 and Mangiferin

The four treatments viz., 60 ppm and 40 ppm, GA_3; 60 ppm and 40 ppm, Mangiferin along with control were evaluated on four cyto-sterile lines viz., IR 58025A, PMS 2A, PMS 3A and PMS 10A. The data on eight characters which may influence out crossing were recorded and evaluated on four cyto-sterile lines. In general the mean performance of all the four CMS lines (Table 4) revealed that all the treatments scored much higher values than control for the characters like duration of florets opening, angle of opened florets, panicle exsertion, spikelet length and grain yield per ha. Keeping these performances in view the treatments for their effectiveness were

Table 4: Comparative effect of best four treatments on the seed yield (*Grain yield per ha in kg)* of Male Sterile lines on A/B seed production plots.

Sl. No.	Treatments	IR58025A	PMS 2A	PMS 3A	PMS 10A	Mean CMS
1	GA_3 60 ppm	3260 (1)	174	189	300	983
2	Mangiferin 60 ppm	2167	238	531	177	778
3	GA_3 40 ppm	2300	241	115	149	701

4	Mangiferin 40 ppm	2400	100	236	272	752
5	Control	2367	203	223	88	720
Mean over treatments		2467	191	239	157	

ranked as 1) GA_3 60 ppm, 2) Mangiferin 60 ppm, 3) GA_3 40 ppm, 4) Mangiferin 40 ppm, and 5) control. Over all the seed yield of CMS line in A×B seed production plot is most important indicator of out crossing potential. In CMS line IR 58025A is the best line followed by PMS 3A, PMS 2A and PMS 10A (Table 4). Further, the GA3 60 ppm was best combination followed by Mangiferin 60 ppm (Table 4). One of the interesting observations was that GA_3 affected more to plant height than mangiferin. Similar observations were made by Singh and Sahoo (1997 and 1998). Spikelet length was not much affected by application of different treatments only exception was 60 ppm GA_3. It was clearly established by this experiment that mangiferin as well as GA_3 have positive effect on floral and related traits and also they helped in better seed setting on all the four cyto-sterile lines. Singh and Sahoo (1997) reported similar findings on single cyto-sterile lines IR 58025A. Cyto-sterile line IR-58025A also scored much higher values for grain yield per ha, spikelet length and plant height. Similar trends were observed when control data were evaluated, indicating equal response to all the four cyto-sterile lines. For grain yield per ha (mean over five treatments) IR-58025A/B (2499 kg) recorded much higher values than PMS-10A (157 kg), PMS-3A (239 kg) and PMS-2A (191 kg). Similar trends were observed when control data were compared for the four cyto-sterile lines indicating towards pronounced out crossing potential in cyto-sterile line IR 58025A. Virmani (1994) have also reported high about out crossing potential of IR 58025A in Indian conditions.

C. Screening of Rice Germplasm for floral traits influencing out crossing in Rice

A cyto-sterile line with high out crossing potential will certainly economize the cost of hybrid seed production in rice. Under this study, the rice germplasm was screened for the genotypes having useful floral traits which may influence out crossing in rice and will serve as potential and prospective maintainers and restorers. Isolation of prospective maintainer with useful floral characteristics may lead to development of cyto-sterile lines with high out crossing potential through back crossing programme. Seventy five genotypes were screened and evaluated for floral and related traits like; duration of opening of florets, angle of opened florets, percentage of exserted stigma, spikelet length, anther length, stigma length, panicle length, number of effective tillers per plant, grain yield per plant and plant height. The useful genotypes screened under this study have been listed in table 5. Analysis of variance recorded highly significant treatment difference for all the traits indicating the presence of sufficient degree of variation for these traits for selection among the cultivars. The present finding is in agreement with Singh (1995). The evaluation of mean performance of above ten characters led to screening of genotypes like Aditya, VL Dhan-8, Vibhava, Pratibha, Kunti, Surajmukhi, Vikas, etc. having multiple floral traits which affect the out crossing in positive direction. Singh (1995) made similar observations while screening fifty genotypes of rice for various floral and related attributes. He observed that genotypes

Table 5: Genotype Screened for floral traits having high out crossing potential in 75 Rice Germplasm.

	Characters	**Genotypes**
1	Duration of floret opening	Aditya, IET-101102, IET-10307, IET-10311,Vibhava, IET-10322
2	Angle of opened florets	VL Dhan 63, VL Dhan 8, IET-101111, IET-101112, IET-101115, IET-101116
3	Percentage of exserted stigma	IET-10317, IET-10319, IET-101114, IET-101119, Vibhava, Pratibha
4	Anther length	IET-101110, IET-101113, Ravi, IET-10315, BK-190
5	Spikelet length	IET-101115, IET-101116, IET-101104, IET-101117, IET-101106
6	Stigma length	IET-101115, IET-101104, IET-101107, IET-101111, IET-101114.

like Rambhog, Sugandha, HPR-167, IRT-05476, China-9, Sukhawana and Dee-Geo-Woo-Gen having multiple floral traits with high out crossing potential. Mahadevappa (1994) also screened rice genotypes for important floral traits found Pokkali, PMS-9A, PMS-10A, Basmati, V20B, Jyothi, IR-42 and TRB-63, etc. having multiple floral traits, with high out crossing potential. Singh (2009, 2012 and 2014) has also reported about floral traits and growth hormones which can together increase the out crossing of CMS lines and ultimately increase and economies the seed yield of A/B lines. With this experiment, it may safely be concluded that the glycine do help in influencing the seed yield and important floral traits of cyto-sterile lines; New growth hormone mangiferin has established its positive impact on seed production of cyto-sterile lines; All the four cyto-sterile lines responded equally well to the use of out crossing promoters on floral attributes but over all IR 58025 A/B was significantly best, this indicated that there is something more than just floral characteristics which increases the out crossing. There is wide range of variability available in rice germplasm. There is a need to develop male sterile and maintainer lines in the genetic background of rice varieties having potential for high out crossing rate.

REFERENCE

Panse,V.G.and Sukhatme P.V. (1954). Statistical methods for agricultural workers- 2nd Edn. pp 381, ICAR, New Delhi.

Singh, Rajesh and Sahoo, S.K. (1996). Effect of GA_3, boric acid and urea spray on hybrid rice seed production in rice (*Oryza sativa* L.). Abst. 102. In DAE-BRNF Symposium on nuclear techniques in increasing crop and animal productivity held at BARC, Mumbai, October 7-9.

Singh, Rajesh and Sahoo, S.K. (1997). Artificial and natural parameters influencing out crossing of cytosterile line in rice (*Oryza sativa* L.). In International Symposium on the Genetics and Exploitation of heterosis in Maize and other crops. CIMMYT, Mexico A68: 162-163.

Singh, Rajesh and Sahoo, S.K. (1988). Evaluating hybrid seed production technology along with new growth hormone ‘Mangiferin’. International Rice Res. News., 23 (1): 23.

Singh, Rajesh (2009). Hybrid Rice Seed Production. *MM Technical Bulletin* No. **2.**

Singh,Rajesh (2012). Hybrid Rice and Maize Seed Production. *MM Technical Bulletin* No **4.**

Singh, R. (2012). Exploitation of heterosis in rice, Journal *of Biotechnology and crop Science*. **2 (2)**: 16-26.

Singh,Rajesh (2014).Cultivation of Hybrid Rice: A complete Package. *Indian Research J of EE,* Vol. **14** (2&3) 6

Singh Rajesh and Lekha Ram (2012). Ideal Hybrid Rice Seed Production Package: An Overview, Indian Research J of EE: Sp Issue Vol **(2)**:244-251

Virmani, S.S. (1994). Heterosis and Hybrid Rice Breeding. Springer Verlag, Berlin.

Virmani, S.S. (1996). “Hybrid Rice” Adv. Agron.,57: 377-462.

Authors Profile

Dr Rajesh Singh is Professor in the Department of Genetics and Plant breeding since 2006. He has to his credit over 200 publications including 21 books and bulletins. Dr Singh has also developed about 16 rice and maize varieties at National level. He has completed over 25 projects with total turnover of Rs 65 crores. He has over 24 years teaching and research experience in field of Breeding and Biotechnology (Molecular Breeding) of Maize, Rice, Sugarcane Crops and Crop Genetic Resources.

Mr Brijesh Singh received the B.Sc. (Ag.) and Post Graduation from Banaras Hindu University, Varanasi. He has specialization in rice breeding with special reference to hybrid rice breeding. In fact he also worked on similar topic as presented in current paper. The part of post graduate work is also been reported in this paper.

Lekha Ram received the B.Sc. (Ag.) and M.Sc. (GPB) degree from MGCGVV, Chitrakoot, Satna, India and BHU, Varanasi, India in 2001 and 2003, respectively. Currently doing research on QPM conversion programme in Department of Genetics and Plant Breeding, BHU, Varanasi, India. Mr lekha Ram has published over 10 papers and His research interest includes breeding for nutritional quality improvement in cereals and legumes. Mr Lekha Ram during the process of completion of post graduation helped in data generation of current research publication.