**The Journal of Animal & Plant Sciences, 25(6): 2015, Page: 1693-1698**
**ISSN: 1018-7081**

# EFFECTS OF WA CYTOPLASM ON VARIOUS QUALITY CHARACTERISTICS OF RICE HYBRIDS

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## ABSTRACT

The differences between twenty pairs of $AF_1$ (CMS line x restorer) and $BF_1$ (maintainer line x restorer) cross combinations were evaluated to appraise the WA cytoplasmic influence on various quality traits of rice hybrids. The cytoplasmic influence for different traits was found to be highly cross-specific, depending on the nuclear background of CMS line and fertility restorer. Most of the traits were not significantly affected by the sterile cytoplasm in majority of the cross combinations. Only in some crosses, the cytoplasm has significant influence for majority of the traits studied. Most significant effect of the sterility inducing cytoplasm was reduction in length of cooked kernel, followed by decrease in amylose content. Male sterility inducing cytoplasm had no effect on aroma.

**Key words:** CMS line, maintainer, restorer, WA cytoplasm, quality traits, rice hybrids.

## INTRODUCTION

Exploitation of heterosis has been contemplated as a potential strategy for yield enhancement in several crop species. Among various crops in which hybrid varieties are used commercially, rice ranks very high. The average yield of hybrid rice is at least 15-20 percent higher than that of inbred rice and it has been anticipated that hybrid rice technology will play a key role in ensuring food security worldwide in the future decades (Sabar and Akhter, 2003). Among the various approaches for commercial production of hybrid rice, three line (CGMS) system has become a practical option. In this system, cytoplasmic male sterility (CMS) has an essential role to play in hybrid development.

Although, research on the commercial utilization of heterosis in rice has made tremendous gains during the last few decades, it is still in its stage of infancy due to the lack of desirable quality in $F_1$ produce. Nowadays, quality considerations assume enhanced importance due to improved standard of living, imposing increased human demand for high quality rice. In the near future, grain quality will be even more important as once the very poor, many of whom depend on rice for their staple food, become better off and begin to demand for higher quality rice (Welch and Graham, 2002). Thus, the most important challenge in rice hybrid breeding is to ascertain that the heterotic rice hybrids possess grain quality that is at least comparable, if not superior, to that of popular inbred varieties grown by farmers.

Quality concerns in hybrid rice assume enhanced importance mainly due to two reasons; first, the marketable produce is a bulk of segregating endosperms of $F_2$ generation, and second, the apprehension regarding an effect of sterility inducing cytoplasm on physico-chemical grain quality traits. The possible negative influence of sterility inducing cytoplasm on quality of produce obtained from $F_1$ plant, which derives its cytoplasm from the seed parent (CMS line), may affect consumer acceptance (Hariprasanna *et al.*, 2006).

Only a handful of studies have been carried out regarding the effect of male sterile cytoplasm on grain quality traits. Hariprasanna *et al.* (2006) observed favourable as well as unfavourable cytoplasmic effects on the physico-chemical grain quality traits in hybrid rice. The magnitude of effect varied, depending upon the type of sterile cytoplasm and parental combination. Rani (2008) also reported the similar findings while analyzing the effects of male sterile cytoplasm on grain yield and quality characters in rice hybrids. The effect of cytoplasm on various traits has also been reported in crops like tobacco (Chaplin and Ford, 1965), maize (Duvick, 1958) and Sorghum (Quinby, 1970). Though these studies have reported both favourable and unfavourable effects of sterile cytoplasm on different traits, replete knowledge of cytoplasmic influence on quality traits of rice is lacking.

Moreover, among the various types of cytoplasmic male sterility, the wild abortive (WA) system is widely used, accounting for about 90% of the rice hybrids produced in China and 100 per cent of the hybrids developed outside China (Sattari *et al.* 2007). The present investigation was carried out to study the effect of WA cytoplasm on various quality traits of rice hybrids.

## MATERIALS AND METHODS

The present study was carried out over two seasons *viz., kharif*-2012 and *kharif*-2013 at the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (UP). The site of study is situated at $25^0$ 18´ N latitude and $83^0$ 03´ E longitude at an elevation of 80.71 m above mean sea level.

Three cytoplasmic male sterile (or A) lines (IR-58025A, IR-68897A and Pusa 6A) having WA cytoplasm as a source of male sterility as well as their respective maintainer (or B) lines were used as female parents in crossing programme. Eight genotypes (Sanwal Basmati, Pusa Sugandh-2, Pusa Sugandh-3, Pusa Sugandh-5, Pusa 2517-2-51-1, HUR-JM-59221, Pusa-44 and Pusa Basmati-1121) identified as restorer (R) lines were used as male parents in the hybridization programme. All the genotypes (A, B and R lines) were obtained from 'All India Coordinated Rice Improvement Project (AICRIP)' at the Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (U.P.).

During *kharif*-2012, the three CMS lines were crossed with the eight identified restorers to generate the set of 20 rice hybrids. Six restorers (Sanwal Basmati, Pusa Sugandh-2, Pusa Sugandh-3, Pusa Sugandh-5, Pusa 2517-2-51-1 and HUR-JM-59221) were crossed with all the three CMS lines. Moreover, Pusa-44 and Pusa Basmati-1121 were crossed with IR-58025A and IR-68897A, respectively. Corresponding B x R crosses were produced in addition to A x R crosses. In *kharif*-2013, the seed of A x R and B x R hybrids generated during previous season were raised at a standard spacing of 20 x 15 cm in 5 m rows in randomized block design with three replications. Single seedling per hill was planted and recommended package of practices followed to raise a good crop. Data on grain quality characters for A x R ($AF_1$) and B x R ($BF_1$) hybrids was worked out in the laboratory. The Cytoplasmic Effect was calculated as proposed by Sheng and Li (1988).

$$\text{Cytoplasmic Effect} = \frac{\overline{AF_1} - \overline{BF_1}}{\overline{BF_1}} \times 100$$

Where, $\overline{AF_1}$ and $\overline{BF_1}$ are the mean values of A x R and B x R, respectively. The statistical significance of deviation was calculated following 't' test for difference between two means from two independent samples.

$$t = \frac{\overline{X}_1 - \overline{X}_2}{[(S_1^2/r_1) + (S_2^2/r_2)]^{1/2}}$$

Where, $S_1$ and $S_2$ are the variances of mean values $\overline{X_1}$ and $\overline{X_2}$ over replications $r_1$ and $r_2$, respectively.

The calculated 't' value was compared with 't' tabulated at 0.05 and 0.01 levels of significance and appropriate degrees of freedom. The significant deviation was attributed to sterility inducing WA cytoplasm. Accordingly, the cytoplasmic effect was determined as significant or non significant for the different traits studied.

## RESULTS AND DISCUSSION

The performance of $AF_1$ (CMS line x restorer line) hybrid is expected to be equivalent to that of cross generated by the pollination of respective maintainer line with the same restorer ($BF_1$). If the performance of $AF_1$ differs from that of $BF_1$, it may be attributed to the sterile cytoplasm derived from the CMS line as the latter does not involve this. The difference in the performance or in other words the deviation ($AF_1$-$BF_1$), if significant and consistent may be due to the sterility inducing cytoplasm. In the present investigation, 20 pairs of rice hybrids were evaluated to study the cytoplasmic effect for various quality attributes (Table 1).

In case of hulling recovery, two crosses based on IR-58025A and only one cross based on Pusa 6A were observed to show the significant negative value of cytoplasmic influence. For milling recovery, only two hybrids both based on IR-68897A revealed the significant negative value of cytoplasmic effect. However, none of the crosses were observed to show significant cytoplasmic effects for head rice recovery. Hariprasanna *et al.* (2006) reported that the cytoplasmic influence calculated for hulling recovery, milling recovery and head rice recovery were largely non significant. However, Shivani *et al.* (2007) reported that hulling, milling and head rice recovery percentages were lower in A x R crosses as compared to their corresponding B x R crosses. Yi and Cheng (1992) also found that milling quality traits in rice show cytoplasmic effects. Rutger and Bollich (1985) had pointed out that milling recovery in rice hybrids was low due to the CMS lines. Rani (2008) also reported that sterile cytoplasm exerted negative impact on head rice recovery in general and all the $AF_1$ hybrids resulted from IR-58025A were found to be inferior to corresponding $BF_1$ hybrids for the trait.

For kernel length before cooking, five cross combinations from each of CMS lines IR-58025A and IR-68897A, and four hybrids based on Pusa 6A revealed significant negative estimate of cytoplasmic effect. The WA cytoplasm was observed to reduce kernel breadth before cooking significantly in one hybrid based on IR-68897A and four hybrids based on CMS line Pusa 6A. Hariprasanna *et al.* (2006) observed that only few hybrids exhibited significant negative influence of sterile cytoplasm for kernel length and kernel breadth among the WA-CMS based crosses. However, in another study by the same researcher (Hariprasanna, 1998), both positive and negative influences of WA cytoplasm on kernel breadth were reported. Shivani *et al.* (2007) observed negative effects of male sterility inducing cytoplasm for kernel length in different cross combinations. However, Rani (2008) reported that positive effects were predominant on kernel length due to male sterile cytoplasm.

The trait kernel length after cooking was most affected by the male sterile cytoplasm and all the 20 cross combinations were observed to show significant negative cytoplasmic effect. This is in accordance with the reports of Shivani *et al.* (2007) who also observed negative effects of male sterility inducing cytoplasm on kernel length after cooking for various cross combinations. For kernel breadth after cooking, three hybrids based on IR-58025A, and four cross combinations from each of CMS lines IR-68897A and Pusa 6A revealed significant negative effect of sterility inducing cytoplasm. Hariprasanna *et al.* (2006) reported that CMS effect on kernel dimensions after cooking depends on the cytoplasmic nuclear interaction, which makes it cross-specific.

For kernel length/breadth ratio before cooking, one cross combination based on CMS line Pusa 6A revealed significant positive cytoplasmic effect, while another cross from the same CMS line exhibited negative effect of male sterile cytoplasm. Hariprasanna *et al.* (2006) reported that kernel length/breadth ratio before cooking revealed positive as well as negative cytoplasmic effects depending on the cross combination, which confirms the present findings. Shivani *et al.* (2007) observed negative effects of male sterility inducing cytoplasm for kernel length/breadth ratio in different cross combinations. However, Rani (2008) reported that positive effects were predominant on kernel length/breadth ratio due to male sterile cytoplasm. For kernel length/breadth ratio after cooking, one cross combination based on CMS line IR-58025A revealed the significant negative influence of male sterile cytoplasm, while another cross from the same CMS line exhibited the positive effect of sterility inducing cytoplasm. Two hybrids based on CMS line IR-68897A revealed significant positive cytoplasmic influence for length/breadth ratio of cooked kernel. Hariprasanna *et al.* (2006) reported that WA cytoplasm in the CMS lines Pusa 3A and IR-58025A did not negatively influence the appearance of cooked kernel as indicated by the non-significant differences between the $AF_1$ (A x R) and $BF_1$ (A x R) hybrids for kernel length/breadth ratio after cooking.

The elongation ratio was found to reveal non-significant cytoplasmic effect for all the cross combinations. Hariprasanna *et al.* (2006) also reported that the effect of WA cytoplasm for kernel elongation upon cooking was not significant in majority of the cross combinations studied. However, Shivani *et al.* (2007) reported that elongation ratio was positively influenced by the sterility inducing cytoplasm in various cross combinations. In case of elongation index, one hybrid from each of the three CMS lines was found to exhibit the significant positive value of cytoplasmic effect in present study. However, Hariprasanna *et al.* (2006) observed that none of the crosses studied exhibited significant difference for elongation index between $AF_1$ and $BF_1$, and hence negligible cytoplasmic effect was reported for the trait.

The alkali spread value (ASV) which also gives the measure of gelatinization temperature, do not differ significantly between $AF_1$ and $BF_1$ in majority of the cross combinations. In only one cross combination (IR-68897A x Pusa Sugandh-5), the alkali spread value showed significant negative influence of WA cytoplasm. Previous studies by Bong (1988), Yi and Cheng (1992), and Holguin *et al.* (1995) have indicated that the cytoplasmic factors or maternal effect did not influence the gelatinization temperature (or alkali spread value) in rice hybrids. However, Shivani *et al.* (2007) reported that nucleo-cytoplasmic effects for gelatinization temperature were cross specific.

Except for one, all the other hybrids showed significant negative value of cytoplasmic effect for amylose content. Thus, amylose content was highly affected by WA cytoplasm, next only to kernel length after cooking. Shivani *et al.* (2007) also reported that amylose content of A x R hybrids was 1-2% less than their corresponding B x R hybrids and thus, supporting the present findings. In the present study, aroma was not at all affected by the male sterile cytoplasm. Earlier report of Shivani *et al.* (2007) that male sterility inducing cytoplasm had no effects on aroma supports the present findings.

To summarise the findings of present investigation, the WA cytoplasmic influence for different traits was found to be highly cross-specific, depending on the nuclear background of CMS line and fertility restorer. Most of the traits were not significantly affected by the sterile cytoplasm in majority of the cross combinations. Only in some crosses, the cytoplasm has significant positive influence on elongation index. However, the traits, hulling recovery, milling recovery, kernel length before cooking, kernel breadth before cooking, kernel length after cooking, kernel breadth after cooking, alkali spread value and amylose content were found to be negatively influenced by the sterility inducing cytoplasm for single (in case of alkali spread value) to all (in case of kernel length after cooking) cross combinations. For kernel length/breadth ratio before and after cooking, there were both favourable as well as unfavourable cytoplasmic effects depending upon the parental combination. The traits, head rice recovery, elongation ratio and aroma were not significantly influenced by male sterile cytoplasm. Most significant effect of the sterility inducing cytoplasm was reduction in length of cooked kernel followed by decrease in amylose content. The reduction in length of cooked kernel is undesirable as long slender grain type rice varieties fetch maximum premium in the world market. The reduction in amylose content may lead to the production of low amylose rices, which cook moist and sticky, and are not preferable

**Table 1. Cytoplasmic effect (%) for different quality traits**

| Cross Combination | HR | MR | HRR | KLBC | KBBC | KLAC | KBAC | L/B BC | L/B AC | ER | EI | ASV | AC | A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IR-58025A&B x Sanwal Basmati | -2.43 | -2.05 | -1.65 | -1.44 | -1.05 | -1.72$^{**}$ | -1.27 | -0.39 | -0.46 | -0.28 | -0.07 | -2.18 | -1.89$^{*}$ | 0.00 |
| IR-58025A&B x Pusa Sugandh-2 | -1.61 | -2.86 | 1.79 | -1.59$^{**}$ | -1.31 | -2.38$^{**}$ | -1.09 | -0.28 | -1.30$^{*}$ | -0.80 | -1.02 | 1.45 | -1.17$^{*}$ | 0.00 |
| IR-58025A&B x Pusa Sugandh-3 | -1.59 | 1.60 | 1.52 | -1.70$^{*}$ | -1.39 | -1.44$^{*}$ | -2.38 | -0.31 | 0.97 | 0.26 | 1.28 | -0.58 | -1.04$^{*}$ | 0.00 |
| IR-58025A&B x Pusa Sugandh-5 | -1.60 | -2.58 | -2.64 | -1.62 | -2.16 | -1.41$^{*}$ | -4.15$^{**}$ | 0.56 | 2.86$^{**}$ | 0.21 | 2.30$^{*}$ | 1.80 | -1.71$^{*}$ | 0.00 |
| IR-58025A&B x Pusa 2517-2-51-1 | 0.31 | -2.39 | -3.68 | -1.30$^{*}$ | -0.77 | -1.91$^{**}$ | -1.27 | -0.53 | -0.64 | -0.61 | -0.11 | -0.96 | -1.51$^{**}$ | 0.00 |
| IR-58025A&B x HUR-JM-59221 | -0.81 | -1.34 | -1.47 | -1.18$^{*}$ | -1.57 | -2.37$^{**}$ | -2.48$^{*}$ | 0.40 | 0.12 | -1.20 | -0.29 | -1.32 | -1.62$^{*}$ | 0.00 |
| IR-58025A&B x Pusa-44 | -0.94 | -1.42 | -0.48 | -1.65$^{*}$ | -1.72 | -1.68$^{**}$ | -2.24$^{*}$ | 0.07 | 0.58 | -0.04 | 0.50 | -0.31 | -1.00 | 0.00 |
| IR-68897A&B x Sanwal Basmati | -1.76$^{**}$ | -2.47$^{*}$ | -0.21 | -2.64$^{**}$ | -1.93 | -1.47$^{**}$ | -4.09$^{**}$ | -0.72 | 2.74$^{**}$ | 1.20 | 3.49$^{*}$ | 1.11 | -1.42$^{**}$ | 0.00 |
| IR-68897A&B x Pusa Sugandh-2 | -0.86 | -1.87 | -3.09 | -1.02 | -2.28 | -1.54$^{*}$ | -3.08$^{*}$ | 1.28 | 1.58 | -0.52 | 0.28 | -1.29 | -2.01$^{**}$ | 0.00 |
| IR-68897A&B x Pusa Sugandh-3 | -1.83 | -0.25 | -0.33 | -1.31$^{*}$ | -1.33 | -2.27$^{**}$ | -1.43 | 0.02 | -0.85 | -0.97 | -0.87 | 2.56 | -1.09$^{**}$ | 0.00 |
| IR-68897A&B x Pusa Sugandh-5 | -2.26$^{**}$ | -2.06$^{*}$ | -4.40 | -1.37$^{*}$ | -1.84 | -1.30$^{**}$ | -1.53 | 0.48 | 0.24 | 0.07 | -0.24 | -2.05$^{**}$ | -2.03$^{**}$ | 0.00 |
| IR-68897A&B x Pusa 2517-2-51-1 | -1.01 | 0.27 | -1.16 | -1.33$^{*}$ | -1.79$^{*}$ | -1.52$^{*}$ | -2.30$^{*}$ | 0.47 | 0.80 | -0.20 | 0.32 | -1.39 | -2.13$^{**}$ | 0.00 |
| IR-68897A&B x HUR-JM-59221 | -1.80 | -2.36 | -0.64 | -1.41 | -1.02 | -1.52$^{**}$ | -1.28 | -0.39 | -0.24 | -0.11 | 0.15 | 0.64 | -2.10$^{**}$ | 0.00 |
| IR-68897A&B x Pusa-1121 | -1.79 | 0.63 | -0.60 | -1.45$^{*}$ | -1.29 | -1.51$^{**}$ | -2.49$^{**}$ | -0.16 | 1.01$^{**}$ | -0.06 | 1.17 | -1.79 | -1.55$^{**}$ | 0.00 |
| Pusa 6A&B x Sanwal Basmati | -1.17 | -1.58 | -2.06 | -0.91 | -3.20$^{**}$ | -2.39$^{**}$ | -3.43$^{**}$ | 2.36$^{**}$ | 1.08 | -1.49 | -1.25 | -1.16 | -1.76$^{**}$ | 0.00 |
| Pusa 6A&B x Pusa Sugandh-2 | -2.68$^{*}$ | -1.66 | -2.70 | -1.58$^{*}$ | -2.14$^{*}$ | -1.29$^{**}$ | -1.04$^{**}$ | 0.58 | -0.25 | 0.04 | -0.75 | -1.58 | -1.38$^{**}$ | 0.00 |
| Pusa 6A&B x Pusa Sugandh-3 | 0.36 | -1.18 | -2.68 | -1.32$^{*}$ | -1.81$^{*}$ | -1.19$^{*}$ | -1.22 | 0.50 | -0.03 | 0.44 | -0.54 | -1.67 | -1.78$^{*}$ | 0.00 |
| Pusa 6A&B x Pusa Sugandh-5 | -1.43 | -1.42 | -3.20 | -1.63$^{*}$ | -2.11$^{*}$ | -1.89$^{*}$ | -2.52 | 0.50 | 0.64 | -0.32 | 0.06 | 0.55 | -2.07$^{**}$ | 0.00 |
| Pusa 6A&B x Pusa 2517-2-51-1 | -2.52 | -2.38 | -2.94 | -1.64$^{**}$ | -0.39 | -1.21$^{*}$ | -1.12$^{*}$ | -1.26$^{**}$ | -0.08 | 0.44 | 1.19$^{*}$ | 0.21 | -1.13$^{**}$ | 0.00 |
| Pusa 6A&B x HUR-JM-59221 | -0.99 | -3.09 | 3.39 | -1.17 | -1.33 | -1.84$^{**}$ | -2.25$^{*}$ | 0.17 | 0.42 | -0.67 | 0.25 | -0.64 | -1.67$^{**}$ | 0.00 |

[HR= Hulling recovery, MR= Milling recovery, HRR= Head rice recovery, KLBC= Kernel length before cooking, KBBC= Kernel breadth before cooking, KLAC= Kernel length after cooking, KBAC= Kernel breadth after cooking, L/B BC= Kernel length/breadth ratio before cooking, L/B AC= Kernel length/breadth ratio after cooking, ER= Elongation ratio, EI= Elongation index, ASV= Alkali spread value, AC= Amylose content and A= Aroma; $^{*}$, $^{**}$ = Significant at 0.05 and 0.01 levels, respectively] especially in Indian context. Male sterility inducing cytoplasm had no effects on aroma.

Both favourable and unfavourable influence of male sterile cytoplasm has been reported by various workers. The nature and magnitude of cytoplasmic effect vary with sterile cytoplasm, parental combination and trait under study. Sun *et al.* (2006) investigated the genetic effects of male sterile cytoplasms on major characters of rice hybrids and reported that effects of different sterile cytoplasms were different for different traits. Rani (2008) reported both positive and negative influence of male sterile cytoplasm while analyzing the effects of male sterile cytoplasm on grain yield and quality characters in rice hybrids. Hariprasanna *et al.* (2006) observed favourable as well as unfavourable cytoplasmic effects on the physico-chemical grain quality traits in hybrid rice. However, Wang *et al.* (1997) and Qin *et al.* (2013) reported that majority of CMS sources have unfavourable effects on yield related traits and that the unfavourable effects vary among the sources of male sterility. It could be feasible to reduce or overcome the unfavourable effects of cytosterility by choosing appropriate parental lines (Kadoo *et al.,* 2002). Some of the previous studies have revealed that cytoplasmic influence on quality traits is not a major constraint in hybrid rice breeding, though there can be certain specific instances for significant impact of male sterile cytoplasm (Shu *et al.,* 1998 and Xu *et al.,* 1998).

The cytoplasmic influence might be the result of specific nucleo-cytoplasmic interactions. Several rice researchers have opined for the existence of possible interaction between nuclear genome and male sterile cytoplasm for different agronomic traits. Virmani (1996) proposed that cytoplasm as well as nucleo-cytoplasmic interactions influence the heterosis for yield, yield attributes and other agronomic traits. Faiz *et al.* (2007) reported that inconsistent behaviour of the $F_1$ hybrids was the result of nucleo-cytoplasmic interactions rather than the negative effect of the male sterile cytoplasm itself. The development of CMS lines possessing different sources of male sterility in the same nuclear background (alloplasmic CMS lines) and crossing these with a single pollen parent will help in better evaluation of cytoplasmic as well as nucleo-cytoplasmic interaction effects. Hybrids developed using the alloplasmic CMS lines would help in determination of cytoplasmic effect in a more reliable manner and may assist in the development of rice hybrids with less or no undesirable cytoplasmic effects. To minimise the cytoplasmic effects, each cross combination irrespective of the cytosterile source should be evaluated and only those crosses selected where cytoplasmic influences are absent or less predominant. Thus, studies on the effect of male sterile cytoplasm on various traits provide practical information for breeding CMS lines and their appropriate selection to mate with specific restorers for improvement of various quality traits in rice hybrids.

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