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Effects of male sterility inducing cytoplasm on morpho-physiological and biochemical characters in rice (*Oryza sativa* L.)

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Abstract

Three sets of A × R (AF_1) and B × R (BF_1) hybrids were evaluated to study the influence of male sterility inducing (WA) cytoplasm on various morpho-physiological and biochemical characters in rice (*Oryza sativa* L.). The nature and magnitude of cytoplasmic effects varied with character and cross combination. The cytoplasm negatively influenced plant height, spikelet fertility, average panicle weight, 1000-grain weight, biomass yield, harvest index and grain yield, while enhancing number of productive tillers and growth duration. The cytoplasm negatively influenced physiological characters like CGR, RGR, NAR, LAI and LAD and biochemical characters like α-amylase activity in germinating seeds and nitrate reductase activity at various stages of crop growth. It could be feasible to reduce or overcome the negative effects of cytosterility by choosing appropriate parental lines.

Key words : Rice, WA cytoplasm, sterile cytoplasm, effect on physiolgical and biochemical characters

Introduction

Availability of stable cytoplasmic male sterility and fertility restoration system is vital for commercial exploitation of heterosis in any crop. With the discovery of the 'wild-abortive' (WA) male sterility inducing cytoplasm from *Oryza sativa* f. *spontanea* and subsequent development of three-line hybrids made a breakthrough in exploitation of heterosis in rice [1]. The WA cytoplasm has been found to be the most stable [2] and hence about 95% of the rice hybrids world-over are based on this single source of cytosterility. Besides the fear that increased genetic homogeneity of a vital character like cytoplasmic male sterility could make it vulnerable to diseases and pests, some investigations indicated that the sterility inducing cytoplasm influences expression of certain agronomic characters in addition to inducing male sterility. However, such reports are scanty with respect to physiological and biochemical characters, which are equally important in expression of heterosis. Additionally, it has been reported that effects of the cytoplasm vary with cytoplasmic source and cytoplasm-nuclear interactions. Hence it is important to understand the nature and magnitude of influence of the cytoplasm on various morpho-physiological and biochemical characters for their successful manipulation. Such an understanding would facilitate selection of desirable heterotic combinations with the least negative effect of the cytoplasm. Therefore, the present investigation was undertaken to study the effects of sterility inducing cytoplasm on various yield parameters and physiological and biochemical characters influencing grain yield.

Materials and methods

Three sets of hybrids were evaluated to study the effects of WA cytoplasm on various characters. First set involved the hybrids PMS 2A/Pusa 1127 (designated as AF_1-1) and PMS 2B/Pusa 1127 (designated as BF_1-1), while second set contained the hybrids Pusa 5A/Pusa 1124 (AF_1-2) and Pusa 5B/Pusa 1124 (BF_1-2). Whereas third set comprised of the hybrids IR 58025A/PRR 78 (AF_1-3) and IR 58025B/PRR 78 (BF_1-3). The cytoplasmic male sterile lines (A lines), PMS 2A, Pusa 5A, IR 58025A, their respective maintainer lines (B lines) and the restorers (R lines), Pusa 1127, Pusa 1124 and PRR 78 were grown in Kharif 1999 at Indian Agricultural Research Institute (IARI), New Delhi and crosses (A × R and B × R) were afftected to produce three sets of hybrids by hand emasculation and pollination. In Kharif 2000, the hybrids were grown in randomized complete block design with two replications at IARI. Each hybrid was represented by four rows of 3.5 m at a spacing of 20 × 15 cm in both replications. Observations were recorded on yield components and physiological and biochemical characters on five randomly selected plants from middle two rows in each replication. Observations on yield and yield components were recorded at maturity while growth parameters and certain other characters were recorded at different

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growth stages viz seedling, tillering, flowering and maturity. Mean of observations of the five plants was used for further analysis.

The nature and magnitude of effects of the cytoplasm were worked out as percent deviations in the means of AF_1 (A × R hybrid) and BF_1 (B × R hybrid) separately in each set according to the following formula [3]:

$$\text{Deviation (\%)} = [(\overline{AF}_1 - \overline{BF}_1)/\overline{BF}_1] \times 100$$

Significance of the deviations was tested using the 't' test.

Growth parameters : Crop growth rate (CGR, mg/plant/day), relative growth rate (RGR, mg/g/plant/day) and net assimilation rate (NAR, mg/dm^2/day) were calculated for following stages: a) seedling to tillering, b) tillering to flowering and c) flowering to maturity, CGR was also calculated for whole growth duration i.e. seedling to maturity. Following formulae were used to calculate these parameters [4].

$$CGR = \frac{W_2 - W_1}{t_2 - t_1}$$

$$RGR = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}$$

$$NAR = \frac{(W_2 - W_1)(\log_e A_2 - \log_2 A_1)}{(t_2 - t_1)(A_2 - A_1)}$$

where W_1, W_2 and A_1, A_2 are the plant dry weight and leaf area at times t_1 and t_2 respectively.

Leaf area index (LAI) : The leaf area index was calculated using the following formula at different stages of crop growth *viz.*, tillering, flowering and maturity.

$$LAI = \frac{\text{Leaf area (dm}^2)}{\text{Land area covered by plant (dm}^2)}$$

Leaf area duration (LAD) : The leaf area duration was computed at two growth stages *viz.*, tillering to flowering and flowering to maturity by multiplying the mean LAI with number of days under each growth period according to the following formula [5].

$$LAD = \frac{LAI_1 + LAI_2}{2} \times (t_2 - t_1)$$

where, LAI_1 and LAI_2 are the leaf area indices at times t_1 and t_2 respectively.

Estimation of α-amylase activity and nitrate reductase activity : Standard protocols outlined by Thimmaiaha [6] were followed to estimate the α-amylase activity in germinating hybrid seeds. The avtivity was expressed as mg of maltose produced per seed per minute (mg/seed/min). The activity of the enzyme nitrate reductase was estimated at seedling, tillering, flowering and maturity stages and expressed as μ moles of KNO_2 formed per gram of fresh tissue per hour (μ moles KNO_2/g fr.wt./hr).

Results and discussion

Mean performance of hybrids of the three sets is presented in Table 1, while the nature and magnitude of effects of sterility inducing cytoplasm worked out as per cent increase or decrease in performance of the AF_1s over performance of BF_1s are presented in Table 2. Comparison of the three A × R hybrids having the 'WA' cytoplasm, with the version of the same hybrids in normal cytoplasm, B × R hybrids, revealed significant differences between them for 28 of 34 characters (some characters studied at different growth phases) (Table 2).

Significant reduction in plant height was observed in case of all the three AF_1s because of reduced length of the uppermost internode, which is due to negative influence of WA cytoplasm. Negative effects of the sterility inducing cytoplasm on plant height have also been reported earlier [7, 8]. The WA cytoplasm exerted positive influence on number of productive tillers. All the three AF_1s had higher number of productive tillers compared to their counterparts; but the effect was significant only in AF_1-2. However, sometimes high tillering observed in rice hybrids leads to high spikelet sterility due to non-synchrony of late emerging tillers and imbalance of source:sink relationships. Conversely, lack of proper fertility restoration and the resulting high sterility may result into diversion of photosynthates to vegetative growth of late emerging tillers. These tillers may have panicles with high spikelet sterility. Hence there is a need to give due consideration to both the number of productive tillers and spikelet fertility.

Highly significant positive effect of the sterility inducing cytoplasm on days to 50% flowering was observed in all the three hybrids, which was undesirable. Delayed flowering in the AF_1s could be traced to their female parents (A lines), which also flowered late compared to their respective maintainer lines (data not presented). Similar results were also obtained by Wan [7] and Saran and Sahai [9]. However, flag leaf area and number of spikelets per panicle did not exhibit significant effect of the sterility causing cytoplasm.

Spikelet sterility is one of the constraints in hybrid rice breeding program as it affects economic yield considerably. Weak or varying level of fertility restoration

Table 1. Mean performance of the hybrids for various characters

Characters	PMS 2A/ Pusa 1127	PMS 2B/ Pusa 1127	Pusa 5A/ Pusa 1124	Pusa 5B/ Pusa 1124	IR 58025A/ PRR 78	IR 58025B/ PRR 78
Plant height (cm)	82.60	87.70	95.50	101.80	102.00	107.20
Number of productive tillers	12.20	11.10	12.50	10.90	11.90	11.60
Days to 50% flowering	94.50	89.50	93.00	91.00	91.00	86.00
Flag leaf area (cm^2)	57.97	58.73	62.07	63.06	64.76	64.25
Spikelets per panicle	210.30	220.90	208.60	221.90	228.50	243.40
Spikelet fertility (%)	61.75	70.79	68.03	69.30	66.97	71.40
Average panicle weight (g)	1.67	1.94	1.91	2.36	2.16	2.32
1000-grain weight (g)	17.65	18.13	23.09	24.05	21.54	22.25
Biomass yield (g)	38.35	42.28	45.45	48.26	52.73	52.90
CGR (mg/day/plant)						
Seedling-tillering	320.98	361.77	419.98	438.10	484.38	539.25
Tillering - flowering	386.36	335.42	375.56	392.23	311.60	443.83
Flowering - maturity	468.87	568.45	509.84	631.49	608.59	529.68
Whole duration	315.19	350.86	366.50	400.57	396.05	418.32
RGR (mg/g/plant/day)						
Seedling - tillering	38.29	46.05	46.83	52.28	51.60	57.88
Tillering - flowering	17.21	15.13	14.38	15.46	11.62	15.72
Flowering - maturity	14.85	17.39	13.79	16.54	16.05	13.29
NAR (mg/dm^2/day)						
Seedling - tillering	146.60	156.67	166.82	170.73	200.25	213.46
Tillering - flowering	29.24	24.32	24.53	25.15	20.46	28.02
Flowering - maturity	46.76	54.64	47.58	57.72	49.88	42.23
LAI						
Tillering	3.90	4.18	4.76	4.85	4.49	4.75
Flowering	4.95	5.05	5.47	5.57	5.73	5.86
Maturity	2.13	2.26	2.18	2.23	2.76	2.87
LAD						
Tillering - flowering	68.17	66.48	92.10	107.33	109.46	103.96
Flowering - maturity	97.33	113.37	118.53	115.01	114.73	124.27
α-Amylase activity (mg/seed/min)						
at 24 hrs	12.83	13.33	14.00	14.50	13.33	14.17
at 48 hrs	18.33	20.00	20.00	20.17	19.50	20.17
at 72 hrs	23.50	24.83	29.50	30.00	26.83	27.33
NRA (μ moles NO_2/g fr.wt./hr)						
Seedling	625.10	740.00	733.50	714.00	769.00	677.00
Tillering	979.50	1,077.00	1,004.00	1,081.00	1,098.50	1,067.00
Flowering	1,033.00	984.00	1,188.00	1,134.00	1,107.00	1,131.30
25 days after flowering	265.30	260.40	233.90	245.00	370.50	369.00
Grain yield (g)	14.16	16.33	19.04	21.06	20.38	21.72
Harvest index	0.38	0.39	0.43	0.45	0.39	0.41

leads to increased spikelet sterility resulting in reduced grain yield. In addition, with the WA cytoplasm reduced panicle exertion is a problem even with complete fertility restoration, which leads to basal spikelet sterility [10, 11]. In the present investigation, all the AF_1s exhibited negative deviation for spikelet fertility; however, the effect was significant only in AF_1-1. Developing a hybrid combination involving CMS lines with improved panicle exertion and restorers giving high fertility restoration might help in improving basal spikelet fertility of the resulting hybrid and thus contributing towards increased grain yield.

Interestingly, average panicle weight of the hybrid Pusa 5A/Pusa 1124 (AF_1-2) was significantly less than BF_1-2, even though spikelet fertility levels of the two were nearly same. Additionally, although the hybrid AF_1-1 had significantly less spikelet fertility than BF_1-1, its effect on average panicle weight, though considerably high (–13.70%), was non-significant. However, as clear from Table 1, this was due to more number of tillers in AF_1-2 (12.50) as against 10.90 in BF_1-2. This was the main reason for reduction in average panicle weight of AF_1-2 and not spikelet sterility. Whereas in AF_1-1, both reduced spikelet fertility and higher number of tillers contributed to reduction in average panicle weight.

The inverse relationship between number of productive tillers and average panicle weight supports observations of Rao [12] that higher number of secondary and tertiary tillers contributes little to grain yield and actually has negative effect. The inverse

Table 2. Nature and magnitude of effects (%) of sterility inducing cytoplasm on various characters

Characters	PMS 2A/ Pusa 1127	Pusa 5A/ Pusa 1124	IR 58025A/ PRR 78
Plant height (cm)	–5.82*	–6.19**	–4.85**
Number of productive tillers	9.91	14.68*	2.59
Days to 50% flowering	5.59**	2.20**	5.81**
Flag leaf area (cm^2)	–1.30	–1.58	0.80
Spikelets per panicles	–4.80	–5.99	–6.12
Spikelet fertility (%)	–12.76**	–1.83	–6.21
Average panicle weight (g)	–13.70	–19.23*	–6.95
1000-grain weight (g)	–2.61	–3.98**	–3.19**
Biomass yield (g)	–9.29*	–5.83	–0.31
CGR (mg/day/plant)			
Seedling - tillering	–11.27**	–4.14	–10.18**
Tillering - flowering	15.19**	–4.25*	–29.79**
Flowering - maturity	–17.52**	–19.26**	14.90**
Whole duration	–10.17**	–8.51**	–5.32**
RGR (mg/g/plant/day)			
Seedling - tillering	–16.85**	–10.43**	–10.85**
Tillering - flowering	13.69**	–6.98**	–26.09**
Flowering - maturity	–14.62**	–16.63**	20.82**
NAR (mg/dm^2/day)			
Seedling - tillering	–6.43	–2.29	–6.19
Tillering - flowering	20.21**	–2.49	–26.99**
Flowering - maturity	–14.42	–17.57	18.11
LAI			
Tillering	–6.69*	–1.86	–5.61**
Flowering	–1.98**	–1.74	–2.05**
Maturity	–5.98**	–2.38**	–3.56**
LAD			
Tillering-flowering	2.54*	–14.19**	5.29
Flowering - maturity	–14.14**	3.06	–7.68**
α-Amylase activity (mg/seed/min)			
at 24 hrs after seed soaking	–3.75*	–3.47*	–5.93**
at 48 hrs after seed soaking	–8.35**	–0.84	–3.31*
at 72 hrs after seed soaking	–5.34**	–1.66	–1.83
NRA (μ moles KNO_2/g fr.wt./hr)			
Seedling	–15.53**	2.73	13.59**
Tillering	–9.05**	–7.12**	2.95
Flowering	4.98**	4.76**	–2.15*
25 days after flowering	1.88*	–4.53	0.41
Grain yield (g)	–13.34	–9.58	–6.18
Harvest index	–3.59	–4.36	–6.28

*,** = Significant at 5% and 1% levels, respectively.

relationship between number of productive tillers and spikelet fertility was also reported by He and Shen [11] who stated that sometimes high tillering observed in rice hybrids is due to sterility.

Negative influence of the cytoplasm on 1000-grain weight and biomass yield were cross specific. Reduction in 1000-grain weight was highly significant in AF_1-2 and AF_1-3. While, for biomass yield, only AF_1-1 exhibited significant reduction (–9.29%).

The WA cytoplasm exerted significant negative effects on crop growth rate (CGR) and relative growth rate (RGR) in all stages of crop growth. However, the effects varied with cross combination as well as growth stage. The negative influence of the WA cytoplasm on these important growth parameters in AF_1s may have resulted in their poor performance compared to their respective BF_1s. For CGR, the highest negative effect was observed in flowering to maturity phase in both AF_1-1 and AF_1-2. While, in AF_1-3, the negative influence was as high as –29.79% in tillering to flowering phase.

Interestingly, negative influence of the cytoplasm on RGR varied with cross combination as well as growth stage. In AF_1-1, maximum reduction in RGR was observed in seedling to tillering phase, while in AF_1-2 it was during flowering to maturity. Whereas in AF_1-3, the maximum reduction (–29.09%) was observed during tillering to flowering phase. Highly significant cytoplasmic effect on net assimilation rate (NAR) was observed only in tillering to flowering phase, where it ranged from –26.99 in AF_1-3 to 20.21% in AF_1-1.

Presence of sufficient leaf area is essential for realizing optimum level of photosynthesis. Leaf area directly contributes to biomass accumulation and crop growth rate. To achieve required biomass yield, optimum LAI (leaf area index) has to be ensured first. However, negative influence of WA cytoplasm on LAI in AF_1s was observed, which might have resulted in reduced CGR and RGR in these hybrids compared to their respective BF_1s. The negative influence on LAI was significant in all the three growth stages in AF_1-1 and AF_1-3, while in AF_1-2, significant negative effect was observed only at maturity. In case of leaf area duration (LAD), the cytoplasmic effect was not consistent. In AF_1-1 and AF_1-3, the cytoplasm had positive effect during tillering to flowering and highly significant negative effect in later growth phase. While, the opposite effect was noted in AF_1-2, where the influence was highly significant and negative in tillering to flowering phase and positive but non-significant in the later phase.

α-Amylase is a primary enzyme in cereals in conversion of starch to simple sugars that provide energy for the growing seedling. Thus, higher activity of the enzyme ensures faster growth of seedlings giving them initial advantage. In the present investigation, α-amylase activity was studied in germinating hybrid seeds at 24, 48 and 72 hours after seed soaking. It was observed that all the AF_1s exhibited reduced α-amylase activity than the corresponding BF_1s. Significant negative influence of the WA cytoplasm was

observed on α-amylase activity up to 72 hours after seed soaking in AF_1-1, while it was limited to 24 hours in AF_1-2 and 48 hours in AF_1-3.

Nitrate reductase (NR) catalyzes reduction of nitrate to nitrite, the rate limiting step in reduction of nitrate to ammonia. In the present investigation, cytoplasmic influence on nitrate reductase activity (NRA) varied with growth stage and cross combination. In seedling stage, AF_1-1 exhibited negative effect as high as (–15.53%), while in AF_1-3, the effect was significantly positive (13.59%). At tillering stage, both AF_1-1 and AF_1-2 exhibited highly significant negative influence of the WA cytoplasm and AF_1-3 demonstrated non-significant positive influence. However, at flowering, the opposite pattern was observed and AF_1-1 and AF_1-2 exhibited highly significant positive influence and AF_1-3 exhibited significant negative influence of the WA cytoplasm on NRA. Whereas, NRA at 25 days after flowering was significantly higher in AF_1-1 compared to BF_1-1. This indicates cross specific nature of influence of the WA cytoplasm on various characters.

Despite significant negative effects of the sterility inducing cytoplasm on various characters, the overall effects on grain yield per plant and harvest index, although negative, were not significant. This could be due to character compensation. Negative effects of the WA cytoplasm on grain yield ranged from –6.18% to –13.34%, while those on harvest index ranged from –3.59% to –10.42%.

A perusal of the Table 2 reveals another interesting fact. For some characters like LAD in tillering to flowering phase and NRA at 25 days after flowering, it was observed that lower values of cytoplasmic influence on those characters in a set (involving one AF_1 and one BF_1) was significant, however higher values in other set(s) were non-significant. This could be because the AF_1s were compared with BF_1s of the same set for calculating the 't' value. In other words, the comparisons were not done on pooled basis, by comparing all the AF_1s with all the BF_1s. As the AF_1 and BF_1 in each set had different magnitude of variation, it resulted in a different significance value for each set. Hence, for example, for LAD at tillering to flowering phase, the value of 2.54% in AF_1-1 was significant, while the higher value of 5.29% in AF_1-3 was non-significant for negative effect of the sterility inducing cytoplasm.

Conclusions

From the present study it is inferred that, the nature and magnitude of effects of the WA cytoplasm varied with character in question and also the cross combination. In general, the sterility inducing cytoplasm negatively influenced characters like plant height, spikelet fertility, average panicle weight, 1000-grain weight, biomass yield, harvest index and grain yield, while it enhanced number of productive tillers and growth duration. Negative effect of the WA cytoplasm was evident in case of all the physiological and biochemical characters studied, in various growth stages. For grain yield, the least negative effect of the cytoplasm was observed in AF_1-3 (IR 58025A/PRR 78), while for harvest index, AF_1-1 (PMS 2A/Pusa 1127) exhibited the least negative effect. Since some of the effects were obviously the outcome of the extent of sterility, it is possible to reduce or overcome the negative influence of cytosterility by choosing appropriate restorers and CMS lines that suppress fertility restoration the least.

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