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# Partial conversion of elite maintainer genotype mas99 into new male sterile line suitable for aerobic and drought conditions through backcross breeding in rice

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**Abstract**
Two populations were developed and advanced to $BC_3$ generation using IR70369A and KCMS31A CMS lines as donors and MAS 99 as recurrent parent. In $BC_3F_1$ generation, the populations were evaluated with parents for morpho-floral traits under aerobic field condition. In both crosses, the mean values of number of tillers/plant (22 & 27), plant height at maturity (74 & 73cm), days to 50% flowering (95 & 97 days) were found comparable to the recurrent parent with superior floral traits such as pollen sterility (100%), stigma exertion (30% & 25%) and spikelet sterility (100%). IR70369A × MAS99 cross derived plants, A04, A35, A47, A65, A84 and KCMS31A × MAS99 cross derived plants, B06, B27, B30, B34 and B44 were identified as superior MAS99 converted CMS plants as indicated by morpho-floral traits and advanced to $BC_4F_1$. Between two crosses, IR70369A × MAS99 cross derived population was superior for days to 50% flowering and stigma exertion rate.
**Key words:** Aerobic rice, backcross, conversion, male sterility, spikelet fertility

## Introduction

Rice is a highly researched crop for crop improvement in the areas of biotic and abiotic stress tolerance, nutritional quality and yield. Curtail in rice yields coupled with water scarcity has led to low production and productivity in rice. Alternate crop cultivation is being practiced among the conventional rice growers off-late due to water stress and low yields though Hybrid rice technology is one of the approaches for breaking the yield plateau (Siddiq, 1997). Availability of suitable hybrids for drought or cultivation for aerobic situations are scarce. The development of potential CMS lines is an important step in the production of promising hybrids for adverse situations. Availability of suitable male sterile line, maintainer (B line) and restorer (R line) in three line hybrid rice breeding is essential (Hittalmani and Shivashanker, 1987). The discovery of a wild-abortive cytoplasmic male sterile line (WA-CMS) in rice at Hainan Island in 1973 resulted in the huge success of three-line hybrid rice breeding in China (Hua *et al.*, 2009). However, limited availability of CMS lines has hindered the exploitation of the hybrid rice technology to the maximum in India. Limited resources of cytoplasmic male sterility and lower variation of CMS lines causes genetic vulnerability to biotic and abiotic stresses (Rumanti *et al.*, 2011). Therefore, to diversify CMS lines breeders can transfer male sterile trait to the existing B lines through consecutive backcrosses. Previous work in our research group showed that genotype MAS 99 is a maintainer line for cytoplasmic male sterility of WA type which is promising short duration genotype suitable for aerobic and drought conditions (Satheesh, 2015). CMS lines developed so far are suitable for

lowland irrigated puddled condition. Hence, we made an attempt to develop CMS lines suitable for aerobic condition. Most of the hybrids in rice were developed using IR58025A and IR62829A CMS lines leading to narrow genetic base hybrids thereby, become vulnerable to biotic and biotic stresses (Hooker 1974). To alleviate these critical situations, we need to diversify CMS lines which are suitable for various conditions by converting identified maintainer lines into CMS line. Therefore, it is necessary to transfer available CMS system into elite breeding lines to develop suitable hybrids for specific situations (Kumar *et al.*, 1996). Similarly, Ali and Khan (1996) successfully transferred CMS genes from IR58025A and IR62829A into Basmati line 47456 in Pakistan.Vasoukolaei *et al.* (2010) converted Iranian rice cultivars into cytoplasmic male sterile lines through repeated back crossing. Whereas, Ali *et al.* (2013) evaluated newly developed quasi-CMS lines, forwarded further to use and develop superior aromatic rice hybrids. The present study was undertaken to generate new CMS line in the nuclear background of MAS 99 suitable for aerobic condition which can be further utilized for development of new rice hybrids.

## Materials and methods

The material for the present study composed of two male sterile lines derived from wild abortive sources 'IR70369A' and 'KCMS31A'. A locally adapted short duration genotype i.e MAS 99 which has deep roots, tolerant

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to intermittent drought developed by MAS lab, University of agricultural sciences, Bangalore was used as recipient line. During *kharif* 2012, MAS99 genotype was crossed with IR70369A and KCMS31A male sterile lines and their hybrid combinations were observed for pollen fertility and spikelet fertility during summer 2013. The pollen sterility was examined using acetocarmine stain (2%). The sterile plants were tagged and back crossed with male parent MAS 99 as recurrent parent. Subsequently in every season phenotypic foreground selection was carried out by testing pollen sterility to confirm the donor parent trait introgression, then further backcross was affected. Backcrossing was continued for both seasons in 2013 and 2014. The scheme of back cross followed to develop these quasi- CMS lines was given in Fig. 1. The recommended package of practices was followed during crop growth period under aerobic condition. In $BC_1F_1$ and $BC_2F_1$ generation plants that synchronised in flowering and were similar in plant height compared to MAS99 (recurrent parent) were selected and forwarded to next generation. In $BC_3F_1$ generation, about 100 plants (A1-A100) derived from IR70369A × MAS99 cross and 50 plants (B1-B50) of KCMS31A × MAS 99 cross were evaluated along with their parents for morpho-floral traits. The observations were recorded as per SES (1996) on individual plants for 13 characters viz., Plant height at 45 days, days to fifty percent flowering, pollen sterility (%), style length (mm), stigma length (mm), stigma exertion percentage (%), SPAD reading, plant height at maturity (cm), panicles plant$^{-1}$, panicle exertion percentage (%), panicle length (cm), number of spikelets panicle$^{-1}$ and spikelet fertility (%). The pollen and spikelet fertility were determined as the ratio of fertile pollens to the total pollens and as the ratio of filled spikelets to the total spikelets respectively and expressed in percentage. Length of stigma and style were measured using micrometry and expressed in mm. The stigma exsertion was calculated by the ratio of spikelets with exerted stigma to the total number of spikelets and expressed in percentage. The percentage of length of panicle that emerges out from flag leaf to the total length of panicle was considered as panicle exertion percent. Panicles emerging from the sheath were bagged with butter paper bags prior to anthesis to prevent cross pollination. Bagged panicles were harvested to assess spikelet fertility. The trait means of $BC_3F_1$ plants of both crosses were compared with parents to analyse the recovery of recurrent parent (MAS 99) genome. Two tail t-test was carried out to compare statistically. The superior CMS plants were identified and advanced to $BC_4F_1$.

**Results and Discussion**

In test cross nursery, IR 70369A/MAS 99 and KCMS31A/MAS99 crosses were identified as 100 % sterile as per the pollen and spikelet fertility test results in summer 2013. It was noticed that Pollen fertility test results are in line with spikelet fertility (Naresh Babu *et al.*, 2010). In $BC_1F_1$ and $BC_2F_1$ generation, all the progenies were completely male sterile this confirmed the introgression of male sterile trait. Similar results were reported by Ahmadikhah *et al*. (2015). Pollen and Spikelet fertility test revealed that in IR70369A × MAS 99 derived population, 97% of the plants were completely male sterile and In KCMS31A × MAS 99 derived population, 90% of the plants were completely male sterile in $BC_3F_1$ generation. The frequency of male sterile plants was higher in IR70369A × MAS99 than in KCMS31A × MAS99 cross. The comparison of sterile and fertile pollen and spikelets are presented in Fig. 2. In IR70369A× MAS 99 derived population mean values of traits such as plant height at 45 days (37.54cm), days to fifty percent flowering (94.80 days), SPAD reading (34.65), total tillers (22.01), panicles plant$^{-1}$ (19.93), plant height at maturity (73.68 cm), panicle length (17.73 cm), spikelet panicle$^{-1}$ (130.20) were comparable with recurrent parent mean values whereas for style length (0.93mm), stigma length (1.07 mm), stigma exertion (30.19 %), days to maturity (128.20 days), panicle exertion (64.91%), were significantly different from recurrent parent (Table 1). This inferred that requirement of additional backcrosses to make them comparable to recurrent parent. In KCMS31A× MAS 99 derived population trait means of total tillers (22.06), SPAD reading (33.24), panicles plant$^{-1}$ (20.20), panicle length (17.66 cm), plant height at maturity (72.53 cm) and spikelet panicle$^{-1}$ (130.28) were statistically comparable with recurrent parent, whereas traits means of plant height at 45 days (36.46 cm), days to fifty percent flowering (96.90 days), style length (0.93 mm), stigma length (1.06 mm), stigma exertion rate (24.78%), days to maturity (132.38 days) and panicle exertion (64.21%) differing significantly from recurrent parent (Table 1). In both crosses, the mean values of traits such as days to fifty percent flowering, plant height at maturity, number of tillers per plant, though statistically significant difference from recurrent parent, practically found comparable. Another important desirable trait is stigma exertion has values comparable to donor parent. In both the crosses though some of the plants carried higher percentage of recurrent parent genome additional backcrosses is required for complete retrieval of entire recurrent parent genome and to stabilize the population. Among IR70369A × MAS99 cross derived plants, A04, A35, A47, A65 and A84 were superior and in KCMS31A × MAS99 cross derived plants B06,

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B27, B30, B34 and B44 were identified as superior plants. Stigma exertion and panicle exertion of these plants was comparable to checks besides 100% pollen and spikelet sterility (Table 2). Between the two populations, IR70369A × MAS99 cross derived population was superior for days to 50% flowering and stigma exertion rate.These CMS lines can be used in future for the development of superior hybrids suitable for aerobic and drought condition. Similarly new CMS lines were developed in rice through back crossing by Dalmacio *et al*. (1995); Manonmani *et al.* (2006); Pradhan and Jachuck, (2008); Xian-Hua *et al*. (2013). The investigation concludes new short duration quasi-CMS lines suitable for aerobic and intermittent drought conditions were developed by converting B-line MAS 99B in to A-line MAS 99A through introgression of male sterility from IR70369A and KCMS31A by consecutive backcrossing.The selected lines need to be further stabilised.

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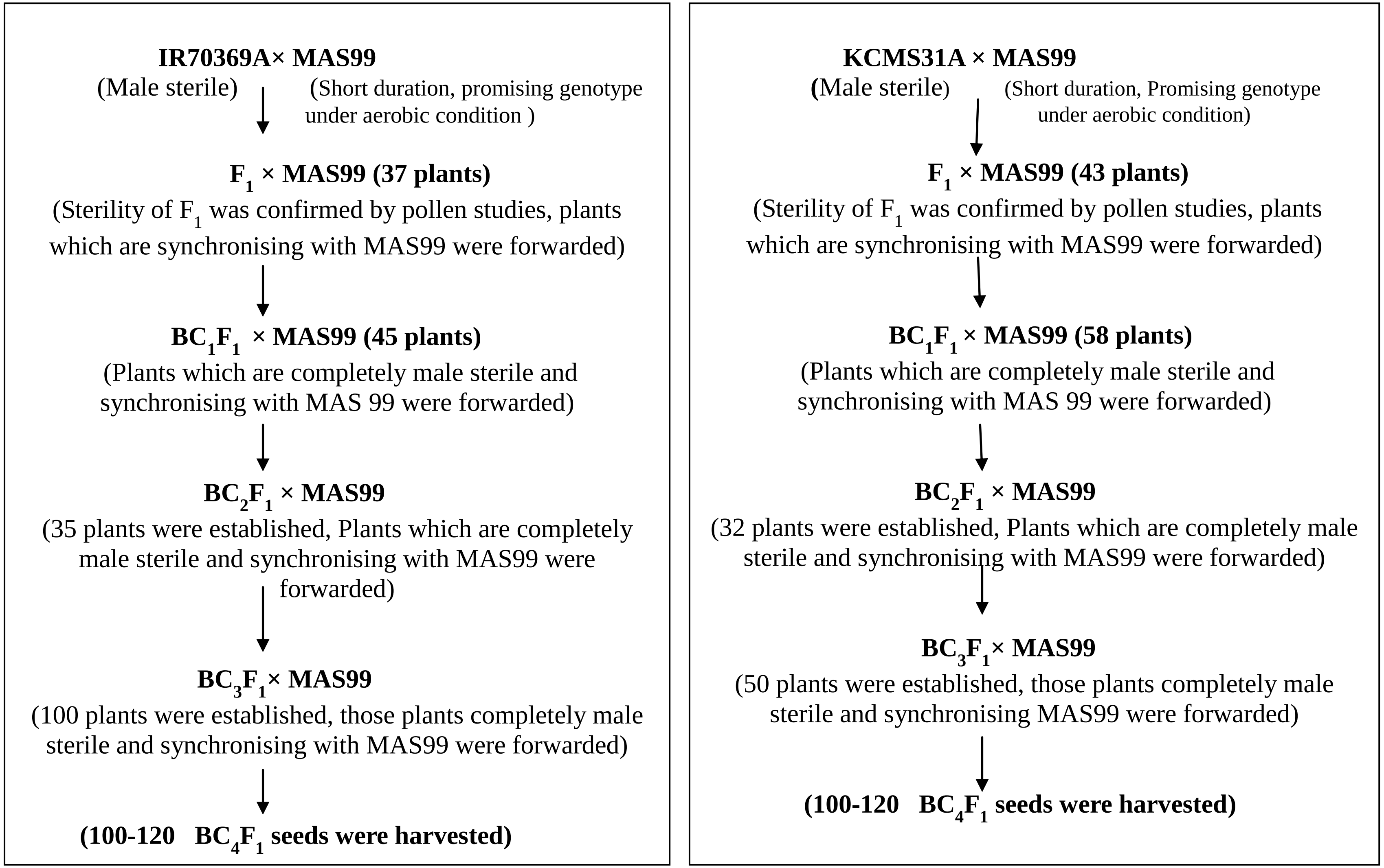


**Fig 1**. Scheme of backcross followed to develop new CMS line from two crosses in rice

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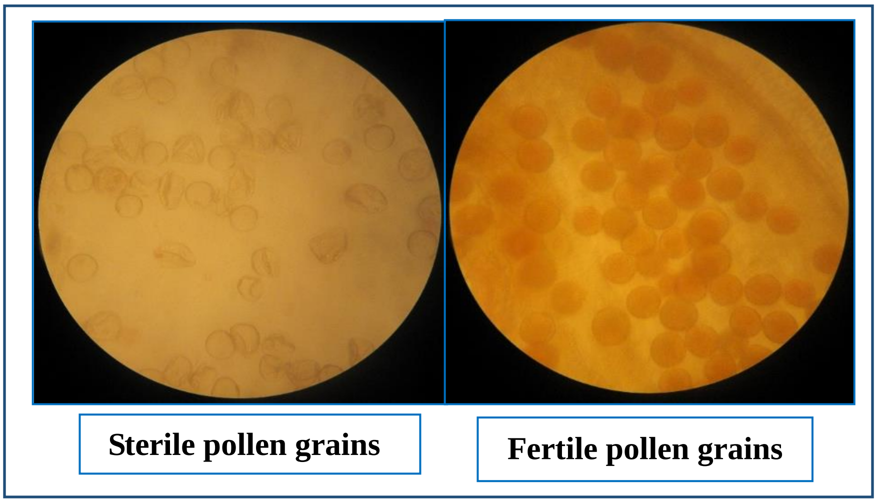
**(Fig. A)**




**(Fig. B)**

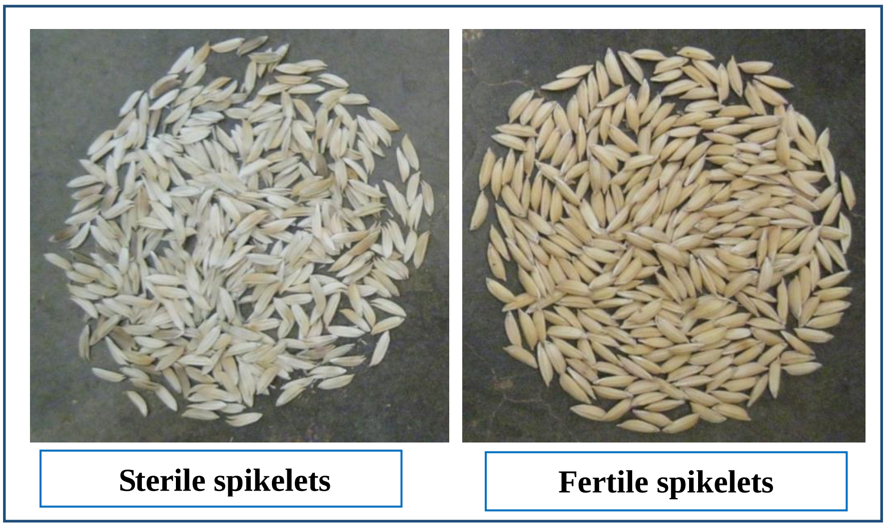



**Fig 2.** Comparison of sterile and fertile pollen grains (Fig .A), sterile and fertile spikelets (Fig. B)

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**Table 1.** Comparison of Parental trait means with $BC_3F_1$ populations derived from IR70369A × MAS99 and KCMS31 × MAS99 crosses in rice

| SL.NO. | TRAITS | IR70369A | MAS99 (RP) | $BC_3F_1$ | RP-$BC_3F_1$ | P(T<=t) two-tail | KCMS31A | $BC_3F_1$ | RP-$BC_3F_1$ | P(T<=t) two-tail |
|---|---|---|---|---|---|---|---|---|---|---|
| 1) | Plant height at 45 days(cm) | 32.90 | 37.20 | 37.54 | -0.34 | 0.00 | 35.20 | 36.46 | 0.74 | 0.00 |
| 2) | Days to 50% flowering | 104.20 | 94.80 | 94.78 | 0.02 | 0.07 | 107.60 | 96.90 | -2.10 | 0.00 |
| 3) | Style length (mm) | 0.95 | 0.88 | 0.93 | -0.05 | 0.02 | 0.91 | 0.93 | -0.05 | 0.02 |
| 4) | Stigma length (mm) | 1.20 | 1.14 | 1.07 | 0.07 | 0.00 | 1.18 | 1.06 | 0.08 | 0.00 |
| 5) | Stigma exertion (%) | 34.95 | 2.32 | 30.19 | -27.87 | 0.00 | 26.00 | 24.78 | -22.46 | 0.00 |
| 6) | SPAD reading | 32.94 | 37.50 | 34.65 | 2.85 | 0.00 | 31.86 | 33.24 | 4.24 | 0.00 |
| 7) | Days to maturity | 134.40 | 127.30 | 128.20 | -0.90 | 0.00 | 139.40 | 132.38 | -5.08 | 0.00 |
| 8) | Total tillers | 30.50 | 23.90 | 22.01 | 1.89 | 0.06 | 26.90 | 22.06 | 1.84 | 0.08 |
| 9) | Panicles plant$^{-1}$ | 17.10 | 19.90 | 19.93 | -0.03 | 0.95 | 16.20 | 20.20 | -0.30 | 0.80 |
| 10) | Plant height at maturity (cm) | 66.80 | 74.60 | 73.68 | 0.92 | 0.14 | 68.90 | 72.53 | 2.07 | 0.05 |
| 11) | Panicle length (cm) | 17.46 | 19.33 | 17.73 | 1.60 | 0.17 | 16.58 | 17.66 | 1.67 | 0.14 |
| 12) | Panicle exertion (%) | 77.77 | 78.01 | 64.91 | 13.09 | 0.01 | 87.69 | 64.21 | 23.48 | 0.01 |
| 13) | Spikelet panicle$^{-1}$ | 133.40 | 129.20 | 130.20 | -1.00 | 0.76 | 129.60 | 130.28 | -1.08 | 0.80 |

**P<0.05: significant at 5%, P<0.01: significant at 1%**

**Table 2.** Superior MAS 99 converted CMS plants identified in $BC_3F_1$ populations derived from IR70369A × MAS99 and KCMS31A × MAS99 crosses in rice

| SL.no | Plant code no. | Pollen sterility (%) | Days to 50 % flowering | Stigma exertion rate (%) | Total tillers plant$^{-1}$ | Plant height at maturity (cm) | Panicle exertion (%) | Spikelet sterility (%) |
|---|---|---|---|---|---|---|---|---|
| | **IR70369A × MAS99** | | | | | | | |
| 1 | A04 | 100.00 | 94.00 | 33.55 | 30.00 | 75.00 | 66.22 | 100.00 |
| 2 | A35 | 100.00 | 93.00 | 27.04 | 25.00 | 74.00 | 72.25 | 100.00 |
| 3 | A47 | 100.00 | 93.00 | 32.19 | 24.00 | 71.00 | 79.50 | 100.00 |
| 4 | A65 | 100.00 | 91.00 | 33.96 | 27.00 | 76.00 | 65.54 | 100.00 |
| 5 | A84 | 100.00 | 97.00 | 28.78 | 23.00 | 75.00 | 65.07 | 100.00 |
| | **KCMS31A × MAS99** | | | | | | | |
| **SL.no** | **Plant code no.** | **Pollen sterility (%)** | **Days to 50 % flowering** | **Stigma exertion rate (%)** | **Total tillers plant$^{-1}$** | **Plant height at maturity (cm)** | **Panicle exertion (%)** | **Spikelet sterility (%)** |
| 1 | B06 | 100.00 | 96.00 | 23.02 | 30.00 | 75.00 | 59.70 | 100.00 |
| 2 | B27 | 100.00 | 99.00 | 25.26 | 29.00 | 71.00 | 73.09 | 100.00 |
| 3 | B30 | 100.00 | 98.00 | 25.36 | 30.00 | 74.00 | 73.20 | 100.00 |
| 4 | B34 | 100.00 | 99.00 | 27.77 | 25.00 | 75.00 | 72.90 | 100.00 |
| 5 | B44 | 100.00 | 96.00 | 30.38 | 28.00 | 76.00 | 66.04 | 100.00 |
| **Check CMS lines** | | | | | | | | |
| 1. | **IR70369A** | 100.00 | 104.20 | 34.95 | 30.50 | 66.80 | 77.02 | 100.00 |
| 2. | **KCMS31A** | 100.00 | 107.60 | 26.00 | 26.90 | 68.90 | 88.23 | 100.00 |

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