

# Comparative analysis of restoration behavior of *milo* (104A, 401A) and *maldandi* (M31-2A) based male sterile lines in sorghum [*Sorghum bicolor* (L.) Monech]

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## Short Report

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## Abstract

In sorghum, *milo* source of male sterility has been used for developing all the hybrids so far and it created the narrow cytoplasmic base. In addition, it also narrows down the nuclear diversity which may be the invitation for the outbreak of pests and diseases in the near future. To handle this hazard new diverse sources like  $A_2$ ,  $A_3$ ,  $A_4$ , *maldandi*, *VZM* and  $G_1$  have been discovered. The availability of restorers on these sources is very difficult hence their utilization is also a challenging task. The present study was, therefore, carried out to test different sorghum lines for restoration behavior on two *milo* lines (104A and 401A) and one *maldandi* (M31-2A) with more emphasis on *maldandi* source because of its added advantage of enhancing grain size and pest tolerance. In rabi-2017, 125 genotypes were tested for the restoration behavior on these sources, and five restorers viz., IS 19389, IS 995, IS 28389, IS 26046 (BRJ 67 – 8) and DSMR-8 restored fertility on 104A, two lines viz., IS 29335 and IS 26046 restored on 401A, while on M31-2A six lines viz., IS 19975, BRJ-67-4 (DSMR-4), BRJ 67 – 8 (DSMR-8), BRJ 67 – 16, BRJ 67 – 19, BRJ 67 – 21 restored the fertility. Only two lines DSMR-8 and DSMR-4 exhibited stable, consistent and strong restoration (> 90% seed set) across the seasons (*rabi*-2017 and *khari*-2017) and years (2017 and *rabi*-2018). All the used MS lines exhibited the restoration complexity in the following order – 104A < 401A < M31-2A.

## Introduction

Sorghum [*Sorghum bicolor* (L.) Monech] is the fifth major cereal crop of the world following wheat, rice, maize, and barley in terms of production and utilization. Sorghum grain is a dietary staple for millions of people in semi-arid areas of Asia and Africa where drought stress causes frequent failures of other crops. In Africa and Asia, sorghum grain is mainly used as food, while in the United States and Australia it is used to feed cattle (Reddy et al., 2013). Due to its versatile use as a source of food, feed, fodder and fuel, it is under cultivation in tropical, subtropical and even in temperate regions of the world as great millet. The world's sorghum production of 57.50 million tonnes comes from the area of 40.28 m ha with the productivity of 1.43 tonnes per hectare during 2019-20 (Foreign Agricultural Service/ USDA, 2020).

All the hybrids released commercially to date share the same sterile cytoplasm of *milo*, discovered by Stephens and Holland in 1954. This situation can be an invitation for huge damage in the near future as evidenced by the repeated use of Texas cytoplasm in maize (Levings, 1990).

Many researchers have reported that most of the lines show the fertile reaction on *milo*( $A_1$ ) male sterile source, hence is easy compared to others for hybrid production. The new diverse sterile cytoplasmic sources viz.,  $A_2$ ,  $A_3$ ,  $A_4$ , *maldandi*, *VZM* and  $G_1$  were identified to overcome the threat of narrow cytoplasmic base. Due to the complex mechanism of restoration on non-*milo* cytoplasm, the restoration is difficult and research is also lacking in this area. Kishan and Borikar (1989) concluded that most of the genotypes from the Indian programme give a restorer reaction against *milo* cytoplasm thus, identification of suitable restorer for *milo* ( $A_1$ ) cytoplasm is quite easy. However, the *milo* shows poor grain quality, susceptibility to shoot fly, poor fertility restoration in hybrids under *rabi* environment. The  $A_2$ ,  $A_3$  and  $A_4$  cytoplasm are the available sources but for this, restoration appeared difficult. Because of these limitations,  $A_2$ ,  $A_3$ ,  $A_4$  and other cytoplasmic male sterile lines have not been used for commercial exploitation. Use of indigenous *maldandi* source of male sterility, instead of exotic *milo* appears to be the best option. But there are no stable and consistent fertility restorers on this cytoplasm. Hence the present study was undertaken to identify the strong and stable restorers across the seasons and years.

## Material And Methods

A total of 125 genotypes, comprising 68 (Set-I) and 57 (Set-II) were used as the pollen parents (Table 1 & 2). The Set-I is a part of minicore and the Set-II is comprised of derived lines of mutation treatment of BRJ-67 and BRJ-198. The fertility restoration of both sets was assessed on two MS lines of *milo* ( $A_1$ ) viz., 104A & 401A and one *maldandi* ( $A_{4M}$ ) viz., M31-2A. A set of 204 crosses [(68 + 57) x 3 CMS lines] were developed during *summer* and *khari* season of 2017.

The experiment was conducted at Botany Garden, Agriculture college, UAS, Dharwad during *rabi*-2017, *khari*-2018 and *rabi*-2018. Each hybrid was evaluated in a single row of 3 meters with an intra-row spacing of 15 cm and inter row spacing of 45 cm. The hybrid plants were covered with brown paper bags to avoid cross pollination at physiological maturity. Selected panicles were harvested, observed physically for seed set and *per cent* seed set was calculated using the following formula (Kishan and Borikar, 1989)

$$\text{Seed set per cent} = \frac{\text{Total number of seeds/panicle}}{\text{Total number of spikelets/panicle}} \times 100$$

Based on the seed set *per cent* the genotypes were grouped into different categories of restoration as described by Biradar *et al.* (1996).

## Results And Discussion

### Set-I: Fertility restoration behavior of minicore genotypes

In set-I, out of 68 hybrids generated by crossing with the first *milo* based CMS line (104A), 15 crosses exhibited satisfactory (>60 %) seed setting, while 20 hybrids were lacking in seed setting and characterized as perfect maintainers (Table 3). Four genotypes viz., IS 19389, IS 995, IS

28389 and IS 26046 were characterized as strong restorers as they exhibited 97.99 % mean seed set. Nearly 35 *per cent* of lines were found to be partial restorers and have an average seed set *per cent* of 23.82. The mean seed set percentage of moderate restorers (9) was 71.22 *per cent*. Nine lines showed low restoration with the mean seed set *per cent* of 4.89. Of the 68 hybrids obtained by crossing with the second *milo* based CMS line (401A), 14 hybrids showed greater than 60 *per cent* seed set, while 19 hybrids showed no seed set and turned to be perfect maintainers. Only two genotypes, IS 29335 and IS 26046 were recorded as strong restorers and showed a 94.69 % mean seed set. The highest proportion of the lines (31.34%) were found to be partial restorers with an average 24.28 *per cent* seed set. A total of 11.94 *per cent* (8) of the total lines evaluated were moderate restorers and revealed 69.47 *per cent* seeds set. Thirteen lines exhibited low restoration with a mean seed set of 6.81 *per cent*. Only two hybrids, out of 68, exhibited more than 60 *per cent* seed setting on selfing on the *maldandi* male sterile line and only one line IS 19975 was found to be a strong restorer with 100 *per cent* seed set. The highest proportion of the lines (72.06 %) were turned to be perfect maintainers and were lacking in seed set. Only one line was recorded as the moderate restorer with a 61.54 *per cent* seed set. Eleven and six genotypes were found to be partial restorers (22.79 %) and low restorers (4.74 %), respectively.

The present study revealed the complex behavior of restoration on *maldandi* compared to the *milo*. If we compare all the CMS lines used in the order of complexity for the availability of restorers it would be 104A < 401A < M31-2A, whereas the complexity for the availability of perfect maintainers would be M31-2A<401A<104A.

#### **Set-II: Fertility restoration behavior of derived lines of BRJ-67 and BRJ-198.**

Of the 57 hybrids, 104A (*milo*), a total of 48 lines showed greater than 60 *per cent* seed set, whereas only one line turned to be perfect maintainers (Table 4). The strong restoration (91.68%) was exhibited by only one genotype, (BRJ 67-8) DSMR-8. Around 54.39 % of lines showed moderate restoration with a 74.05 *per cent* mean seed set. Partial restorers (8) exhibited mean seed set percentage of 49.13 and none of the lines showed low restoration. A total of 47 hybrids, on the second *milo* based CMS line (401A), showed more than 60 *per cent* seed set, whereas none of the lines were recorded as perfect maintainers. The strong restoration was not observed on 401A. The majority (38) of the lines were noticed as moderate restorers and exhibited 69.82 % seed set. The mean seed set percentage of partial restorers (9) was 52.52 %. Only one line showed low restoration with mean seed set *per cent* of 7.87. The hybrids on *maldandi* male sterile line (M31-2A), 32 out of 57 hybrids exhibited greater than 60 *per cent* seed set on selfing. Out of these, five genotypes *viz.*, BRJ-67-4 (DSMR-4), BRJ 67-8 (DSMR-8), BRJ 67-16, BRJ 67-19, BRJ 67-21 were considered as strong restorers as they restored an average 94.29 *per cent* fertility. The highest proportion of the lines 35.09 % were found to be perfect maintainers with a zero *per cent* seed set. Fifteen lines were recorded moderate restorers with 70.85 *per cent* seed set. Two and three lines were turned to be partial and low restorers with 27.18 and 4.66 *per cent* seed set, respectively.

Across two sets, only one line *viz.*, DSMR-8 restored the fertility on both *milo* and *maldandi* male sterile lines. This indicates that the restorers on *maldandi* may not always restore the fertility on *milo*. Overall the study indicates that restoration on *maldandi* male sterile line is complex compared to *milo* and between two *milo* lines *viz.*, 104A and 401A, the 401A is more complex compared to 104A. Further from the two sets, 18 lines (Table 5) were selected and repeated testing was made during *khari*-2018 and *rabi*-2018. Across three seasons two lines *viz.*, DSMR-8 and DSMR-4 (Table 6) were found very strong restorers across two cytoplasm and three lines.

## **Conclusion**

In this experiment, a greater number of restorers were recorded for 104A and 401A which had the blood of *milo* system, revealing the easy availability of restorers for this system compared to *maldandi* system. All the CMS lines used for this study would be in the order of 104A < 401A < M31-A for the complexity of the availability of restorers and for perfect maintainers the order would be M31-2A < 401A < 104A.

## **Declarations**

### **Acknowledgements**

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### **Competing interests**

The authors declare that they have no competing interests.

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## Tables

Due to technical limitations, Tables 1 and 2 are only available as a download in the Supplemental Files section.

**Table 3: Classification of restoration based on mean seed set *per cent* in minicore in *rabi*-2017**

Restoration class	Seed set <i>per cent</i>	Diverse sources of cytoplasm								
		<i>Milo</i>						<i>Maldandi</i>		
		104A			401A			M31-2A		
		No. of genotypes	Seed set <i>per cent</i>	Proportion of lines	No. of restorers	Seed set <i>per cent</i>	Proportion of lines	No. of restorers	Seed set <i>per cent</i>	Proportion of lines
Strong restoration	> 90 %	4	97.99	5.97	2	94.69	2.99	1	100	1.47
High restoration	80 to 90 %	2	86.29	2.99	4	85.23	5.97	0	-	0.00
Moderate restoration	60 to 80 %	9	71.22	13.43	8	69.47	11.94	1	61.54	1.47
Partial restoration	10 to 60 %	23	23.83	34.33	21	24.28	31.34	11	22.79	16.18
Low restoration	< 10 %	9	4.89	13.43	13	6.81	19.40	6	4.74	8.82
No seed set	0 %	20	0.00	29.85	19	0.00	28.36	49	0.00	72.06

**Table 4: Classification of restoration based on mean seed set *per cent* in derived lines of BRJ-67 and BRJ-198 in *rabi*-2017**

Restoration class	Seed set <i>per cent</i>	Diverse sources of cytoplasm								
		<i>Milo</i>						<i>Maldandi</i>		
		104A			401A			M31-2A		
		No. of genotypes	Seed set <i>per cent</i>	Proportion of lines	No. of restorers	Seed set <i>per cent</i>	Proportion of lines	No. of restorers	Seed set <i>per cent</i>	Proportion of lines
Strong restoration	> 90 %	1	91.68	1.75	0	-	0.00	5	94.29	8.77
High restoration	80 to 90 %	16	85.11	28.07	9	84.03	15.79	12	84.56	21.05
Moderate restoration	60 to 80 %	31	74.05	54.39	38	69.82	66.67	15	70.85	26.32
Partial restoration	10 to 60 %	8	49.13	14.04	9	52.52	15.79	2	27.18	3.51
Low restoration	< 10 %	0	-	0.00	1	7.87	1.75	3	4.66	5.26
No seedset	0 %	1	0.00	1.75	0	-	0.00	20	0.00	35.09

**Table 5: Selected lines identified as a restorer (>85 %) on M31-2A in *rabi*-2017 and *kharif*-2018**

S. No.	Genotypes	Per cent restoration		S. No	Genotypes	Per cent restoration		S. No	Genotypes	Per cent restoration	
		Rabi-2017	Kharif-2018			Rabi-2017	Kharif-2018			Rabi-2017	Kharif-2018
1	IS 19975	100.00	78.16	7	BRJ-67 -3	84.92	58.37	13	BRJ-67 -30	83.76	82.73
2	BRJ-67 -8	99.58	99.36	8	BRJ-67 -25	89.56	84.61	14	BRJ-67 -15	82.89	75.16
3	BRJ-67 -4	95.70	96.81	9	BRJ-67 -27	87.60	82.08	15	BRJ-67 -10	82.33	78.62
4	BRJ-67 -21	94.62	86.49	10	BRJ-67 -18	88.90	83.60	16	BRJ-67 -22	81.35	69.46
5	BRJ-67 -19	91.35	87.15	11	BRJ-67 -29	85.64	81.85	17	BRJ-67 -12	81.23	52.46
6	BRJ-67 -16	90.18	86.24	12	BRJ-67 -20	85.36	79.59	18	BRJ-67 -6	81.23	73.12

Table 6: Consistent lines across the season for fertility restoration behavior against M31-2A in *rabi-2018*.

S. No.	Restorer	M31-2A	S. No	Restorer	M31-2A
1	BRJ-67-8 (DSMR-8)	98.94	2	BRJ-67-4 (DSMR-4)	97.42

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table1and2.docx](#)