



Research Article

Early generation combining ability of *maldandi* restorers in *rabi* sorghum [*Sorghum bicolor* (L.) Moench]

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Abstract

In this study, a reported restorer on maldandi cytoplasm DSMR 8 was used to identify combining ability in early segregating generation. A large F_2 population of the cross M31-2A \times DSMR 8 was raised. Among them, fifteen superior restoring plants were selected and forwarded to the F_3 generation. These fifteen F_3 plants were crossed to three male sterile lines having both *milo* and *maldandi* cytoplasmic sources (M31-2A, 401A, and 104A) in $L \times T$ design. Among lines, MR 4 showed significant positive *gca* effects for six traits. Among 45 crosses 18 recorded high *sca* and the remaining 27 crosses showed low *sca*. The crosses M 31-2A \times MR 12 and M 31-2A \times MR 14 showed maximum overall *sca* status as they were having significant positive *sca* effects. Out of 15 lines, only five lines viz., MR 4, MR 12, MR 1, MR 13, and MR 2 could be forwarded to the next generation as these lines were transgressive segregants for combining ability because of good *gca* and better per se performance when crossed to *maldandi* cytoplasm.

Keywords early segregating generation, maldandi cytoplasm, restoration reaction

Introduction

India pledged to achieve carbon neutrality by 2070 to prevent the irreversible effects of climate change. To combat this, C_4 plants have their role as these plants need optimal temperature and have higher photosynthetic efficiency, making them mandatory crops available for cultivation in the future. Sorghum [*Sorghum bicolor* (L.) Moench] is a C_4 plant with increased photosynthetic efficiency [1]. It has acclimatized to a wide range of conditions over the world, from desert and semiarid areas to tropical ones. It plays a crucial function in providing micronutrients at a low cost in addition to food and fodder. This is essential in a nation like India, where 25% of people live in poverty. Recently, The Global Hunger Index 2022 placed India at 107th position out of 121 countries. However, with its sustainable cheap cost and increased micronutrient production, this crop solves the problems of climate change, malnourishment, and to some extent poverty. To modernize and revitalize these crops, FAO designated 2023 as the "International Year of Millets". The first instance of cytoplasmic male sterility (CMS) in sorghum was found when the "*kafir*" nuclear genome was inserted into a "*milo*"

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cytoplasmic background, which is unsuitable [2]. This discovery of A₁ CMS and its subsequent exploitation for hybrid development has enhanced sorghum cultivation because F₁ hybrids outperform traditional landraces in grain yield by 50-60%. Out of the available male sterile sources, including A₂, A₃, A₄ (A₄ *maldandi*, A₄ VZM, A₄, and G₁) [3], A₅, A₆, and KS for both the *kharif* and *rabi* seasons, only Milo (A₁) has been employed to date for the creation of commercial hybrids.

Due to its great yield, hybrids became quite popular in *kharif*. However, over time, the *kafir* nucleus and milo cytoplasm combined to impair grain quality and make it more vulnerable to pests, disease, grain mould, and seasonal temperature fluctuations. These problems made even *rabi* sorghum hybrids unacceptable. So far, no attempts have been undertaken to produce male sterile lines employing indigenous cytoplasm with *rabi*-adopted characteristics and counterpart restorers. The *milo* source of male sterility is relatively more sensitive to cold temperatures and shoot flies [4-5]. To improve the frequency of hybrids with shoot fly resistance in the post-rainy seasons, shoot fly resistance should be required in at least in seed parents or both parents. *Maldandi* (A₄) appears to be the most promising of these other sources during the *rabi* season, as it influences grain size [6] and shoot fly tolerance [4].

The identification of maintainers and restorers on various male sterility sources, such as *milo* (401A and 104A), and particularly on *maldandi* (M31-2A), becomes crucial and will serve as the basis for the successful use of these various CMS sources to increase production while also enhancing pests and diseases resistance. The introduction and use of a new source of male sterility, as well as the identification of restorers and maintainers, not only increases cytoplasmic diversity but also increases nuclear diversity in those cross combinations, broadening the pool of parents from which can be created new hybrids. To achieve this, it is vital to determine the restoration ability of various sources of male sterility. However, *maldandi* cytoplasm has a scarcity of prospective restorers and their stability. Exploiting hybrid vigour and expanding the genetic basis would be made easier with the identification of such restorers. In the basic crossover, an identified restorer on *Maldandi* DSMR 8 was used as a parent. The current investigation seeks to assess the combining ability of the *Maldandi* restorer in early segregating generation for yield and yield attributing characteristics.

Methodology

The identified restorer DSMR 8 was crossed with *maldandi* male sterile line. Thus obtained hybrid was forwarded to F₂ in summer 2019. Around 300-400 F₂ plants were grown and selfed. Among them, the top 15 plants which are presumed to be restorer and agronomically superior were selected randomly and forwarded to F₃ in *rabi* 2019. Further, these 15 plants were used as pollen parents and crossed with male sterile lines viz., M31-2A, 104 A, and 401 A in L × T design during Summer 2020. Obtained 45 hybrids, 18 parents along with two checks were evaluated in RBD design during *rabi* 2020 (Table 1). All the necessary agronomical practices were followed. Mean values of the top five plants were taken and subjected to statistical analysis using the gpbstat package in RStudio. The F₃ plants of the cross M31-2A × DSMR 8 were named MR (*maldandi* restorer). The detailed flow of the work is mentioned in Figure 1.

Results and Discussion

For all the traits, the mean sum of squares (MSS) of crosses was significant. The traits like days to 50 % flowering, panicle length, and panicle weight showed significant MSS among the lines, and only panicle length showed peak MSS among the testers (Table 2). The assessment of *sca* and *gca* effect of crosses and parents helps in the selection of good combining parents and combinations, respectively. Hybrids, parents, and checks were subjected to combining ability analysis and results are depicted in Tables 3 and 4.

Table 1. List of genotypes used for Line × Tester analysis

SN.	Superior F ₃ lines	Females		
1	MR1	SN.	CMS	Origin
2	MR 2	16	104A (<i>milo</i>)	India
3	MR 3	17	401A(<i>milo</i>)	India
4	MR 4	18	M 31-2A (<i>maldandi</i>)	India
5	MR 5	Checks (Varieties)		
6	MR 6	SN.		Origin
7	MR 7	19	M 35-1	India
8	MR 8	20	PKV Kranthi	India
9	MR 9			
10	MR 10			
11	MR 11			
12	MR 12			
13	MR 13			
14	MR 14			
15	MR 15			

MR: Maldandi Restorer

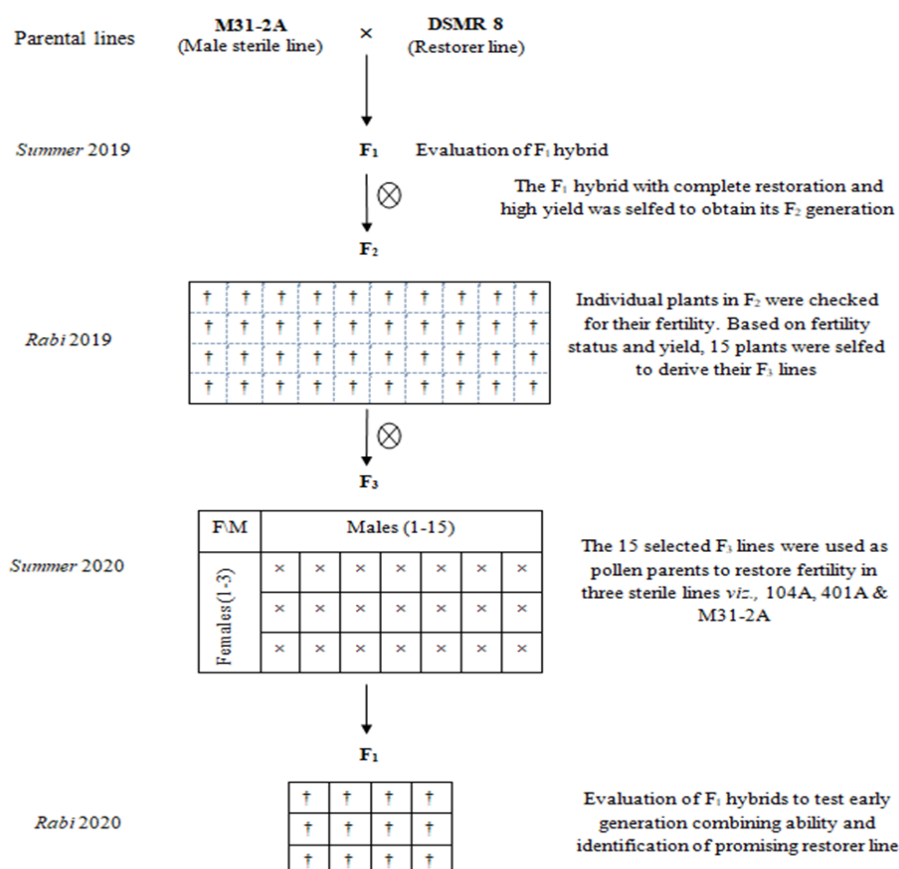


Figure 1. Schematic representation of the methodology followed to test the early generation combining ability

Maturity related traits

Early flowering and early maturing sorghum genotypes are important in *Rabi* sorghum as they escape the terminal drought. Usually, sorghum maturity has a direct effect on total biomass production and grain yield [7]. Among lines, MR 4 was the most suitable for developing high-yielding and early maturing hybrid in *rabi* sorghum as it showed positive significant *gca* effects for panicle width, panicle weight, and grain yield per plant along with negative significant *gca* effects for earliness. The line with negative *gca* for maturity and positive *gca* for productivity traits is most important as genetically and physiologically it will be most efficient [8]. Among hybrids, viz., M 31-2A × MR 12, M 31-2A × MR 14, 104 A × MR 8, and 104 A × MR 2 had negative significant *sca* effect for earliness and positive significant *sca* for grain yield indicating they are a good combination for both the traits in a desirable direction.

Table 2. ANOVA for combining ability for yield and yield components in derived F₁'s of maldandi restorer
F₃ lines against different cytoplasm

Sources of variation		Days to 50 % flowering	Plant height (cm)	Number of leaves	Panicle length (cm)	Panicle width (cm)	Panicle weight (g)	Grain yield plant(g) ⁻¹	100 seed weight (g)
Replications	1	0.001	154.71	2.84*	1.97**	8.10	4.89	0.92	59.21
Crosses	44	30.76**	1077.93**	2.88**	0.46**	1595.77**	11.05**	2.00**	1501.12**
Line effect	14	63.13**	1366.73	4.04	0.83**	2315.54	18.65*	2.50	2200.47
Tester effect	2	13.76	580.41	0.27	0.78*	1113.01	10.82	0.55	424.67
Line × Tester effect	28	15.80**	969.06**	2.49**	0.16	1270.36**	7.26**	1.86**	1228.33**
Error	44	4.705	48.05	0.29	0.12	33.60	3.28	0.82	60.84
Total	89	17.53	558.40	1.60	0.28	805.62	7.14	1.41	772.87
S. Em ±		1.59	4.40	0.40	1.11	0.54	4.76	3.59	0.22
CV		3.01	3.18	5.84	8.38	11.03	7.70	6.88	6.37

Morpho-physiological traits

In sorghum, plant height affects grain yield and overall biomass production [9]. In *rabi* sorghum, fodder yield is as significant as grain yield, hence an optimal plant height and the maximum number of leaves are desired. Among testers, M 31-2A was a good general combiner for both the number of leaves and plant height. In the case of lines viz., MR 2, MR 4, and MR 8 were good combiners for both traits showing significant positive *gca* effect. Plant height and fodder yield are generally positively associated and are of prime importance in developing hybrids for *rabi* season. Among 45 crosses M 31-2A × MR 7, M 31-2A × MR 12, M 31-2A × MR 14, 104 A × MR 2, and 401 A × MR 6 exhibited significant *sca* for both the traits. Twelve crosses had a positive significant *sca* effect for plant height and nine crosses for the number of leaves per plant.

Grain yield and its components

None of the parents in this study were effective combiners for all the yield and yield attributing traits. However, the tester, M 31-2A was a good general combiner for grain yield per plant, panicle weight, and 100 seed weight. Among lines, MR 4 was found to be a good general combiner for panicle width, panicle weight, and grain yield. Improvement in panicle weight would automatically result in an improvement in grain yield and its component traits [10]. Out of 45 crosses, the present study of *sca* effects revealed superiority of several crosses as many as 3, 8, 14, 1, and 15 crosses exhibiting good specific combining abilities concerning panicle width, panicle length, panicle weight, 100 seed weight, and grain yield, respectively.

Table 3. Estimates of *gca* effects of *maldandi* restorer F₃ lines along with different CMS tester for yield and its attributing traits in *rabi* sorghum

SN.	Parents	Days to 50 % flowering	Plant height (cm)	Number of leaves	Panicle length (cm)	Panicle width (cm)	Panicle weight (g)	Grain yield plant (g) ⁻¹	100 seed weight (g)
	Testers								
1	M 31-2A	1.18**	9.35**	0.25*	-0.68 *	0.15	20.01**	19.34**	0.35**
2	104 A	2.05**	-3.54**	-0.07	0.66*	-0.06	-15.95**	-13.9 **	-0.20**
3	401 A	-3.23**	-5.81**	-0.178	0.02	-0.08	-4.05*	-5.35**	-0.14*
	CD at 5%	0.82	2.24	0.20	0.12	1.87	0.58	0.29	2.51
	F₃ Lines								
1	MR 1	-2.19	-11.14**	-0.61 *	-1.1	-0.09	11.14**	18.31**	0.01
2	MR 2	-1.8	7.189 **	1.38**	-0.19	0.51	14.97**	19.81**	0.21
3	MR 3	-1.04	18.02**	0.72**	0.16	0.4	2.47	-0.85	-0.08
4	MR 4	-2.85**	8.02**	0.72**	-0.02	0.91**	9.97**	7.64**	-0.1
5	MR 5	0.29	-8.97**	-0.27	1.51*	-0.15	-2.02	8.97**	0.15
6	MR 6	-1.04	-16.14**	-0.94**	-1.52*	-0.88**	-8.52**	-8.18**	-0.08
7	MR 7	0.65	-18.64**	0.22	0.56	0.15	-2.18	-6.68**	-0.17
8	MR 8	0.63	8.02**	0.05	2.48**	-0.45	2.81	-0.52	0.28*
9	MR 9	1.93*	6.35*	0.88**	0.93	0.93**	2.14	-11.85**	-0.26
10	MR 10	-1.68	-6.64*	0.38	0.03	0.65	4.81	6.97**	0.06
11	MR 11	-0.68	2.68	-0.44	0.9	0.1	-1.18	-1.85	0.32*
12	MR 12	0.2	3.68	-0.44	-0.32	0.26	2.64	6.14**	0.08
13	MR 13	3.07 **	3.35	-0.44	-1.31*	-0.85*	-10.02**	-12.18**	0
14	MR 14	0.87	7.18**	-0.44	-1.24	-0.55	-6.85 *	-5.52*	-0.09
15	MR 15	3.62**	-2.97	-0.77**	-0.87	-0.91**	-20.18**	-20.18**	-0.35*
	CD at 5%	1.85	5.02	0.46	1.31	0.64	5.62	4.19	0.27

Overall combining ability status

The overall combining ability status of parents and hybrids was estimated as suggested by Deepalakshi and Ganesamurthy [11]. Among testers, M 31-2A showed high *gca* status by showing significant positive *gca* effects in desirable direction for all traits except earliness and panicle width. Among lines, MR 4 showed significant positive *gca* effects for all traits except test weight and panicle length. Similarly, lines viz., MR 1, MR 2, MR 3, MR 5, MR 8, and MR 9 were found to be having positive *gca* effects for 2, 4, 2, 2, 3, and 3 traits respectively. The cross M 31-2A × MR 12, M 31-2A × MR 14, and 104 A × MR 2 exhibited positive *sca* effects for maturity, physiological, and yield-related traits revealing high *sca* status. Among 45 crosses 18 recorded high *sca* status and the remaining 27 crosses showed low *sca* status. The crosses M 31-2A × MR 12 and M 31-2A × MR 14 showed maximum overall *sca* status as they were having significant positive *sca* effects.

Transgressive segregants on *maldandi* cytoplasm

Based on the yield per se top five F₁'s on *maldandi* cytoplasm were shown in Figure 2. The parental lines (F₃) of these superior per se performing crosses on *maldandi* cytoplasm were forwarded to the next generation. In *maldandi* cytoplasm crosses having parental lines (F₃) viz., MR 4, MR 12, MR 1, MR 13 and MR 2 were found to be having high per se performance for grain yield, and these crosses identified as potential transgressive segregants for combining ability. Thus, out of 15 lines, only these five lines could be forwarded to the next generation as these lines showed good *gca* and better per se performance when crossed on *maldandi* cytoplasm. Interestingly, MR 4 was found to be a genetically and physiologically potential line for the development of *maldandi* based hybrids because of its earliness, wider panicle, along with good grain yield.



Table 4. Estimates of *sca* effects of crosses for yield and its attributing traits in derived F_1 's of maldandi restorer F_3 lines against different cytoplasm

SN.	Crosses	Days to 50 % flowering	Plant height (cm)	Number of leaves	Panicle length (cm)	Panicle width (cm)	Panicle weight (g)	Grain yield plant (g) ⁻¹	100 seed weight (g)
1	M 31-2A × MR 1	0.33	-20.18**	-1.25**	-2.06	1.300*	-0.34	1.32	0.45
2	M 31-2A × MR 2	2.45	-31.02**	-0.76	-1.98	-0.40	-3.18	-5.68	-0.05
3	M 31-2A × MR 3	-1.40	-19.35**	-0.09	-1.33	0.12	1.32	0.49	0.31
4	M 31-2A × MR 4	-2.50	20.64**	0.91*	-2.15	0.20	44.32**	48.98**	-0.44
5	M 31-2A × MR 5	0.86	-22.35**	-2.08**	4.57**	0.77	-6.68	-11.34**	-0.752 **
6	M 31-2A × MR 6	-3.327 *	-30.18**	-2.42**	-0.15	-0.50	-19.67**	-12.67**	-0.562 *
7	M 31-2A × MR 7	2.52	24.81**	0.91*	-0.48	-0.48	10.9*	15.3**	0.13
8	M 31-2A × MR 8	6.03**	-14.35**	-0.92*	-3.64 **	-0.93	-54.01**	-46.34**	-0.486 *
9	M 31-2A × MR 9	-1.26	-5.19	-0.26	-1.10	-1.31 *	13.65**	-13.51**	0.16
10	M 31-2A × MR 10	-2.61	20.31**	0.24	-0.20	0.97	10.98*	8.156 *	0.39
11	M 31-2A × MR 11	-0.62	-11.52*	1.07*	-0.56	-0.98	-22.01**	-28.01**	0.19
12	M 31-2A × MR 12	-3.83*	37.47**	1.57**	3.65**	1.00	30.15**	39.48**	0.14
13	M 31-2A × MR 13	3.54*	7.81	0.58	1.15	-0.03	-2.68	0.82	0.47
14	M 31-2A × MR 14	-4.20*	43.97**	1.57**	3.07**	0.91	17.15**	20.15**	0.31
15	M 31-2A × MR 15	4.02*	-0.86	0.91*	1.20	-0.62	-20.01**	-17.17**	-0.26
16	104 A × MR 1	-1.04	2.71	1.07*	2.58*	0.31	8.62	5.16	-0.15
16	104 A × MR 2	-4.36**	11.87**	1.07*	2.67*	0.81	45.28**	48.15**	0.04
18	104 A × MR 3	0.84	13.54**	-0.25	1.37	0.42	-15.11**	-15.17**	0.38
19	104 A × MR 4	1.20	-3.95	-0.25	2.00	0.41	-17.21**	-18.17**	0.14
20	104 A × MR 5	-1.08	20.54**	0.74	-1.18	-1.02	-6.21	3.49	0.64**
21	104 A × MR 6	0.79	-2.29	0.41	1.00	0.36	2.29	-1.34	0.10
22	104 A × MR 7	1.12	25.2***	0.74	-0.08	-0.47	-14.04**	-9.34*	-0.30
23	104 A × MR 8	-5.91**	6.04	0.41	3.50**	0.73	35.95**	31.98**	0.29
24	104 A × MR 9	2.29	7.71	0.08	-2.20	1.39*	-23.37**	-13.17**	0.08
25	104 A × MR 10	1.91	-13.28**	-0.92*	-2.39*	-1.67**	-23.04**	-23.01**	-0.27
26	104 A × MR 11	1.42	-12.12**	-0.09	-1.66	0.73	-11.04*	-11.67**	-0.36
27	104 A × MR 12	2.02	-21.12**	-1.08**	-2.94*	-0.59	-8.38	-15.17**	-0.08
28	104 A × MR 13	1.14	-9.28*	-0.59	-0.59	0.33	3.79	6.16	0.07
29	104 A × MR 14	2.07	-24.12**	-1.08**	-1.43	-1.03	2.62	-4.01	0.19
30	104 A × MR 15	-2.41	-1.46	-0.26	-0.65	-0.71	20.45**	16.15**	0.01
31	401 A × MR 1	0.71	17.47**	0.18	-0.52	-1.611 **	-8.28	-6.48	-0.30
32	401 A × MR 2	1.92	19.14**	-0.32	-0.69	-0.41	-42.11**	-42.47**	0.02
33	401 A × MR 3	0.55	5.81	0.34	-0.04	-0.54	14.38**	14.68**	0.07
34	401 A × MR 4	1.30	-16.68**	-0.66	0.14	-0.61	-27.11**	-30.81**	0.30
35	401 A × MR 5	0.22	1.81	1.34**	-3.39**	0.26	12.88*	7.85*	0.11
36	401 A × MR 6	2.54	32.47**	2.01**	-0.86	0.14	17.38**	14.02**	0.46
37	401 A × MR 7	-3.63*	-50.02**	-1.65**	0.56	0.96	3.06	-5.98	0.18
38	401 A × MR 8	-0.12	8.31	0.51	0.14	0.21	18.05**	14.35**	0.20
39	401 A × MR 9	-1.03	-2.52	0.18	3.29**	-0.08	9.72	26.68**	-0.25
40	401 A × MR 10	0.70	-7.02	0.68	2.59*	0.71	12.05*	14.85**	-0.12
41	401 A × MR 11	-0.80	23.64**	-0.98*	2.23	0.26	33.05**	39.68**	0.17
42	401 A × MR 12	1.81	-16.35**	-0.49	-0.71	-0.41	-21.77**	-24.31**	-0.06
43	401 A × MR 13	-4.68**	1.48	0.01	-0.56	-0.29	-1.11	-6.98	-0.53*
44	401 A × MR 14	2.14	-19.85**	-0.49	-1.64	0.11	-19.77**	-16.14**	-0.49*
45	401 A × MR 15	-1.61	2.31	-0.66	-0.56	1.32*	-0.44	1.02	0.25
	CD 95% SCA	3.21	8.71	0.80	0.46	7.27	2.27	1.12	9.73

*, ** significant at 5 and 1 per cent respectively



Figure 5. Top five hybrid combinations on *maldandi* cytoplasm for yield and yield attributing traits

Among the 45 hybrids evaluated, the top ten hybrids based on their grain yield and yield-attributing traits are depicted in Table 5.

Table 5. Top ten hybrids based on yield *per se* and their *sca* and *gca* status in derived F₁'s of *maldandi* restorer F₃ lines against different cytoplasm

SN	High yielding hybrids	Grain yield per plant (g)	Tester	Line	<i>sca</i> effect	Days to 50 per cent flowering	Plant height (cm)	Number of leaves	Panicle length (cm)	Panicle width (cm)	Panicle weight (g)	100 seed weight (g)
1	M 31-2A x MR 4	149.50	19.34**	7.64**	48.98**	70.00	230.00	12.00	16.00	8.50	162.50	5.02
2	M 31-2A x MR 12	138.50	19.34**	6.14**	39.48**	71.73	242.50	11.50	21.50	8.65	141.00	5.77
3	104 A x MR 2	127.50	-13.98**	19.81**	48.15**	70.06	207.50	12.50	22.00	8.50	132.50	5.23
4	M 31-2A x MR 1	112.50	19.34**	18.31**	1.32	73.50	170.00	8.50	15.00	8.60	119.00	6.02
5	M 31-2A x MR 10	108.50	19.34**	6.97**	8.15*	71.06	215.00	11.00	18.00	9.00	124.00	6.01
6	M 31-2A x MR 14	107.50	19.34**	-5.52*	20.15**	72.03	252.50	11.50	20.00	7.75	118.50	5.77
7	M 31-2A x MR 2	107.00	19.34**	19.81**	-5.67	76.00	177.50	11.00	16.00	7.50	120.00	5.71
8	M 31-2A x MR 7	101.50	19.34**	-6.68**	15.32**	78.53	207.50	11.50	18.25	7.05	117.00	5.52
9	M 31-2A x MR 3	92.50	19.34**	-0.85	0.48	72.92	200.00	11.00	17.00	7.90	112.00	5.78
10	104 A x MR 8	91.00	-13.98**	-0.52	31.98**	70.95	202.50	10.50	25.50	7.45	111.00	5.56

*, ** significant at 5 and 1 per cent respectively



Hybrids viz., M 31-2A × MR 4 and M 31-2A × MR 12 were early (70 days and 71.73 days, respectively) maturing and have shown a sufficient number of leaves (12 and 11.50, respectively) ensuring the highest photosynthates accumulation. In addition, they have shown promising grain yield which can be advanced further to gain transgressive segregates. Further, the restoration is governed by major and minor genes, screening and selecting over the generation will reduce the environmental influence [12-14]. From the performance of F₁ hybrids derived from MR lines, it was logically evident that only superior MR Lines should be forwarded to F₄. Isolation of such superior plants will eliminate tedious process of advancing of huge junk material to its succeeding generations. Performing the hybridization using plants selected in early segregating generations would result in the churning of restoration genes because only superior and good restorer plants were selected in every generation by rejecting inferior and bad restorer plants.

Conclusion

The study confirmed that the method of testing the combining ability of *maldandi* restorers in *rabi* sorghum can be efficiently adopted in early segregating generations to improve combining ability status and identify the effective restorer lines. Based on combining ability, superior general and specific combiners identified can be utilized in varietal and hybrid development. The suggested approach is efficient and effective to test combining ability and identify restorer lines against *maldandi* source of male sterility in sorghum.

Conflict of interest

The authors declare no conflicts of interest.

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