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RESEAERCH ARTICLE

Identification of B and R lines on *maldandi* cytoplasm and assessing their genetic diversity in *rabi* sorghum [*Sorghum bicolor* (L.) Moench]

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Abstract

Fifty-four superior minicore collections were crossed with *maldandi* based male sterile line (M31-2A) to study restoration patterns and to classify maintainer (B) and restorer (R) lines. Of 54 mini core collections, only one came out as a strong restorer (IS 19450), 12 partial restorers and 41 maintainers. Further, these 13 partial restorers (12 partial restorers and 1 strong restorer) and 41 maintainers along with M35-1 were subjected to diversity analysis for eleven characters. A total of 55 genotypes were clustered into seven clusters. Out of which, cluster–I and cluster-II had 34 and 16 genotypes, respectively, and five were solitary clusters having single genotypes. The highest inter-cluster distance was noted between cluster-VI and VII followed by cluster V and VI, and cluster-II and VII, indicating ample diversity available among them. Therefore, the genotypes of these clusters can be used as parents for crossing in the hybridization program to obtain desirable and excellent segregants. However, divergent maintainers against the strong restorer (IS 19450) can be used for the development of *maldandi* based hybrids.

Keywords: Maintainers, restorer, *maldandi* cytoplasm, genetic diversity

Introduction

India committed to becoming a carbon-neutral state by 2070 to avoid the irreversible effects of climate change. C_4 plants have a role in adapting to climate change as these plants need optimal temperature and have higher photosynthetic efficiency, making them mandatory crops to grow in the future. Sorghum [*Sorghum bicolor* (L.) Moench] is a C_4 plant with higher photosynthetic efficiency (Reddy et al. 2009). It adapted to a wide range of conditions over the world, from desert and semiarid areas to tropical ones. Sorghum is the fifth-largest cereal crop in the world in terms of production volume, after wheat, rice, maize, and barley. 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, India was ranked 107th out of 121 nations in the Global Hunger Index 2022. However, with its sustainable, cheap cost and increased micronutrient production, this crop solves the problems of climate change, malnourishment, and to some extent, poverty. FAO designated 2023 as the "International Year of Millets" to modernize and revitalize these crops.

The first instance of cytoplasmic male sterility (CMS) in sorghum was found when the nuclear genome of "*kafir*" was inserted into an unsuitable cytoplasmic background of "*milo*" (Stephens and Holland 1954). This discovery of A_1 CMS and its subsequent exploitation for hybrid development has revolutionized sorghum agriculture because F_1 hybrids outperform traditional landraces in grain yield by 50-60%. Out of the available male sterile sources, including A_2, A_3, and A_4 (A_4 *maldandi*, A_4 VZM, A_4, and G_1), A_5, A_6, and KS for

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both the *kharif* and *rabi* seasons, only *Milo* (A_1) 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 yet, no attempts have been undertaken to produce male sterile lines employing indigenous cytoplasm with rabi-adopted characteristics and counterpart restorers. According to Dhillon et al. (2005) and Downes (1972), the *milo* source of male sterility is relatively more sensitive to cold temperatures and shoot flies. To increase the frequency of hybrids with shoot fly resistance in the post-rainy seasons, shoot-fly resistance should be required in both parents or at least in seed parents. *Maldandi* (A_4) appears to be the most promising of these other sources during the *rabi* season, as it influences grain size and shoot fly tolerance.

The identification of restorers and maintainers on various sources of male sterility, 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 resistance to pests and diseases. The introduction and use of a new source of male sterility and 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 to create new hybrids. To achieve this, it is vital to determine the genetic diversity between maintainers and restorers. However, *maldandi* cytoplasm has a scarcity of prospective restorers and their stability. Exploiting hybrid vigour and expanding the genetic basis would be easier with identifying such restorers. Further, diversity studies among the identified maintainers and restorers for yield traits will help in diversifying the cytoplasmic base. Therefore, in the present study, an effort has been made to identify maintainers and restorers and to asses genetic diversity between them for yield traits.

Materials and methods

The experimental material comprised 54 superior ICRISAT minicore collections. All these genotypes along with male sterile line M31-2A were sown for hybridization during *kharif* 2019. The obtained 54 F_1's were evaluated for fertility restoration patterns during *rabi* 2019. Five plants from each row of F_1's were selfed randomly with brown bags before flowering at about the boot leaf stage to avoid cross-pollination. Around 25 to 30 days after flowering (physiological maturity), each panicle was observed visually for seed set under selfing and the percent seed set was calculated using the following formula (Kishan and Borikar 1989). Based on the seed set percent the genotypes were grouped into different categories of restoration as described by Biradar (1996). Based on F_1's seed set, percent lines were classified as maintainers (0%) partial restorers (1 to 89%) and restorers (> 90%).

$$\text{Seed set } \textit{per cent} = \frac{\text{Total number of seeds}}{\text{Total number of spikelet's}} \times 100$$

Further, all 54 minicores along with M35-1 were sown in two replications in randomized block design to assess genetic diversity during *rabi* 2020 (Fig. 1). Average values of the characters over two replications were used for statistical analysis. The standard technique of D^2 statistic was followed to assess genetic divergence among the test genotypes. The Tocher method given by Rao (1952) was adopted for grouping genotypes into different clusters. Further, individual D^2 values against all the genotypes were calculated using R-Studio to identify divergent maintainers concerning strong restorer (IS 19450) on *maldandi* cytoplasm.

Results

Restoration pattern, geographical origin, and racial background of minicore Out of 54 hybrids used only IS 26046, IS19450, and IS 29269 were found to be reliable restorers (> 60% seed setting) on *maldandi* cytoplasm. However, among the lines used 13 were grouped as partial restorers (1 to 100% seed setting), and 41 lines were grouped as perfect maintainers (0% seed setting) as depicted in Table 1. Most of the restorers and partial restorers were from South Africa (3), India (2), Mali (2), and Botswana (2), whereas maintainers belong to 27 countries, most of them were from China (6), India (4), and Yemen (3) while, identified strong restorer (IS 19450) was from Botswana. The distribution of partial restorers, complete restorers and maintainers were plotted in the world map Figs 2 and 3, respectively. The fertility restorers were restricted to only Africa and Asian continents, whereas maintainers were distributed across America, Africa, Asia and parts of European continents. Overall, the distribution ranged from tropical to temperate climatic conditions, as depicted in Figs. 2 and 3. As per the racial background of the lines, most of the partial restorers belong to *guinea* (3), *guinea- caudatum* (2), and *kafir* (2) while maintainers belong to 13 different races among them *caudatum* (11) and *caudatum* derived hybrid races (8) were most as depicted in Table 2.

Genetic divergence among the minicore collections

All the 55 genotypes were grouped into 7 clusters according to their D^2 values. Most of the genotypes were in cluster I (34) and cluster II (16); the other five were solitary clusters possessing single genotypes. The intra and inter-cluster

distances are presented in Table 3. The range of intra-cluster distance was noticed from 0.00 to 48.39. The highest intra-cluster distance was displayed by cluster-I (48.39) followed by Cluster-II (45.82). The range of inter-

cluster distances was noticed from 80.63 to 595.43. The highest inter-cluster distance was noted between cluster-VI and VII (595.43) followed by cluster-V and VI (435.03), and cluster-II and VII (360.98). Apart from this, individual D^2 values for each genotypic combination were calculated by using R-studio. Among them, top divergent maintainers with respect to restorer on *maldandi* cytoplasm (IS 19450) were listed in Table 4. Among the 11 traits studied 1000 seed weight and plant height came out to be the highest contributors to the divergence.

The cluster means of all the 11 characters presented in Table 5 revealed that the analysis of data had appreciable contrast among the clusters for most of the characters investigated. Cluster-III showed the maximum mean for panicle width, while cluster-IV displayed the maximum mean for primaries per panicle and 1000 seed weight. Similarly, cluster-V showed the maximum mean for panicle weight, number of seeds per panicle, and grain yield per plant, while cluster-VI showed the maximum mean for plant height, peduncle length, and panicle length.

Discussion

The availability of restorer on *maldandi* was found to be difficult and more complex than other cytoplasm (Sandeep *et al.* 2020 and Verma *et al.* 2022). In the current study, getting a single restorer with 99% seed set out of 54 genotypes was also possible. However, many genotypes that are classified as partial restorers have shown varied degrees of restoration (4.55 to 99%) when crossed with a single cytoplasm indicating the presence of single or multiple restorer genes (*Rf*) or their penetrance and expressivity differs with different breeding lines besides modifiers causes on differential

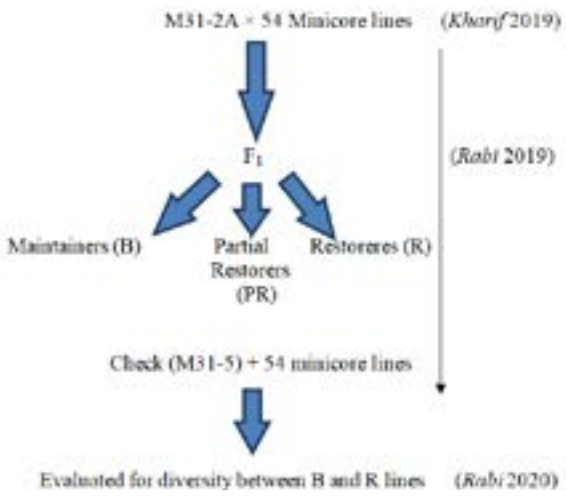

Fig. 1. Schematic representation of the experiment conducted across different season

Fig. 2. Geographical distribution of partial restorers and restorers on *maldandi* cytoplasm

Fig. 3. Geographical distribution of maintainers on *maldandi* cytoplasm

restoration on different breeding lines (Pande *et al*. 1990). Similarly, in rice the interplay between the nuclear genes of the CMS lines and the restorer genes of male parents could explain the variable fertility pattern of breeding lines when crossed with the same WA-CMS line source (Viraktamath 2010). So, based on previous works of literature and present findings it could be concluded that the nuclear background of *ms* line influences the expression of restoration and seed setting. It could also be stated that additional minor genes and environmental factors (rainfall and humidity) affected fertility restoration and led to gradation in the restoration in addition to major genes (Kariyannanavar et al. 2023). The genotypes IS 29269 (86.65%), IS 26046 (61.20%), IS 5919 (35.47%) and IS 26025 (33.45%) have been grouped as partial restorers because of partial seed setting. This partial seed setting may depend upon the strength of minor genes governing restoration. Promising restorers can be obtained by inducting these minor genes into the identified restorer. This will ensure the stability of the restorers by reducing the environmental influences.

Further, these partial restorers and maintainers were subjected to diversity. The genotypes in cluster-I have the highest diversity among their genotypes consequently, these genotypes can potentially be utilized in different breeding programs for developing new varieties. Likewise, cluster-VI showed the highest inter-cluster distance with cluster-VII, indicating to use of the genotypes in these clusters for hybridization to generate fresh variability and to obtain potential

Table 1. Restoration status of the superior minicore against *maldandi* (M 31-2A) cytoplasmic sources of male sterility

S. No.	Genotypes	Seed set per cent Maldandi	S. No.	Genotypes	Seed set per cent Maldandi	S. No	Genotypes	Seed set per cent Maldandi
		M31-2A			M31-2A			M31-2A
1	IS 26617	0	19	IS 24175	0	37	IS 4698	0
2	IS 25249	0	20	IS 24348	0	38	IS 9745	0
3	IS 28313	0	21	IS 25989	0	39	IS 10302	0
4	IS 29335	0	22	IS 29654	0	40	IS 12735	0
5	IS 29392	0	23	IS 29914	0	41	IS 14290	0
6	IS 30466	0	24	IS 30383	0	42	IS 19445	4.55
7	IS 27912	0	25	IS 30451	0	43	IS 27887	10.46
8	IS 2397	0	26	IS 30536	0	44	IS 33353	8.45
9	IS 8012	0	27	IS 31043	0	45	IS 24462	9.45
10	IS 14861	0	28	IS 24139	0	46	IS 24492	10.77
11	IS 15478	0	29	IS 4515	0	47	IS 4060	10.55
12	IS 15945	0	30	IS 12937	0	48	IS 5919	35.47
13	IS 19389	0	31	IS 28614	0	49	IS 26025	33.45
14	IS 12804	0	32	IS 995	0	50	IS 26046	61.25
15	IS 29468	15.66	33	IS 2413	0	51	IS 19975	10.53
16	IS 7987	0	34	IS 2872	0	52	IS 19450	99.00
17	IS 22616	0	35	IS 602	0	53	IS 29269	86.61
18	IS 22720	0	36	IS 4581	0	54	IS 30466	0

Table 2. Distribution of genotypes from mini-core and derived lines in seven different clusters

Cluster No.	Name of genotypes	No. of genotypes	Origin	Races
1	IS 2872 (M), IS 4581 (M), IS 10302 (M), IS 29914 (M), IS 24348 (M), IS 27912 (M), IS 30536 (M), IS 2413 (M), IS 19389 (M), IS 25249 (M), IS 12804 (M), IS 29392 (M), IS 30466 (M), IS 26617 (M), IS 12308 (M), IS 29654 (M), IS 30451 (M), IS 26046 (PR), IS 4698 (M), IS 24462 (PR), IS 30383 (M), IS 24175 (M), M-35, IS 4515 (M), IS 14290 (M), IS 29335 (M), IS 31043 (M), IS 5919 (PR), IS 8012 (M), IS 29468 (PR), IS 2397 (M), IS 33353 (PR), IS 19445 (PR) , IS 22616 (M)	34	Egypt, India (6), Thailand, Zimbabwe, South Africa (2), Korea, Iran, Bangladesh, Ethiopia, Turkey, Lesotho, China (4), Madagascar, Mali, South Africa, Tanzania, Botswana (2), Swaziland, Uganda, Japan, Lesotho, Kenya, Myanmar	Caudatum- bicolor (7), Durra (5), Caudatum (7), Kafir-caudatum, Bicolor (4), Durra-bicolor, Kafir (3), Kafir-bicolor, Guinea (2), Kafir-durra, Guinea-caudatum
2	IS 26025 (PR), IS 27887 (PR), IS 28614 (M), IS 12937 (M), IS 24139 (M), IS 25989 (M), IS 9745 (M), IS 24492 (PR), IS 995 (M), IS 28313 (M), IS 4060 (PR), IS 15945 (M), IS 15478 (M), IS 14861 (M), IS 22720 (M), IS 19975 (PR)	16	Mali (2), South Africa, Yemen (2), Ethiopia, Tanzania, Sudan, South Africa, USA, India, Cameroon (3), Somalia, Senegal	Guinea (4), Caudatum-bicolor (2), Durra-caudatum (2), Kafir (2), Caudatum (2), Durra-bicolor, Guinea- caudatum (2), Durra
3	IS 19450 (R)	1	Botswana	Gunia- kafir
4	IS 29269 (PR)	1	Switzerland	Gunia- caudatum
5	IS 12735 (M)	1	Yemen	Caudatum- bicolor
6	IS 602 (M)	1	USA	Bicolor
7	IS 7987 (M)	1	Nigeria	Gunia

M = Maintainer R = Restorer PR = Partial restorer

Table 3. Average intra and inter cluster distances (D^2) for seven clusters of sorghum

	I	II	III	IV	V	VI	VII
I	**48.39**	106.87	92.7	80.63	135.8	247.89	161.78
II		**45.82**	160.67	169.04	323.55	143.96	360.98
III			**0**	190.02	172.42	333.55	107.5
IV				**0**	168.24	232.44	245.48
V					**0**	435.03	87.49
VI						**0**	595.43
VII							**0**

Bold values indicate intra cluster distance values

Table 4. Top 10 divergent maintainers with respect to identified restorer

S. No.	Restorer	Maintainers	D^2 value
1	IS 19450	IS 26025	541
2	IS 19450	IS 602	667.11
3	IS 19450	IS 25989	416.23
4	IS 19450	IS 14861	420.06
5	IS 19450	IS 29269	380.05
6	IS 19450	IS 24492	329.28
7	IS 19450	IS 27887	353.3
8	IS 19450	IS 28614	322.89
9	IS 19450	IS 995	314.61
10	IS 19450	IS 25249	333.22

Table 5. Cluster means for eleven characters among seven clusters in sorghum

Cluster No.	No. of genotypes	Days to 50% flowering	Plant height (cm)	Number of leaves	Peduncle length (cm)	Panicle length (cm)	Panicle width (cm)	Panicle weight (g)	Primaries $Panicle^{-1}$	1000 seed weight (g)	Number of seeds $panicle^{-1}$	Grain yield $plant^{-1}$ (g)
I	34	78.84	224.47	8.57	40.94	22.25	41.29	81.75	62.52	35.37	2085.6	72.35
II	16	71.5	236.62	8.45	48.63	23.52	33.24	49.87	57.7	22.73	1736.4	39.73
III	1	76.5	235.5	10.5	22.00	13.51	60.85	86.07	38	26.5	2806.5	71.53
IV	1	77.5	226	7.75	39.5	36.5	44.41	69.23	90	46.15	1294.2	54.51
V	1	77.25	210.5	7.53	45.00	29.00	40.54	149.25	41.01	43.5	3000.3	142.25
VI	1	66.5	280.25	7.56	59.12	52.38	23.55	41.68	51.5	19.44	1778.5	32.13
VII	1	95.5	278.75	11.5	30.75	11.86	57.8	127.03	54.75	41.03	2652.8	113.03

Transgressive segregants. Further, individual D^2 values showed divergent maintainers, namely, IS 602, IS 26025, and IS 14861 with respect to strong restorer (IS 19450) can be used as a parent in the development of *maldandi* based hybrids and these hybrids will be having the dual advantage of shoot fly resistance and good grain size because of *maldandi* cytoplasm.

In the current study, the genotypes (maintainers and partial restorers) from different countries clustered together, or indigenous genotypes segregated in different clusters suggesting there was no association between genetic and geographic diversity because genotypes originated in different locations falling in the same cluster due to their common pedigree or allelic constitution Rao et al. (1989). Similarly, maintainers and partial restorers, when subjected to diversity shared the same clusters, which means both maintainers and partial restorers fall on the same cluster rather than segregating into different clusters, indicating the lack of relationship between phenotypic traits and restoration behavior as clustering was done by using phenotypic traits. Identification of restorer on *maldandi* cytoplasm is complex because of minor genes and several environmental factors. The identified restorer (IS 19450) can be used as a parent in the development of *maldandi* based hybrids by crossing with diversified maintainers. Further, it can be used to diversify the cytoplasmic base.

Authors' contributions

Conceptualization of research (MCW, BDB, VSK); Designing of the experiments (MCW, BDB, SNC, VSK); Contribution of experimental materials (BDB); Execution of field/lab experiments and data collection (PK, R); Analysis of data and interpretation (PK, PKN, R, LKV); Preparation of the manuscript (PK, BDB, R).

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