Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 7 [3] February 2018 : 105-112

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
NAAS Rating 4.95

ORIGINAL ARTICLE **OPEN ACCESS**

Fertility Restoration Pattern of Minicore Collection of *Rabi* Sorghum On Different CMS Lines

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ABSTRACT
To identify sterility maintainers and restorers in sorghum (Sorghum bicolor (L.) Moench), minicore collection germplasm accessions representing variation from 26 countries were crossed with the male-sterile lines milo and maldandi cytoplasm. The F_1 hybrids were classified as male-fertile or male-sterile based on the seed set on bagged ear heads. Among these, 43 (25.59 %) were classified as strong restorers with > 90 % seedset and 22 genotypes (13.10) as maintainers with zero seedset on milo cytoplasm where as on maldandi cytoplasm 19 genotypes (13.87 %) as strong restorers and 37 genotypes (27.01 %) as maintainers in the postrainy season. The maintainers on either of cytoplasm helps in diversification of CMS background where as strong restorers can be used as male parent in hybrid development or can be used as source for transfer for restorer gene into elite genetic background.
Keywords: *Cytoplasm, Maintainers, Restorers, Seedset and Sorghum*

Received 12.05.2018 Revised 29.06.2018 Accepted 01.07.2018

INTRODUCTION

Sorghum is an important crop where exploitation of heterosis led to the green revolution in dryland areas in 1970's. Commercial exploitation of heterosis was possible owing to the availability of a stable and heritable CMS mechanism (Stephens and Holland, 1954), inspite of small bisexual flowers. The commercial hybrids predominantly cultivated all over the globe were based on milo (A_1) cytoplasm. Cytoplasmic uniformity not only restricts the nuclear genetic diversity of male-sterile (A) and restorer (R) lines in hybrid development but creates problems associated with single cytoplasm. In order to broaden genetic and cytoplasmic base of hybrids, there is a need for employing alternate CMS systems in sorghum hybrid development (Praveen *et al*., 2015).

Of the several alternative CMS systems described maldandi cytoplasm appears to be more potential for *rabi* sorghum. *Milo* cytoplasm not only induces the susceptibility to shoot fly but also causes sterility under *rabi* season. Use of indigenous *maldandi* source of male sterility instead of exotic *milo* appears to be the best option. Kishan and Borikar (1989) reported that *maldandi* source of male sterility exhibited larger seed size in hybrids during post rainy season. Dhillon *et al*. (2005) observed that *maldandi* cytoplasm was less susceptible to sorghum shoot fly and can be exploited for producing sorghum hybrids. Infact, Biradar (2011) developed *maldandi* based *rabi* sorghum hybrid SPH1452 (M31-2A x BRJ 62) with all the desirable characters but the level of heterosis was limited to 18-22 per cent. The introduction and utilization of *maldandi* source of male sterility also enhance cytoplasmic diversity and this further widens the choice of parents to develop hybrids. Inspite of all this, the main bottle neck in utilizing this source of male sterility is non availability of stable restorers with good grain quality. Developing stable restorers with good grain quality is pre-requisite for exploiting the *maldandi* source of male sterility. In the present study an attempt was made to understand the genetic diversity existing with respect to restoration system required for milo and maldandi cytoplasm using minicore collection germplasm.

MATERIALS AND METHODS

The male sterile lines utilized as testers in this study were 104A and M31-2A. 104A is based on milo cytoplasmic source and has the distinction of being used in several commercial hybrids. Male sterile line, M31-2A, representing Maldandi cytoplasm has very good grain quality traits like bold size seed, luster and corneous endosperm and resistant to biotic and abiotic stresses. Genotypes from minicore collection were used to identify restorers and maintainers on diverse cytoplasms. All the male parents were crossed to two testers and developed 2 x 168 F_1 bulked seed of every cross (F_1) was planted in a row of 4m length. About 3 heads from each row were bagged 3 days before stigma emergence. The F_1 hybrids were evaluated at botanical garden, Department of genetics and plant breeding, UAS, Dharwad in *rabi* 2015-16. All the recommended agronomic practices were followed to raise a good crop. At maturity, number of seeds were counted out of total number of spikelets per ear head and seedset percentage was calculated.

$$\text{Seed set \%} = \frac{\text{Total number of seeds}}{\text{Total number of spikelets}} \times 100$$ (Kishan and borikar, 1989)

Classification of genotypes in minicore collection was done by following procedure described by Biradar *et al.* (1996) below.

Category	Seed set%
Strong restoration	>90 %
High restoration	80 to 90 %
Moderate restoration	60 to 80 %
Partial restoration	10 to 60 %
Low restoration	<10 %
No seed set	0 %

Table 1: Restoration status of the minicore collection on milo (104A) and maldandi (M31-2A) cytoplasm sources of male sterility.

S.No	Genotypes	Sources of CMS		S.No	Genotypes	Sources of CMS	
		Milo (A_1)	Maldandi (ML)			Milo (A_1)	Maldandi (ML)
75	IS26694	29.65	13.46	112	IS15945	94.58	74.65
76	IS22294	39.42	13.65	113	IS30451	81.23	75.32
77	IS20298	26.25	15.64	114	IS23590	93.51	75.36
78	IS2379	38.59	16.58	115	IS21083	94.56	81.54
79	IS29714	25.65	19.54	116	IS15744	93.24	82.33
80	IS20625	38.65	19.75	117	IS20743	97.65	82.35
81	IS25249	42.16	22.35	118	IS24463	95.64	85.62
82	IS473	28.52	22.35	119	**IS27887**	94.72	91.35
83	IS8777	38.56	22.36	120	**IS28313**	91.65	91.35
84	IS21512	42.12	23.65	121	**IS25989**	94.65	92.32
85	IS23684	38.95	23.65	122	**IS29654**	94.35	92.34
86	IS31706	41.23	23.65	123	**IS24462**	94.36	92.35
87	IS7310	39.65	23.75	124	**IS29269**	98.45	93.24
88	IS25089	42.35	26.64	125	**IS26025**	91.32	93.65
89	IS5295	42.35	29.65	126	**IS17941**	94.35	94.31
90	IS12706	33.45	33.25	127	**IS19450**	92.36	94.32
91	IS4092	48.65	33.25	128	**IS4581**	95.25	94.35
92	IS29627	97.31	54.32	129	**IS22720**	97.56	94.65
93	IS33353	76.32	54.39	130	**IS22616**	95.68	95.65
94	IS4613	72.12	55.32	131	**IS26617**	97.65	95.68
95	IS30460	68.25	56.32	132	**IS24175**	94.65	96.21
96	IS4515	72.35	56.35	133	**IS28614**	92.75	96.24
97	IS31714	73.65	58.31	134	**IS4698**	94.52	96.27
98	IS21645	95.24	58.64	135	**IS32439**	94.52	96.54
99	IS14861	90.35	59.67	136	**IS19389**	93.27	96.54
100	IS602	91.23	61.32	137	**IS31651**	94.25	97.64
101	IS20679	92.36	61.32	138	IS7305	16.54	*
102	IS4060	94.62	62.34	139	IS29772	92.35	*
103	IS29304	74.65	62.34	140	IS24348	94.25	*
104	IS19262	94.25	64.58	141	IS30460	36.98	*
105	IS24492	93.65	69.34	142	IS20816	15.35	*
106	IS19975	94.65	71.24	143	IS26617	6.98	*
107	IS15478	94.65	71.25	144	IS30466	16.95	*
108	IS23891	95.62	72.35	145	IS1212	5.65	*
109	IS995	96.24	72.35	146	IS30451	92.35	*
110	IS11619	94.65	73.24	147	IS24953	26.54	*
111	IS14290	78.95	74.65	148	IS28614	31.25	*

Table 1: Restoration status of the minicore collection on milo (104A) and maldandi (M31-2A) cytoplasm sources of male sterility.

S.No	Genotypes	Sources of CMS	
		Milo (A_1)	Maldandi (ML)
149	IS27887	29.34	*
150	IS30507	12.35	*
151	IS25910	0.00	*
152	IS7679	17.54	*
153	IS20743	26.98	*
154	IS29241	25.68	*
155	IS2413	22.35	*
156	IS4631	5.24	*
157	IS25732	36.25	*
158	IS8012	42.35	*
159	IS9745	0.00	*
160	IS5094	12.35	*
161	IS4515	23.65	*
162	IS2872	13.26	*
163	IS11026	0.00	*
164	IS26737	95.64	*
165	IS995	18.54	*
166	IS3158	23.65	*
167	IS12706	28.54	*
168	IS22720	94.65	*

RESULTS

(a)Milo based F_1's

A total of 168 genotypes crossed on milo CMS line, among the crosses the F_1's corresponding to '51' genotypes exhibited satisfactory (> 60 %) seed setting, while '22' F_1's showed no seed setting. 43 genotypes were found to be strong restorers (> 90 % seed set) on milo source. 25.60 per cent of germplasm lines proved to be strong restorers. Based on number of spikelets revealing seedset, the seed set percentage of each F_1 was determined. The mean seed set percentage of the F_1's produced by strong restorer lines in F_1's was 94.34 %. Most of germplasm lines evaluated were found to be partial restorers with mean seed set percentage of 28.26 % on milo. The proportion of such lines was 46.42 per cent (Table 3.). The mean seed set percentage in F_1's of 7 genotypes was 73.76 per cent (moderate restorers) on milo cytoplasm. The mean seed set percentage of 17 genotypes was 6.39 per cent on milo cytoplasm (poor restorers) (Table 2).

Table 2: Classification of restoration based on mean seed set percentage

Restoration class	Seed set %	Diverse sources of cytoplasm			
		Milo		Maldandi	
		No. of restorers	Mean seed set %	No. of restorers	Mean seed set %
Strong restoration	>90 %	43	94.34	19	94.47
High restoration	80 to 90 %	1	81.23	4	82.96
Moderate restoration	60 to 80 %	7	73.76	15	69.44
Partial restoration	10 to 60 %	78	28.26	39	26.05
Low restoration	<10 %	17	6.39	23	7.04
No seed set	0 %	22	0.00	37	0.00

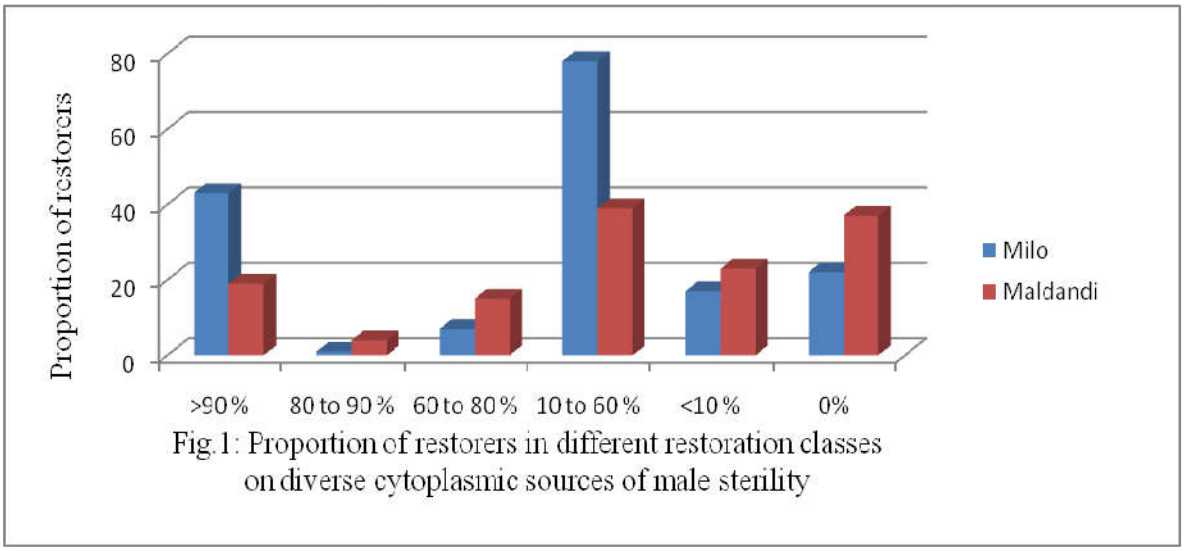

Fig.1: Proportion of restorers in different restoration classes on diverse cytoplasmic sources of male sterility

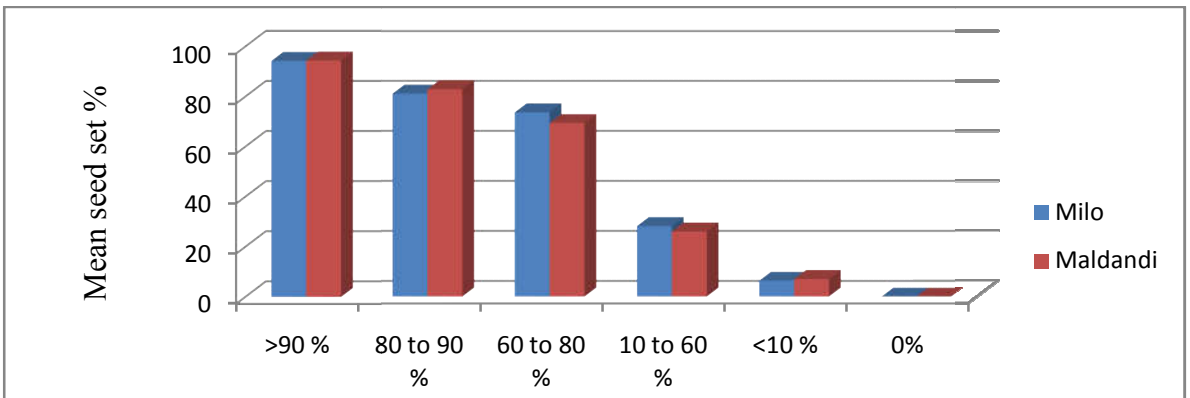

Fig.2: Mean seed set percentage observed in different restoration classes on two diverse sources of male sterility

Table 3: Proportion of lines representing different restoration classes and seedset percentage on diverse cytoplasmic sources

Restoration category	Milo	Maldandi	Common on both cytoplasm
I Strong restorers >90 %			
Number of lines	43	19	19
Proportion %	25.60	13.87	13.87
Mean seed set %	94.34	94.47	94.47
II High restorers 80-90 %			
Number of lines	1	4	0
Proportion %	0.60	2.92	
Mean seed set %	81.23	82.96	
III Moderate restorers 60-80 %			
Number of lines	7	15	0
Proportion %	4.17	10.95	
Mean seed set %	73.76	69.44	
IV Partial restorers 10-60 %			
Number of lines	78	39	31
Proportion %	46.43	28.47	36.04
Mean seed set %	28.26	26.05	27.35
V Low restorers <10 %			
Number of lines	17	23	14
Proportion %	10.12	16.79	53.84
Mean seed set %	6.39	7.04	6.85
VI Maintainers 0 %			
Number of lines	22	37	18
Proportion %	13.10	27.01	43.90
Mean seed set %	0	0	0
Total number of lines tested	168	137	200

(b)Maldandi based F_1's

A total of 137 genotypes crossed on maldandi CMS line, among the hybrids corresponding to 38 germplasm lines showed more than 60 per cent seed setting on selfed ear heads, i.e., only 27.73 per cent of germplasm lines (Table 2). Out of 137 genotypes 19 genotypes were found to be strong restorers which showed > 90 per cent seed set. The mean seed set percentage of the F_1's produced by strong restorers was 94.47 percent. Thirty seven genotypes showed no seed setting in F_1's, of which corresponding male parents were designated as maintainers. Most of germplasm lines evaluated for restoration ability were with mean seed set percentage of 26.05 percent (partial restorers) with proportion of 28.46 percent (Table 3.). The mean seed set percentage of 15 genotypes (moderate restorers) was 69.44 percent. The mean seed set percentage of 23 genotypes (poor restorers) was 7.04 percent on maldandi cytoplasm.

(c)Commonness of restoration on different cytoplasmic sources of male sterility

Out of 168 genotypes tested 19 exhibited more than 90 per cent restoration on milo as well as on maldandi cytoplasm (Table 3.). Similarly 18 genotypes showed no seedset in F_1's of both milo and maldandi cytoplasm. Comparatively with milo cytoplasm number of genotypes with no seedset in F_1's was high in maldandi cytoplasm. The genotypes which showed restoration on maldandi cytoplasm were recorded for strong restoration even on milo cytoplasm also. But the case with strong restorer lines identified on milo cytoplasm is not same with maldandi cytoplasm. Hence considerable differences because of cytoplasms were there.

DISCUSSION

(a) Restorer gene frequency

The availability of restorers determines the extent of the use of various CMS systems in hybrid breeding programme. In the present study restoration frequency on maldandi and milo (A_1) cytoplasm found to be 13.86 per cent and 25.59 per cent respectively (Table. 3). Biradar *et al.* (1996) found restorer frequency on milo of 38.28 per cent and on maldandi of 13.28 per cent out of 128 lines. The work carried out at ICRISAT showed a restoration frequency of 90 per cent on A_1, 50 per cent on A_2, 10 per cent on A_3, and 30 per cent on A_4, when 48 germplasm lines were test crossed onto A_1, A_2, A_3 and A_4 CMS systems (Reddy *et al*., 2003). Hence, considering the restoration frequency, A_1 CMS system provides the widest possible choice in selecting restorers but being maldandi was superior to milo interms of grain quality aspects and other traits, the identified restorers will help in *rabi* sorghum hybrid programme.

Table 4: List of identified genotypes based on fertility restoration of > 60 per cent seed set in F_1 hybrids

1.	**Strong R-lines on both milo and maldandi with >90 % seed set**	
	Name of the genotypes	**Number of genotypes**
	IS29269, IS26617, IS28614, IS27887, IS22720, IS32439 IS17941, IS4698, IS29654, IS19450, IS28313, IS24175, IS26025, IS24462, IS4581, IS31651, IS25989, IS 22616 and IS 19389	19
2	**Strong R-lines on milo only with >90 % seed set**	
	Name of the genotypes	**Number of genotypes**
	IS20743, IS23891, IS995, IS23590, IS15744, IS602, IS29627, IS24463, IS20679, IS14861, IS24492, IS15945 IS21645, IS11619, IS19975, IS19262, IS21083, IS15478 , IS29772, IS24348, IS30451, IS26737, IS22720 and IS20679	24
3	**Restorer lines (R-lines) on both milo and maldandi with 80 to 90 % seed set**	
	Name of the genotypes	**Number of genotypes**
	Milo - IS30451	1
	Maldandi - IS20743, IS15744, IS24463 and IS21083	4
4.	**Restorer lines (R-lines) on both milo and maldandi with 60 to 80 % seed set**	
	Name of the genotypes	**Number of genotypes**
	Milo - IS30460, IS4515, IS4613, IS33353, IS14290, IS31714 and IS29304	7
	Maldandi - IS30451, IS23891, IS995, IS23590, IS602,IS20679, IS14290, IS24492, IS29304, IS15945,IS11619, IS19975, IS19262, IS15478 and IS4060	15

(b) Identification of B- and R-lines

Umadevi *et al.* (2010) reported that the lines identified as effective maintainers can be further back crossed with their respective F_1's to look for completely sterile back cross progenies so that these can be developed as new CMS lines. 22 Genotypes were classified as maintainer lines with zero per cent seedset on both milo and maldandi CMS lines because of lack of restoration ability (Table. 5). Kumar *et al*. (2004) reported in sorghum that sterility in F_1 when we make test crosses indicates the male parent contain recessive genes for restoration but have normal cytoplasm. The frequency of maintainers was highest in

maldandi compared to milo cytoplasm and hence provide the greatest opportunities for genetic diversification of A- lines.

Table 5: List of identified genotypes based on fertility restoration of zero per cent seedset in F_1 hybrids

1.	**Maintainer lines (B-lines) on both milo and maldandi with zero per cent seedset**	
	Name of the genotypes	**Number of genotypes**
	IS18039, IS20195, IS29187, IS10969, IS29441, IS23586, IS19445, IS28389, IS14010, IS3971, IS30092, IS13893, IS24218, IS23644, IS17980, IS12883, IS20632 and IS23521	18
2	**Maintainer lines (B-lines) on milo with zero per cent seedset**	
	Name of the genotypes	**Number of genotypes**
	IS18039, IS20195, IS29187, IS10969, IS29441, IS23586, IS19445, IS28389, IS11919, IS14010, IS15931, IS3971, IS30092 IS13893, IS29091, IS24218, IS23644, IS17980, IS12883, IS20632, IS23521 and IS24139	22
3	**Maintainer lines (B-lines) on maldandi with zero per cent seedset**	
	Name of the genotypes	**Number of genotypes**
	IS16151, IS24348, IS32787, IS8012, IS9745, IS2389, IS4360, IS18039, IS16382, IS29335, IS20195, IS29914, IS29187, IS6421, IS29091, IS10969, IS15931, IS29441, IS23586, IS19445, IS28389, IS14010, IS29606, IS30572, IS3971, IS30092, IS13893, IS24218, IS12804, IS2382, IS23644, IS17980, IS11919, IS12883, IS31446, IS20632 and IS23521	37

A total of 43 genotypes acted as strong restorers (> 90 % seedset) on milo and 19 genotypes acted as strong restorers on maldandi cytoplasm (Table 4). The differential restoration of genotypes on diverse CMS sources depends upon number of restorer genes in nuclear background.

In the present study the genotypes which showed restoration on maldandi also showed restoration on milo also but not converse which means more number of restorer genes could be involved for restoration on maldandi. Elkonin *et al*. (1998) reported in sorghum that fertility restoration was controlled by one or two dominant genes depending on the nuclear background of the male parents. Klein *et al*. (2001) reported that single Rf_1 gene plays important role in inducing fertility restoration on milo cytoplasm. Dandin *et al.* (2014) reported that maldandi based male sterile lines require one or two independent major restorer genes for fertility restoration.

On milo cytoplasm 16 genotypes were found to be strong restorers with > 90 per cent seedset but on maldandi those 16 genotypes showed moderate restoration with 60-80 per cent seedset (Table 1.). Genotypes like IS29627, IS21645 and IS14861 showed strong restoration (>90 %) on milo and on maldandi they found to be partial restorers with 10-60 per cent seedset. On milo 78 genotypes found to be partial restorers with 10-60 per cent seedset but on maldandi 38 genotypes only found to be partial restorers. Reddy *et al*. (2003) reported that test crosses with a partial seedset on all the bagged panicles indicates the corresponding male parents are neither as complete (>90 % seedset) nor as complete maintainers (0 % seedset).

These findings shows differences of fertility restoration across diverse cytoplasmic sources even the pollinator parent was same. These differences could be because of differences in cumulative action of modifier genes or weak restorer gene to the particular cytoplasm. Shalini *et al*. (2015) reported in rice that the variation in fertility restoration could be because of modifier genes/weaker restorer gene. Reddy *et al*. (2003) reported in sorghum that cumulative action of restorer genes and modifier genes results variation in fertility restoration.

Umadevi *et al.* (2010) reported in rice that in some cases, the same genotype can behave as a restorer for one CMS line and as a maintainer for the other CMS line. In the present study genotypes like IS24348, IS8012, IS16382, IS29914, IS6421 and IS30572 behaved as effective maintainer for maldandi with zero per cent seedset and partial restorers for milo with 10 to 60 per cent.

(c) Differential response of same set of strong restorers (>90 % seedset)

Regarding common restoration behaviour of the genotypes on diverse cytoplasms, out of 43 strong restorers on milo cytoplasm, 19 genotypes exhibited simultaneous restoration on maldandi also. Pattanashetti *et al*. (2002) reported that, out of 27 genotypes, only three genotypes *viz.,* BRJ 67, CR 9 and BRJ 62 have been found to be common restorers on milo (104A) and maldandi (M31-2A) sources of cytoplasm.

While out of 43 genotypes, 24 showed restoration on milo cytoplasm only but not on maldandi. This shows these particular genotypes may not contain extra restorer genes which show restoration on maldandi cytoplasm also. Murthy and Gangadhar (1990) studied Segregating progenies with milo (A_1) cytoplasm in F_2 generation and showed that a single gene was responsible for fertility restoration of A_1 male-sterile cytoplasm.

The strong restorer lines (R-lines) identified on maldandi cytoplasm were able to restore on milo also. This shows that these lines contain restorer genes which can show fertility on diverse sources of cytoplasm. These lines may have higher number of genes involving in fertility restoration in maldandi. Dandin *et al*. (2014) reported in sorghum that two independent major Rf genes were involved in fertility restoration on maldandi cytoplasm. These findings also conclude that restorers on maldandi cytoplasm contain more than one Rf gene in its nuclear background but may or may not be restorers of milo cytoplasm.

Lines which commonly restore on milo and maldandi cytoplasm can be crossed to obtain segregates which accumulate genes responsible for restoring on all the two cytoplasms. Common restorers can be used to develop alloplasmic hybrids with diverse cytoplasm and thereby it is possible to overcome the risks associated with the use of single cytoplasmic source for producing hybrid.

(d) Effect of other genes on restoration

In the present study genotypes like IS29627, IS21645 and IS14861 showed strong restoration (>90 %) on milo (Table 1.) and on maldandi they found to be partial restorers with 10-60 per cent seedset. On milo cytoplasm 16 genotypes were found to be strong restorers but on maldandi they showed moderate restoration with 60-80 per cent seedset. For the same set of restorers differential seedset on milo and maldandi cytoplasm indicates there could be involvement of other genes (major/minor). The intraallelic and interallelic interactions between such genes will definitely have role on fertility restoration. Incase of genotypes with strong restoration (> 90 % seedset) on milo and maldandi cytoplasm there could be strong association of number of modifier genes along with the major fertility gene.

On milo cytoplasm 22 genotypes were found to be B-lines with zero seedset per cent but on maldandi 37 genotypes were found to be B-lines. The number of B-lines were low in milo cytoplasm because there could be effect of modifier genes comparatively better than maldandi cytoplasm which results in few seeds on panicle but not on maldandi cytoplasm. Shalini *et al.* (2015) reported in rice that the appearance of partial fertile segregants in crosses with complete restorers suggested the probable role of modifiers in fertility restoration.

On milo 78 genotypes found to be partial restorers with 10-60 per cent seedset but on maldandi 38 genotypes only found to be partial restorers. The number of genotypes with partial were high on milo cytoplasm rather than maldandi cytoplasm which shows the high effect of modifier genes on milo cytoplasm whereas low number of genotypes as partial restorers on maldandi cytoplasm could be due to poor expression of weak restorer gene or may be more number of modifier genes could be required. Hence, to enhance the restoration potential one has to tap the ability of modifier genes along with major restorer genes in nuclear background. Two or more major genes and more dominant modifiers with additive effect coordinate complete fertility restoration.

CONCLUSION

The maintainers on either of cytoplasm helps in diversification of CMS background where as strong restorers can be used as male parent in hybrid development or can be used as source for transfer for restorer gene into elite genetic background.

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