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Research Article

Identifying the Fertility Restorers and Maintainers from the Local Landraces in Sorghum

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Abstract

Cytoplasmic male sterility system has been widely been used for increasing sorghum yield through heterosis exploitation. To effectively use male–sterility inducing cytoplasm, it is necessary to identify restorers and lines that are suitable for conversion to male sterility. The present study was undertaken to determine the fertility/sterility reactions of a subset of entries from a total of 1019 accessions of two collections (1976 and 2003) of landrace sorghums crossed onto two CMS lines NE223A and Tx623A. The male-sterile lines were Kafir-milo derivatives. Three hundred and forty test-crosses were generated and evaluated in two environments in Niger. Studies revealed four F1 test-crosses (NE223A x L95-1, NE223A x L95-3, NE223A x L119-2 and NE223A x L102-1) with complete sterility across the two locations. The corresponding parental lines were therefore recommended for conversion to male sterile. However, forty (40) additional test-crosses showed male sterility at Maradi only and two others at Konni respectively, whereas 286 and 272 hybrids shed pollen. The pollen parents of the fertile test crosses were classified as fertility restorers. The male-sterility of female NE223A was maintained by more lines (42) than Tx623A (8) while, at the same time, there were more sterile crosses within the 2003 collection than within the 1976 collection. According to the classification of sorghum races, the sub-group guinea-margaritiferum had the highest number of lines without restorer genes.

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop in the world after wheat, rice, maize and barley. In addition to its food, feed and fodder qualities, it has emerged recently as 'fuel' crop. Globally, hybrids are the preferred type of varieties in sorghum as they yield 20 to 30% additional grain and stover over a range of environments compared to open-pollinated varieties and landraces [1]. However, hybrid cultivar development requires additional research investment over those needed for non-hybrid cultivars for developing suitable parental lines [2].

CMS has been successfully used to develop hybrids for increasing crop production worldwide [3]. The use of cytoplasmic genetic male sterility system in sorghum hybrid development is possible only when effective maintainers and restorers are identified. The introduced CMS lines A/BTx623 and NE223A/B used in the sorghum genetic enhancement program in Niger were those introduced from the US during through institutional collaboration. CMS lines introduced from elsewhere may not be well adapted to a given target environment. Therefore, it is very important to identify maintainers and restorers from local germplasm for development of new lines in a hybrid program. Identification of locally adapted maintainers and restorers which show complete sterility and high degree of restoration of CMS lines would be of great importance in commercial hybrid program.

Cytoplasmic Male Sterility system is a physiological abnormality resulting from disharmonious interaction between the cytoplasmic factors, the mitochondrial genetic factors and nuclear genetic factors leading to the production of degenerate or non-viable pollen grains or non-dehiscent anthers with or without functional pollen grains [2]. Development of a hybrid using CMS requires identification of a male sterile line (A-line), a maintainer line (B-line), and a restorer line (R-line). Main characteristics of these lines are A-line is male sterile, used as female in commercial hybrid production. B-line is isogenic line of A-line, and is needed to maintain A-line. B-line is considered as key line without that A-line could not be maintained. R-line is a male fertile line used as pollen parent in commercial seed production plot. Test cross program help identify maintainers as well as restorers. Maintainer lines used for conversion into new CMS lines and restorer lines are subsequently used as male parent in hybrid development program.

Successful use of hybrid vigor largely depends on the availability of locally developed cytoplasmic genetic male sterile (CMS) and restorer lines [4]. Moreover, breeders need wide range of CMS lines to produce desired hybrids. Therefore, it is necessary to transfer available CMS system into local elite breeding lines. So, the use of local CMS line would be helpful to development of adaptable, heterotic hybrids. Toure and Scheuring [5], evaluated the Malian sorghum collection for sterility/fertility reactions. Potential maintainers and restorers were found and were recommended in the development of A-lines for creation of potential hybrid parents. Efforts have been made by sorghum team in Niger in the early 2000 with the development of L28 A/B line, but some flowers still shed pollen during sterilization phase. The present study was undertaken to identify maintainer and restorer lines from two landrace collection trips conducted in 1976 and 2003 [6,7], where a total of 1019 sorghum accessions were collected.

MATERIALS AND METHODS

Germplasm collection and its description

Two reference sorghum collections were used in this study. The first germplasm collection was done in 1976 by FAO and ORSTOM and covered 183 villages. The second collection was done in 2003 and covered 79 villages of the 183 villages visited in 1976. The visited villages during the collection covered the rainfall pattern and the agro-ecological conditions of the country. The method used and the sampling technique was described by Deu et al., and by Bezancon et al. [6,7]. The genetic diversity of the collection was described by Deu et al. [8].

Germplasm evaluation

The 1019 accessions were evaluated at Maradi and Konni. Maradi is at 07.05 E and 13.48 N and Konni is at 05.05E and 13.48N. Maradi and Konni have approximately the same climate of the Sudano-Sahelien zone. Agro-climatic data (rainfall and temperatures) during the period of evaluation are presented in Figure 1.

ATx623 and AN223 were used as female lines and crossed with local inbred lines used as pollinators. The female ATx623 was introduced from Texas A&M through INRAN/INTSORMIL collaboration in 1984. ATx623 is an early maturing variety with 1.60 m tall, white grain and medium and semi-open panicle. This variety is supposed to be a good combiner. AN223 from the other side is from Nebraska and also introduced through INRAN/INTSORMIL collaboration. This female with a medium maturity cycle, is dwarf (1m) with long, big and semi-open panicle, with good grain quality. The male parents were local landraces from two collections made in Niger in 1976 and 2003.

F_1 production

During the rainy season, the female lines ATx623 and AN223 were crossed with a sample of the two collections at Maradi (longitude 07.05 E; latitude 13.48 N). The samples of each collection were planted separately in bands of one hundred rows. Each row was three meters length. In the middle of the samples were planted the two CMS lines, separately in two bands. The samples of 2003 collected accessions were planted in six bands and the samples of the 1976 in 7 bands. Each band was 100 meters long and comprised 96 varieties with two rows used as borders between bands. Each variety was planted in one row of three meters length and 50 cm between hills. A total of 3 ha land area was used. The trial was supplied with 50 kg/ha NPK 15-15-15 and 50 kg/ha urea (N = 46%) applied twice during the growing season. The field was kept weed free by hand weeding two times during the growing period.

The CMS lines used as females (A lines) receive pollen from those accessions which had good synchronization with them to produce F1 hybrids. The mating was a Line x Tester design, the A lines being used as testers. Staggered planting was used to maximize the number of germplasm collections flowering at the same time as the A lines. For this reason, three different dates of sowing of the female lines were used. Supplementary irrigation was applied to avoid drought stress on the germplasm. Heads of the female parents were covered with pollination bags to avoid contamination with unwanted pollen. An average of three crosses was made between the A lines and each accession.

Method of evaluation

A total of 340 F_1 testcrosses obtained from crosses between the local varieties and the two CMS lines were planted during the following rainy season at Maradi with two different dates of sowing and at Konni (one planting date). Each hybrid was planted on a single row of three meters with 50 cm between hills on the same row. The trial was supplied with 50 kg/ha NPK 15-15-15 and 50 kg/ha Urea applied twice during the growing season. The field was kept weed free by hand weeding during the growing period. The methods of evaluation and the selection criteria used were those of Reddy [9].

RESULTS

The 340 F_1 hybrids were successfully synthesized (seed obtained) and evaluated the following raining season for sterility/fertility reaction (Table 1).

Evaluation of the 340 F_1 in the two localities, 286 and 272 hybrids at Maradi and Konni respectively shed pollen and were classified as fertile. Fifty hybrids (50) were sterile: 44 at Maradi and 6 at Konni. Two sterile accessions obtained in Konni were different from those obtained at Maradi. The sterility of hybrids was confirmed with the lack of pollen productivity during the flowering period and the total absence of grain formation at maturity on the panicles when covered in the two localities. The CMS line AN223 gave 42 of the fifty sterile hybrids and with the ATx623 eight (8) were sterile (Table 2).

Four (4) sterile hybrids (AN223 x L95-1, AN223 x L95-3, AN223 x L102-1, AN223 x L119-2) were common between the two localities. According to the classification of Harlan and De Wet [10], there belong to different races of sorghum (Table 3).

At Maradi, thirty six (36) F_1 sterile hybrids (maintainer lines) were obtained with the CMS A- line AN223 and eight (8) sterile hybrids with the CMS A-line ATx623. There were 178 and 108 F_1 hybrid fertile (Restorer lines) with each of the two A-lines respectively. Some semi-sterile lines (10) were also observed with the female AN223 (Table 4).

At Konni, only six (6) F_1 hybrids all coming from the crosses with the female AN223 were sterile (maintainer lines). There were 272 fertile (Restorer lines) in which 168 with the female

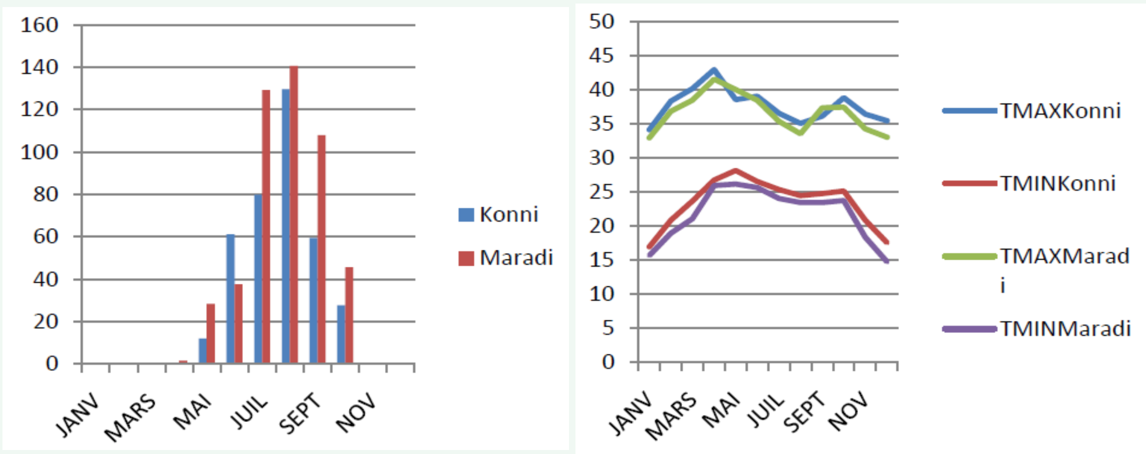

Figure 1 Rains and temperatures repartitions during the year 2009 at Maradi and Konni.

Table 1: Sorghum landraces assessed for fertility reaction when crossed onto CMS lines.

	Number of crosses		
Source of pollinator	**With Female ATx623**	**With Female NE223A**	**Total F_1 hybrids**
1976 collection	72	111	183
2003 collection	44	113	157
Total F_1 Hybrids	116	224	340

Table 2: List of sterile hybrids and their parental lines in Maradi.

	Sterile F_1 hybrid	Landrace name	Race of landrace
1	AN223 x L39-2	KATCHABO	Durra/ Caudatum
2	AN223 x L39-3	KATCHABO	Durra/ Caudatum
3	AN223 x L232-2	JA DAWA	Durra/ Caudatum
4	AN223 x L234-1	KIERMA	Guinea
5	AN223 x L234-2	KIERMA	Guinea
6	AN223 x L234-3	KIERMA	Guinea
7	AN223 x L297-1	MALE	Guinea
8	AN223 x L297-2	MALE	Guinea
9	AN223 x L382-1	SIKOMBE	Guinea/ Caudatum
10	AN223 x L382-2	SIKOMBE	Guinea / Caudatum
11	AN223 x L382-3	SIKOMBE	Guinea / Caudatum
12	AN223 x L516-2	AMO KANA	Guinea / Caudatum
13	AN223 x L535-2	SOKOMBA	Guinea
14	AN223 x L535-3	SOKOMBA	Guinea
15	AN223 x L95-1	Sokomba kirey	Guinea - margaritiferum
16	AN223 x L95-2	Sokomba kirey	Guinea - margaritiferum
17	AN223 x L95-3	Sokomba kirey	Guinea - margaritiferum
18	AN223 x L102-1	Akouya	Caudatum
19	AN223 x L102-2	Akouya	Caudatum
20	AN223 x L119-1	Mallé (tardive)	Guinea - margaritiferum
21	AN223 x L119-2	Mallé (tardive)	Guinea - margaritiferum
22	AN223 x L119-3	Mallé (tardive)	Guinea - margaritiferum
23	AN223 x L156-1	Socomba	Guinea - margaritiferum
24	AN223 x L156-2	Socomba	Guinea - Margaritiferum
25	AN223 x L165-1	Socomba	Guinea - margaritiferum
26	AN223 x L165-2	Socomba	Guinea - margaritiferum
27	AN223 x L165-3	Socomba	Guinea - margaritiferum
28	AN223 x L200-1	Socomba	Guinea - margaritiferum
29	AN223 x L200-2	Socomba	Guinea - margaritiferum
30	AN223 x L200-3	Socomba	Guinea - margaritiferum
31	AN223 x L207-1	Mallé	Guinea - margaritiferum
32	AN223 x L207-2	Mallé	Guinea - margaritiferum
33	AN223 x L207-3	Mallé	Guinea - margaritiferum
34	AN223 x L450-1	Kierma	Guinea - margaritiferum
35	AN223 x L450-2	Kierma	Guinea - margaritiferum
36	AN223 x L450-3	Kierma	Guinea - margaritiferum
37	ATx623 x L217-1	KIERMA	Guinea
38	ATx623 x L217-2	KIERMA	Guinea
39	ATx623 x L377-1	SOKOMBA	Caudatum
40	ATx623 x L377-2	SOKOMBA	Caudatum
41	ATx623 x L377-3	SOKOMBA	Caudatum
42	ATx23 x L508-1	SOKOMBA	Guinea
43	ATx623 x L508-2	SOKOMBA	Guinea
44	ATx623 x L516-1	AMO KANA	Durra/ Caudatum

AN223 and 104 with the female ATx623. Sixty two (62) other hybrids were affected by drought and couldn't be evaluated. No semi-sterile F_1 hybrid was observed in this locality but 62 F_1 hybrids were affected by drought and did not reach the blooming period (Table 5).

During the crossing period, each landrace being a population (family), each cross in the same family is different from another. Evaluating crosses within families, nine of them at Maradi got total sterility in the two repetitions. Eight (8) families were maintainers with the female AN223 and one was maintainer with the female ATx623 (L377**)**. None family got total sterility at Konni. The total sterility within a family implied the sterility of the three crosses that have been made between one tester and the local (Table 6).

When sterile families were partitioned between the different races of sorghum, six of them were Guinea-margaritiferum, one Guinea and one other hybrid Guinea-caudatum. Their complete sterility was obtained with the female AN223. Only one maintainer belonging the natural hybrid Guinea caudatum race was found with the femaleATx623 (Table 7).

Table 3: List of sterile hybrids and their parental lines in Konni.

	Sterile F_1 hybrid	Landrace name	Race of landrace
1	AN223 x L70-1	Hamo kirey	Durra/ Caudatum
2	AN223 x L70-2	Hamo kirey	Durra/ Caudatum
3	AN223 x L95-1*	Sokomba kirey	Guinea- margaritiferum
4	AN223 x L95-3*	Sokomba kirey	Guinea - margaritiferum
5	AN223 x L102-1*	Akouya	Caudatum
6	AN223 x L119-2*	Mallé (tardive)	Guinea - margaritiferum

*common sterile hybrids in the two localities

Table 4: Repartition of hybrids into sterile and fertile at Maradi.

Hybrids	AN223	ATX623	Total
sterile	36	8	
semi-sterile	10	0	10
fertile	178	108	286
Total	224	116	**340**

Table 5: Repartition of hybrids into sterile and fertile at Konni.

Hybrids	AN223	ATX623	Total
Sterile	6	0	6
Semi-sterile	0	0	0
Fertile	168	104	272
Total	174	104	278

Table 6: Number of sterile F1 hybrids within families at Maradi.

Tester	Family	Family name	Race	Sterile
AN223	L39	KATCHABO	Durra/ Caudatum	2
AN223	L232	JA DAWA	Durra/ Caudatum	2
AN223	**L234***	**KIERMA**	**Guinea**	**3**
AN223	L297	MALE	Guinea	2
AN223	**L382***	**SIKOMBE**	**Guinea/ Caudatum**	**3**
ATx623	L516	AMOKANA	Guinea/ Caudatum	1
AN223	L535	SOCOMBA	Guinea	2
ATx623	L217	KIERMA	Guinea	2
ATx623	**L377***	**SOCOMBA**	**Caudatum**	**3**
ATx623	L508	SOCOMBA	Guinea	2
AN223	**L95***	**Socomba Kirey**	**Guinea- margaritiferum**	**3**
AN223	L102	Akouya	Caudatum	2
AN223	**L119***	**Malle**	**Guinea - margaritiferum**	**3**
AN223	L156	Socomba	Guinea - margaritiferum	2
AN223	**L165***	**Socomba**	**Guinea - margaritiferum**	**3**
AN223	**L200***	**Socomba**	**Guinea - margaritiferum**	**3**
AN223	**L207***	**Malle**	**Guinea - margaritiferum**	**3**
AN223	**L450***	**Kierma**	**Guinea - margaritiferum**	**3**

*Families with complete sterility

DISCUSSION

The four local landraces (L95-1, L95-3, L119-2 and L102-1) that exhibited completely sterile crosses with the CMS line NE223A at both locations may be converted into CMS lines. Similar results of effective sterility in a national collection were found by Toure and Scheuring [5], at Sotuba and Babougou in Mali. These results meet the requirements of Reddy et al. [11], that demand that a male sterile line used for a large scale production of hybrids should neither shed pollen, nor set seed when selfed, regardless of location or season. For these reasons, the four pollen parents of these sterile hybrids are going to be transformed into A lines through backcrossing. The existence of A lines with local landraces in the breeding program of Niger will improve sorghum production through high yielding hybrids development.

The discovery of complete fertility restorers and complete sterility maintainers within local germplasm is important in Niger's sorghum breeding program because it helps in the use of landraces in hybrid development. Experimental hybrids were made using local varieties as well as exotic lines as pollinators on seed parents provided by INTSORMIL or ICRISAT. Although well adapted to the environment, most landraces produced tall hybrids which lodged and which are later than their pollinators [12]. There is therefore a need to develop suitable parents and an economical method of hybrid seed production available to small holders [12].

The 280 fertile crosses found differs somewhat from a study in Mali by Toure and Scheuring [13] where out of 227 $F_{1's}$ evaluated, 142 shed pollen and 86 had non-restorer genes. In this study, only fifty F_1 showed male sterility in at least one location and only four were sterile at both locations out of the 340 F_1 hybrids evaluated. This implies that most of the germplasm evaluated in Niger restored fertility.

In addition to the crosses that were sterile at one location but fertile at the other, ten crosses were found to be semi-sterile with the female NE223A at Maradi. These crosses were all discarded from the experiment. This finding is in accordance with Miller and Pickett [14], and Reddy [9], who found that some crosses, were semi-sterile. Male parents that produced semi-sterile crosses were rejected from a breeding program because they serve neither as restorers nor as maintainers [11]. Toure and Scheuring [13], evaluating local germplasm for stability and fertility reaction in Mali found complete stability across the two environments.

Cytoplasmic male sterility is environmentally dependant. The numbers of non-restorer and restorer lines were higher at Maradi than at Konni. CMS stability in relation to temperature is an important factor in the production of hybrid seeds. Maradi and Konni have approximately the same agro-climatic conditions. Average maximum and minimum temperatures during the crop growing period were at Maradi 37.7°C and 24.7°C, and 38.2°C and 25.9°C at Konni. Total rainfall in the two localities was, respectively, 491.4 and 370.1mm during the experiment. Results agreed with those obtained by Reddy et al. [11]. The difference in rainfall was probably high enough to show the differences in sterility across environments. According to Tarumoto et al. [12],

Table 7: Repartition of the maintainer families within races of sorghum according to the CMS lines.

Females	Guinea	Caudatum	Guinea/ Caudatum	Durra/ Caudatum	guinea margaritiferum	Total
AN223	1	0	1	0	6	8
ATx623	0	1	0	0	0	1
Total	1	1	1	0	6	9

the temperature stimulus in the duration of flower development causes phenotypic fluctuation for sterility in A_1 male sterile lines.

Sometimes, during the flowering period when crosses were evaluated for sterility/fertility reactions, there was a risk of contamination which may have affected the crosses. The contamination may be attributed to the fact that covered heads of hybrids were opened for verification of the presence of pollen and at the same time for the evaluation of the anther morphology. This may have resulted in the flowers receiving pollen from other panicles shedding pollen at the time. This probably affected the efficiency of expression of male sterility in some of the hybrids. New methods should therefore be found to facilitate sterility/ fertility evaluation and to render the method more efficient. The proportion of sterile hybrids was 13% at Maradi and 2% at Konni. This proportion may be low according to the numbers of hybrids evaluated (340), but it is a good start for the sorghum program in Niger because it is the first time that this kind of study was conducted.

Three of four sterility maintaining lines (L95-1, L95-3, L119-2) belong to the guinea margaritiferum race and one (L102-1) is caudatum. The race guinea margaritiferum was found to possess lines that efficiently maintained sterility in the A_1 cytoplasm in another study [15]. Backcrossing to transfer genes into the restorer B-lines based on the CMS line NE223A should certainly use this guinea-margaritiferum race however, it is recommended that the sorghum improvement program should place emphasis first on the Caudatum race because it is more productive. Guinea-margaritiferum race varieties are found in the Southwest region of the country and are more adapted to high annual rainfall, whereas the Caudatum races are adapted to drier conditions where most sorghum is produced. However, all sorghum basic botanical races except kafir can be found in Niger [16], and each of them can be used for gene transfer in the presence of sterility restorer genes.

The guinea margaritiferum group is genetically differentiated from all other races, including non-margaritiferum guinea sorghums [6,16,17]. This resulted from a lack of concordance in flowering and strong human selection which could contribute to an efficient reproductive barrier [18-21]. The genetic distinctness of margaritiferums from other guinea sorghums from western Africa is remarkable, since both are interfertile and cultivated in sympatry in the same season by the same farmers [16]. The genetic history of guinea-margaritiferum is enigmatic and still requires further investigation within the species *Sorghum bicolor* [6]. Our study revealed maintainer lines within this race.

CONCLUSION

Four out of 340 F_1 hybrids, (~1%) were found with complete sterility leading to the identification of potential A lines from the conversion of 4 Niger landrace sorghums. The 4 landraces are composed of one from the Caudatum race known for adaptation in the dry environments and 3 from the Guinea Margaritefum race adapted for relatively better growing conditions. The use of adapted A lines will enhance hybrid development for high productivity and better adaptation to local conditions. Forty four (44) hybrids exhibited complete sterility at Maradi and six at Konni. However ten others expressed partial sterility a Maradi. In addition, 286 and 272 lines shed pollen and the respective pollen parents were classified as fertility restorers. The large number of fertile hybrids indicates a predominance of R lines among Niger landraces which may be used to derive improved male parents.

Sterilization of lines will continue with the CMS line NE223A as most of the sterile hybrids came from this female. Both NE223A and TX623A may be used for other crosses within the two collections as a great number of the accessions were not successfully crossed and may be sterilized with one of the CMS sources. Following the breeding work with MDK, this is the very first systematic study of the fertility reaction of sorghum landraces in Niger. The diversity in the landraces suggests in itself that farmers in different agro-ecological zones have specific preferences which are in part related to varietal adaptation. Thus this work may lead to the development of various A lines with specific adaptation which will in turn help produce hybrids for the various sorghum growing communities.

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