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ANTHER PROTEIN STUDIES ON DIVERSE CYTOSTERILES OF SORGHUM

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ABSTRACT

Anther protein banding patterns of six diverse cytoplasmic male steriles, their maintainers, one tester and six hybrids were studied in sorghum. The diverse cytosteriles differed from each other but male steriles with the same cytoplasm had some commonness. The male steriles and their maintainers differed from each other, and the difference could be a useful tool to differentiate the fertility–sterility status of sorghum genotypes. Further, some protein bands present in the hybrids were derived from both the parents.

Key words: Sorghum, cytosteriles, anther proteins, cytoplasm and characterization.

All commercial hybrids of sorghum developed so far are based on milo (A_1) cytoplasm. The need for utilizing non-milo sources of cytoplasm in hybrid production was felt in order to avoid disease and environmental hazards. With this in view, the available diverse cytoplasms has to be characterized for commercial exploitation. Characterization of diverse cytosteriles based on fertility restoration was attempted earlier also [1, 2]. But studies on characterization of diverse cytosteriles based on biochemical markers are limited. An attempt was made using soluble proteins and isozymes from anthers [3] as well as seeds [4, 5] of sorghum. These studies were limited to the indigenous cytoplasmic sources only. The present study aims to characterize diverse cytosteriles of sorghum with the help of anther proteins, using diverse sources of both indigenous and exotic cytoplasms.

MATERIALS AND METHODS

The CMS lines DMS1 A and 111 A belong to A_1 cytoplasmic group, A_2 A and TAM428 A to A_2 cytoplasmic group, IS1112C A to A_3 cytoplasmic group and CSV4 A to the G_1 source of cytoplasm. The respective maintainers of these CMS lines and restorer TNS30 along with the six hybrids obtained by crossing TNS30 to each of the six CMS lines were used for anther protein analysis.

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EXTRACTION OF PROTEIN

One gram of fresh anthers was taken and made into a paste with 0.3 ml of 0.2 M phosphate buffer (pH 7.2) in pestle and mortar. The sample was centrifuged and the supernatant (protein extract) was collected and stored frozen for protein estimation and electrophoresis.

ESTIMATION AND SEPARATION OF PROTEIN

The amount of total protein present in the anther extracts was estimated by the method of Bradford [5]. Polyacrylamide gel electrophoresis was used to separate the anther proteins. The electrophoretic procedure described by Walker [6] was adopted. Sample containing 50-200 μg of protein was loaded in the sample wells (all the samples have equal quantity of protein), electrophoresis was conducted, the gel was stained and destained to visualise as many bands as possible. The relative migration (Rm) of each band was calculated and used for comparison.

RESULTS AND DISCUSSION

The anther protein banding patterns of diverse cytoplasmic male steriles along with maintainers are given in Table 1. The Rm values for protein bands were used for comparison.

Table 1. Anther protein banding patterns of diverse male steriles (A) of sorghum and their maintainers (B)

Rm value	A_2		TAM 428		IS1112C		CSV4		DMS1		111	
	A	B	A	B	A	B	A	B	A	B	A	B
0.1	–	–	+	–	–	–	–	–	–	–	+	–
0.2	–	+	–	–	–	–	–	–	–	–	–	–
0.3	–	–	–	–	–	+	–	–	–	–	–	–
0.4	+	–	–	–	–	–	–	–	–	–	–	–
0.5	–	–	–	–	–	–	–	–	–	–	+	–
0.6	+	+	+	–	–	–	–	–	+	–	+	–
0.7	–	–	–	–	+	+	–	–	–	–	–	–
0.8	–	–	–	–	–	–	–	–	+	–	+	–
1.1	–	–	–	–	–	+	–	–	–	–	–	–
1.2	+	–	+	–	–	–	–	–	–	–	–	–
1.3	–	–	–	–	–	+	–	–	+	+	–	–
1.4	–	–	–	–	–	–	+	+	–	–	–	–
1.5	–	–	–	–	+	–	–	–	–	–	–	–

(Contd.)

Table 1 (contd.)

Rm value	A_2		TAM 428		IS1112C		CSV4		DMS1		111	
	A	B	A	B	A	B	A	B	A	B	A	B
1.6	–	–	+	+	–	+	–	–	–	+	–	–
1.9	–	–	–	–	+	–	–	–	–	–	–	–
2.0	+	+	+	–	–	–	–	–	+	+	+	–
2.1	–	–	–	–	–	–	+	+	–	–	–	–
2.2	–	–	–	–	+	+	–	–	–	–	–	–
2.3	–	–	–	–	+	–	–	–	+	–	–	–
2.4	+	–	–	+	–	–	–	–	–	–	–	–
2.5	–	–	–	–	–	–	–	+	–	–	–	–
2.6	+	–	–	–	+	–	–	–	–	–	–	–
2.7	–	–	–	–	–	–	–	–	+	+	–	–
2.8	+	–	–	–	–	–	+	–	–	–	–	–
3.0	–	–	–	+	–	–	+	+	+	+	+	+
3.2	–	–	–	+	–	+	+	+	–	–	–	–
3.3	+	+	–	–	–	–	–	–	–	–	+	–
3.5	–	–	–	–	+	+	+	–	–	+	–	–
3.7	+	–	–	+	–	–	–	–	–	–	–	+
4.2	+	–	–	–	–	–	–	–	–	–	–	–
4.5	–	+	+	+	–	+	–	+	–	+	+	–
4.7	–	–	–	–	–	–	+	–	–	–	–	–
4.8	+	–	–	–	+	+	–	–	–	–	–	–
4.9	–	–	–	–	–	–	–	–	–	–	+	+
5.0	–	+	+	–	–	–	–	–	+	+	–	–
5.1	–	–	–	–	–	–	+	–	–	–	–	–
5.2	–	+	–	–	+	–	–	+	–	–	–	–
5.3	–	–	+	–	–	–	–	–	–	–	–	+
5.5	–	–	–	–	–	–	–	–	+	–	+	–
5.7	+	+	–	+	+	+	+	–	–	+	+	–
6.0	–	–	+	–	+	+	+	–	–	+	+	–
6.6	–	–	–	–	–	–	–	–	–	–	+	–
6.9	–	–	–	–	+	–	–	–	–	–	–	+
7.3	+	–	–	–	–	–	–	–	–	–	+	+
8.0	+	–	–	–	–	–	–	–	–	–	+	–

(Contd.)

Table 1 (contd.)

Rm value	A_2		TAM 428		IS1112C		CSV4		DMS1		111	
	A	B	A	B	A	B	A	B	A	B	A	B
8.2	–	–	–	–	–	–	–	–	–	+	+	–
8.4	–	+	–	–	–	–	–	–	–	–	–	–
8.5	+	–	+	+	–	+	–	–	–	–	–	–
8.9	–	–	–	–	–	–	–	–	+	–	+	–
9.1	–	–	+	–	–	–	–	–	–	–	–	–
9.2	–	+	–	–	–	–	–	+	–	–	–	–
9.7	+	–	–	–	–	–	–	–	–	–	+	–
10.0	–	–	–	–	–	–	–	–	+	+	–	–

Note. + Presence of band, –absence of band.

COMPARISON OF DIVERSE CYTOPLASMIC MALE STERILE LINES

In general, the comparison of protein patterns showed some basic similarities and differences among the male sterile lines. The similar anther protein patterns of DMS1 A and 111 A confirm close relationship (A_1 cytoplasm) between these two lines. Similar grouping of cytosteriles was reported by Tripathi et al. [7] who classified the lines CK60 B and Nagpur B as carriers of A_1 cytoplasm based on soluble protein from anthers. The protein patterns of the milo (A_1) cytoplasmic male sterile lines DMS1 A and 111 A differ qualitatively from those of other cytosteriles by the appearance of bands with Rm 0.8, 5.5 and 8.9.

The similarity in anther protein patterns of the A_2A and TAM428 A group indicated common genomic relationship between them. These results are in agreement with those of [2, 8], who grouped A_2 type cytosteriles based on fertility restoration studies. The protein banding patterns of A_2 A and TAM428 A showed that they are different from others by the appearance of bands with Rm 1.2 and 8.5, which were useful in grouping them into a single type.

Unique bands with Rm 0.7, 1.5, 1.9 and 2.2 of IS1112C A were useful to distinguish it from that of other groups of male sterile lines and to classify it in a separate group. These results are in conformity with the classification of Worstell et al. [8] in which they grouped IS1112C A as A_3 cytoplasmic group. This was also confirmed by fertility restoration studies [2].

Bands with Rm 1.4, 2.1, 4.7 and 5.1 are unique characteristics of CSV4 A. This enabled us to differentiate CSV4 A (G_1 cytoplasm) from that of other lines. Gangakrishnan and Borikar [2] reported G_1 A as a distinct group based on fertility restoration studies and

classified it as A5 group. Mithila [9] differentiated male sterile lines of diverse cytoplasmic sources on the basis of seed protein banding pattern in pearl millet.

COMPARISON OF MALE STERILE AND FERTILE LINES

A comparison of the protein banding patterns of male sterile lines with their corresponding maintainer lines led to the conclusion that there is a qualitative difference in proteins between these two (Table 1). Similar conclusions were made earlier in sorghum [7]. The difference observed was due to differential cytoplasm even though they were isogenic. There are some common protein bands between the male sterile and their respective maintainers because of their isogenic nature. The critical biochemical tool will be useful in differentiating fertility–sterility status of the genotypes. Also the observed protein band difference in sterile and fertile lines helped to confirm the field results (pollen fertility, seed set) and to get reliable estimate of sterility status. Mithila [9] observed certain additional protein bands in the maintainers which were absent in their male sterile lines in pearl millet.

COMPARISON OF PARENTS AND HYBRIDS

The anther protein banding patterns of male sterile, restorers, and hybrids involving diverse cytoplasms are presented in Table 2.

Table 2. Anther protein banding patterns of male steriles, restorers and hybrids between different parents carrying diverse cytoplasms in sorghum

Rm value	A_2 A	A_2 A x TNS30	TNS30	TAM 428A x TNS30	TAM 428 A	IS1112 CA	IS1112 CA x TNS30	TNS30	CSV4A x TNS30	CSV 4A	DMS1A	DMS1A x TNS30	TNS30	111 A x TNS30	111 A
0.1	–	–	–	+	+	–	–	–	+	–	–	+	–	+	+
0.2	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
0.4	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
0.5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
0.6	+	+	–	–	+	–	–	–	–	–	+	–	–	+	+
0.7	–	+	–	+	–	+	+	–	+	–	–	–	–	+	–
0.8	–	–	–	–	–	–	+	–	–	–	–	–	–	+	+
0.9	–	–	+	–	–	–	–	+	+	–	–	–	+	–	–
1.0	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–
1.1	–	+	–	+	–	–	+	–	+	–	–	+	–	–	–
1.2	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–

(Contd.)

Table 2 (contd.)

Rm value	A_2 A	A_2 A x TNS30	TNS30	TAM 428A x TNS30	TAM 428 A	IS1112 CA	IS1112 CA x TNS30	TNS30	CSV4A x TNS30	CSV 4A	DMS1A	DMS1A x TNS30	TNS30	111 A x TNS30	111 A
1.3	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–
1.4	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–
1.5	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
1.6	–	–	–	–	+	–	–	–	–	–	–	–	–	+	–
1.9	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
2.0	+	+	–	+	+	–	–	–	–	–	+	+	–	+	+
2.1	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–
2.2	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–
2.3	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
2.4	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2.5	–	–	+	–	–	–	–	+	–	–	–	–	+	–	+
2.6	+	–	–	–	–	+	–	–	–	–	–	–	–	–	–
2.7	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–
2.8	+	+	+	–	–	–	–	+	–	+	–	–	+	–	–
3.0	–	–	–	–	–	–	–	–	–	+	+	–	–	–	+
3.2	–	–	–	+	–	–	–	–	–	+	–	+	–	–	–
3.3	+	+	+	–	–	–	–	+	–	–	–	–	+	–	+
3.5	–	–	–	–	–	+	–	–	–	+	–	–	–	–	–
3.7	+	+	–	–	–	–	–	–	–	–	–	+	–	–	+
4.2	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–
4.3	–	–	+	–	–	–	–	+	–	–	–	–	+	–	–
4.5	–	+	–	–	+	–	–	–	–	–	–	+	–	+	+
4.7	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–
4.8	+	+	+	–	–	+	–	+	+	–	–	+	+	–	–
4.9	–	–	–	+	–	–	–	–	–	–	–	–	–	–	+
5.0	–	–	+	–	+	–	–	+	–	–	+	+	+	–	–
5.1	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–
5.2	–	+	–	–	–	+	+	–	–	–	–	–	–	–	–
5.3	–	–	–	–	+	–	–	–	–	–	–	–		–	–
5.5	–	+	+	+	–	–	–	+	–	–	+	+	+	+	+

(Contd.)

Table 2 (contd.)

Rm value	A_2 A	A_2 A x TNS30	TNS30	TAM 428A x TNS30	TAM 428 A	IS1112 CA	IS1112 CA x TNS30	TNS30	CSV4A x TNS30	CSV 4A	DMS1A	DMS1A x TNS30	TNS30	111 A x TNS30	111 A
5.6	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–
5.7	+	+	–	–	–	+	–	–	+	+	–	–	–	–	+
6.0	–	+	+	–	+	+	+	+	+	+	–	+	+	–	+
6.4	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–
6.6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
6.8	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–
6.9	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
7.0	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–
7.3	+	–	+	–	–	–	–	+	–	–	–	–	+	–	+
7.8	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+
8.0	+	–	–	–	–	–	–	–	+	–	–	–	–	–	+
8.1	–	+	+	–	–	–	–	+	–	–	–	–	+	+	–
8.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
8.4	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–
8.5	+	–	+	+	+	–	+	+	+	–	–	+	+	–	–
8.8	–	+	+	–	–	–	–	+	–	–	–	–	+	+	–
9.0	–	+	–	–	–	–	–	–	–	–	+	–	–	–	+
9.1	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–
9.2	–	–	+	+	–	–	+	+	+	–	–	–	+	+	–
9.6	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–
9.7	+	–	–	–	–	–	–	–	–	–	+	–	–	–	+
10.0	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–

Note: + Presence of band, – absence of band, + present only in hybrid.

The hybrids, as expected, had protein bands of both high as well as low molecular weight that were present in their respective parents. Proteins are the primary product of genes. So, the high molecular weight proteins observed in the hybrids of present study were encoded by the female parental genome and the low molecular weight proteins are encoded by the nuclear genome of the male parent. Also, the hybrids showed some additional protein bands which may be the result of interaction between the nuclear and mitochondrial genome of female parent with that of the nuclear genome of male parent.

Some of the protein bands which are missing in the hybrids when compared to parents (A, R) is due to interaction between the nuclear genome of restorer line with the nuclear-mitochondrial genome of male sterile lines. The interaction results in the suppression of certain proteins (missing of protein bands) observed in the present study which are responsible for sterility in the male sterile lines and fertility in the restorer lines. Some of the new proteins observed (extra bands seen) in hybrids of present study may be due to the interaction of both the parental genome resulting in the fertility restoration in hybrids. This biochemical tool may help in identifying the parents of a particular hybrid by comparing the protein banding patterns of the hybrids and different parents.

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