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Amino Acids in Anthers of *Milo* and in Cytoplasmic Genetic Male Sterile Sorghums (*Sorghum bicolor* L. Moench) of Indian Origin

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Summary. Amino acid composition of proteins from anthers of *milo* and Indian origin male steriles were determined. Comparison of amino acid between A and B lines showed lower contents of histidine, threonine, glutamic acid, glycine, leucine and phenylalanine and higher contents of alanine, serine, proline and tyrosine in line A compared to line B. Alanine content in anthers of A lines was more than two fold higher than that in the anthers from B lines. Marked differences in amino acid composition of anthers of A and B lines are suggestive of their involvement in male sterility. Cytoplasmic male steriles of Indian origin M35-1A and M31-2A showed greater similarity but differed from *milo*, VZM2A and B.

Key words: Amino acid – Male sterility – Cytoplasmic male sterile – *Sorghum bicolor* L. Moench – Maintainer – Apomict

Introduction

All commercial hybrids of sorghum developed to date are based on *milo* cytoplasmic male sterility. However, there is a need to identify and utilize alternate sources to safeguard against possible hazards.

Rao (1962), Hussaini and Rao (1964) and Nagur and Menon (1974) reported the occurrence of cytoplasmic-genic male steriles from India. Compared to the *milo-kafir* system, fertility restoration in Indian steriles was difficult and it was, therefore, inferred that these sterility, inducing cytoplasm may be different. Several workers have studied male sterile lines and fertile lines in maize (Fukasawa 1954, Duvik 1965), cotton (Sarvella and Stojanovic 1968) and sorghum (Brooks 1962, Atkins 1970, Atkins and Kern 1972) for such chemical constituents as amino acids, carbohydrates, etc. At both meiosis and after the vegetative division of the microspores (Fukasawa 1954) proline was present in lesser amounts in the anthers of Texas cytoplasm plants than in the anthers of normal cytoplasm plants.

In cotton, Sarvella and Stojanovic (1968) observed higher aspartic acid arginine content in male sterile lines where *hirsutum* genomes were incorporated into *anomalum* and arboreum cytoplasm than the *hirsutum* type itself. Atkins (1970) and Atkins and Kern (1972) observed higher amino acid content in fertile lines. However, the precise difference between different cytoplasmic genetic male steriles from Indian and that of *milo* has not yet been characterised. In the present study the amino acid composition of anther from the *milo* and India origin male steriles has been studied.

Materials and Methods

Diverse male steriles, their maintainers and a apomictic line were included in this study. Seeds of CK60B, VZM-2A, VZM-2B, M35-1A, M35-1B, M31-2A and R473, provided by the All India Coordinated Sorghum Improvement Project (AICSIP), Hyderabad, were grown on IARI farms. Mature anthers were collected before bursting and either stored in liquid nitrogen or analysed immediately.

Protein Hydrolysis and Amino Acid Estimation

Weighed anthers (100 mg in B lines and 300 mg from A lines) were placed into hydrolysis tubes and 6 ml of 6N redistilled HCl was added. The tubes were then evacuated, sealed and hydrolysis was carried out by keeping tubes at 110°C for 24 hours. The hydrolysate was filtered and then freed of acid by repeated flash evaporation at 40°C. The residue was finally dissolved in a small volume 0.1 M sodium citrate buffer, pH 2.0, and amino acid analyses was done employing a TSM amino acid analyzer.

Results

The amino acid (g amino acid per 100 g protein) composition of proteins from anthers of male sterile and main-

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Table 1. Amino acid composition (g amino acid/100 g protein) of protein from anthers of male sterile, maintainer and apomictic sorghum lines

S. No.	Amino acid	CK60B	VZM 2A	VZM 2B	N35–1A	M35–1B	M31–2A	R473
1.	Lysine	7.25	5.90	8.00	6.70	5.90	6.70	9.20
2.	Histidine	1.70	0.90	1.70	–	2.40	1.80	1.80
3.	Argnine	6.78	4.00	4.20	5.90	6.00	6.00	7.00
4.	Aspartic acid	8.56	6.10	9.40	7.60	7.60	7.60	9.20
5.	Threonine	4.68	2.40	3.70	2.80	6.80	2.80	4.10
6.	Serine	5.02	6.90	5.40	8.40	4.80	7.20	6.00
7.	Glutamic acid	9.66	7.00	10.10	8.80	10.10	8.40	11.80
8.	Proline	10.76	13.30	11.10	13.20	11.90	13.20	11.90
9.	Glycine	4.56	4.80	5.30	4.30	5.20	5.20	4.30
10.	Alanine	6.93	16.20	7.40	19.40	7.20	18.40	9.20
11.	Cystine	Trace	Trace	Trace	–	Trace	Trace	–
12.	Valine	4.93	5.10	5.00	6.70	5.40	6.70	6.70
13.	Methionine	2.02	1.50	1.70	1.70	3.40	1.70	3.40
14.	Iso-leucine	4.31	4.10	4.30	4.50	4.50	4.50	6.00
15.	Leucine	6.65	5.40	7.50	6.00	7.50	7.50	9.00
16.	Tyrosine	4.53	5.90	4.20	6.20	4.20	6.20	6.20
17.	Phenylalanine	6.60	2.90	4.20	3.80	5.70	3.80	5.70
18.	Ammonia	3.41	4.30	4.30	3.00	4.50	2.40	2.80

tainer lines is shown in Table 1. Marked differences in the amino-acid composition of anthers from male sterile lines and their maintainers were observed. Comparison of amino acids between sterile and maintainer lines showed higher content of histidine, threonine, glutamic acid, glycine, leucine and phenylalanine and a lower content of serine, proline, alanine and tyrosine in the maintainer lines than in the male steriles. VZM-2B had higher aspartic acid compared to its male sterile VZM-2A while M35-1B had higher methionine and lower valine content than M35-1A anthers. All the A lines were characterized by very high levels of alanine. Alanine concentration was more than two fold higher in sterile lines than in their maintainer lines. Arginine, valine, methionine, isoleucine and ammonia did not show many differences between VZM-2A and VZM-2B. Also, in the case of M35-1A and M35-1B no differences were observed in the levels of arginine, aspartic acid and isoleucine. M35-1A had the highest level of alanine while CK60B had the lowest content. Comparison of the amino acid content of all sterile lines, i.e. VZM-2A, M35-1A, M31-2A, showed the common features of higher alanine, proline and serine content compared to their respective maintainers. Alanine and proline together accounted for 29-32 percent of the total amino acids present in A lines while in the cases of VZM-2B and M35-1B these two amino acids accounted for only 18-19%. Anthers from M35-1A and M31-2A had similar contents of lysine, arginine, aspartic acid, threonine, glutamic acid, proline, valine, methionine, isoleucine, tyrosine and phenyl alanine (Table 1). These results, therefore, indicate that the amino acid composition of M31-2A is very much similar to that found in the anthers of M35-1A. The amino acid composition of M35-1A and M31-2A differed from that of VZM-2A anthers. Both M35-1A, M31-2A anthers had higher contents of lysine, threonine, glutamic acid, alanine, valine, leucine and phenylalanine and lower contents of ammonia, as compared to that found in VZM-2A. This increased concentration of ammonia indicates the presence of higher levels of amides, either asparagine or glutamine, in VZM-2A than in M35-1A, M31-2A. Amino acid composition of anthers of VZM-2B and M35-1B also differed markedly. Lysine, aspartic acid and serine contents were higher while histidine, arginine, threonine, proline, valine, methionine and phenylalanine contents were lower in anthers of VZM-2B as compared to anthers from M35-1B. Methionine content in VZM-2B anthers was half that found in M35-1B anthers. Threonine content in VZM-2B was also nearly half that found in M35-1B anthers. There was not much difference in glycine, alanine, isoleucine, leucine, tyrosine and ammonia levels between M35-1B and VZM-2B anthers. The contents of arginine, threonine, methionine, tyrosine and phenylalanine were higher, while that of lysine, aspartic acid, serine, glutamic acid, glycine, alanine and leucine were lower, in anthers of CK60B than in VZM-2B anthers. Lysine, arginine, aspartic acid and phenylalanine contents were higher and that of histidine, threonine, glutamic acid, proline, glycine, valine, methionine, leucine and ammonia were lower in CK60B anthers than in M35-1B anthers. The amino acid composition of the apomictic line R 473 also differed from other anthers with respect to many amino acids. Lysine, aspartic acid, serine, glutamic acid, proline, alanine, valine, methionine, isoleucine, leucine were higher and threonine, phenylalanine and ammonia lower in R 473 anthers than in anthers of CK60B. Aromatic amino acid contents, including leucine

Table 2. Amino acid content (mg/g anther) from sorghum male steriles

S. No.	Amino acid	CK60B	VZM 2A	VZM 2B	M35–1A	M35–1B	M31–2A	R473
1.	Lysine	3.639	2.352	3.083	2.148	2.841	2.290	3.745
2.	Histidine	0.862	0.358	0.648	Trace	0.965	0.598	0.706
3.	Argnine	3.407	1.595	1.592	1.641	2.812	2.059	3.013
4.	Asparctic acid	4.308	2.430	3.563	2.471	3.389	2.476	3.470
5.	Threonine	2.369	0.981	1.427	1.009	1.392	0.964	1.673
6.	Serine	2.546	2.782	2.106	2.883	2.361	2.682	2.497
7.	Glutamic acid	4.875	2.778	3.924	3.136	4.644	2.740	4.549
8.	Proline	5.400	5.400	4.293	4.652	5.686	4.719	4.974
9.	Glycine	2.277	1.922	2.068	1.504	2.112	1.752	1.614
10.	Alanine	3.468	6.577	2.844	6.861	3.280	6.724	3.827
11.	Cystine	Trace	Trace	Trace	–	Trace	Trace	–
12.	Valine	2.465	2.039	1.885	2.160	2.342	2.429	2.632
13.	Methionine	1.009	0.598	0.634	0.628	1.321	0.653	1.038
14.	Iso-leucine	2.140	1.619	1.688	1.631	2.215	1.698	2.265
15.	Leucine	3.362	2.200	2.920	2.152	3.582	2.420	3.813
16.	Tyrosine	2.304	2.364	1.617	2.054	1.819	2.057	1.944
17.	Phenylalanine	3.321	1.134	1.593	1.161	1.928	1.118	1.959
18.	Ammonia	1.712	0.900	1.648	1.030	2.149	0.818	1.143

and isoleucine, in the apomictic line were comparatively higher than those found in other anthers. Lysine content was highest in R-473 anthers. Amino acid content per unit weight of anthers (mg/g anther) is shown in Table 2 for anthers of male steriles, maintainers and apomictic line, R 473. Amino acid content per unit weight of anther gives an idea of total abundance or lack of amino acid in anthers. A general comparison indicated a greater proportion of amino acids in CK60B, M35-1B and apomictic line, R-473, when compared to male steriles VZM-2A, M35-1A, M31-2A as well as VZM-2B. A comparison of A vs B lines indicated higher contents of lysine, histidine, aspartic acid, threonine, glutamic acid, leucine, phenylalanine and ammonia and lower contents of serine and alanine. Amino acid content in M35-1A had greater similarity to that found in M31-2A whereas that of VZM-2A differed from that of M35-1A and M31-2A with respect to proline, glycine, alanine, valine and tyrosine. CK60B also showed differences with the other maintainer lines VZM-2B, M35-1B as well as with R-473.

Discussion

Cytoplasmic male-sterility in *Sorghum* is fundamentally an aspect of the problem of gene action and nuclear cytoplasmic interaction. In order to find out if the cytoplasmic genetic male sterility could presumably be the result of a relatively simple course involving a deficiency of one or more compounds, amino acid analysis was done. The deficiency might reflect the known functioning of an enzyme system and result in subsequent accumulation of certain metabolites.

Upon compairing amino acids (g/100 g protein) from anthers of A and B lines, lower concentrations of histidine, threonine, glutamic acid, glycine, leucine and phenylalanine and higher concentrations of serine, proline, alanine and tyrosine were observed in A. However, proline and glycine did not show consistent results when amino acids were expressed on anther weight basis. Alanine contents in maintainer lines were less than 50% of that present in sterile lines. Khoo and Stinson (1959) have also observed accumulation of alanine in certain sterile lines of maize. Brooks (1962) also reported higher glycine levels in anthers of sorghum male steriles. However, in the present study male steriles had low glycine contents. The exact role of alanine is not known in inducing male sterility. Because of the predominance of alanine in anthers of sterile lines, it would be interesting to examine the involvement of either alanine or one of its intermediates in determining male sterility. Consistent differences in A and B lines are suggestive of changes in the pattern of amino acid metabolism in sterile anthers, relative to that of fertiles. In general, the amino acid composition of M35-1A anthers had greater similarity to that found in M31-2A anthers. These results further support the similarity observed in M35-1A and M31-2A using isoenzyme pattern mapping (Tripathi 1979).

The amino acid composition from anthers of apomictic line differed considerably from that of sterile and maintainer lines with respect to lysine, aspartic acid, glutamic acid, alanine, isoleucine, leucine, etc. The contents of lysine, leucine, isoleucine, glutamic acid and aspartic acid were higher in anthers of R-473 as compared to anthers of A and B lines. The differences in amino acid composition of sterile and maintainer line and anthers of different cytoplasmic background are suggestive of basic differences in protein quality and quantity.

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