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ULTRASTRUCTURAL CHARACTERIZATION OF PEARL MILLET CYTOPLASMS

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ABSTRACT

The three cytoplasms of pearl millet with single nuclear genotype, L110 (S1), L110 (S2) and L110 (S3) were examined under electron microscope for ultrastructural cytoplasmic variations. Differences among three cytoplasms were observed and it was noted that each cytoplasm showed its structural superiority over the other two in some traits, but showed its inferiority to others in some other ultrastructural traits. This indicated the possibility of improvement and developing commercial lines in S2 and S3 cytoplasms with productivity almost equal to the level of the lines, and hybrids in S1 cytoplasm generally prevalent in India, thus, diversifying cytoplasmic base of the crop.

Key words: Pearl millet, Ultrastructural, genetic vulnerability, male sterility.

The detection and exploitation of cytoplasmic genetic male sterility in pearl millet (*Pennisetum americanum* L. Leeke) by Burton in 1958 [1] has helped in its eventual utilization for grain hybrid production in India, which was the first of its kind released in this crop [2]. Subsequently two other sources of male sterility were searched in the world collection and maintainer and restorer relationships were established [3]. The three cytoplasms were then identified as S1, S2 and S3. Inspite of diverse sources of male sterility only S1 cytoplasm formed the basis for most of released hybrids in India, which is an indication of major genetic vulnerability of present day cultivars. Poor utilization of the other two sources is due to, *first*, their isolation in poor genotypes, *second*, instability of male sterility in most of the combinations and also probably the partial restoration of fertility in crosses with available inbreds which could not be fully explained on the basis of spontaneous mutations. Sectorial chimeras in pollen sterility in the same plant indicate the complexity of spontaneous genetic changes due to cytoplasm and its interaction with nucleus. There is also variation for combining ability for yield and disease resistance between fertile and sterile cytoplasms

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showing the role of the cytoplasm and its interaction with nuclear genotype [4]. The lines L110 (S1), L110 (S2) and L110 (S3) provided excellent material for precisely studying cytoplasmic ultrastructural variations under the control of a single nuclear genotype. The study is also important in relation to crop disasters in pearl millet and some other crop hybrids from time to time due to narrow cytoplasmic base. For this purpose, characterization of cytoplasm at ultrastructural level is crucial and the present study is an effort in this direction.

MATERIALS AND METHODS

The sterile (A) and fertile (B) lines of genotypes L 110 (S1), L 110 (S2) and L 110 (S3) were grown in a pot culture. The leaves for electron microscopy were collected at young leaf (30-day-old = D1), intermediate (40-day-old = D2) and boot leaf (68-day-old = D3) stages. The second leaf from top at D1, D2, and the boot leaf itself (D3) were taken. Pieces about 2 x 1 or 2 x 2 mm in size were cut from leaves and fixed first in 8% glutaraldehyde and then in 1% osmium tetraoxide and were later embedded in plastic capsules with resin mixtures using standard procedures which formed blocks after polymerization. The sections of the order of 300-400 Å thickness were cut with LKB Ultratome (Ultratome 1) and were stained in 1% uranyl acetate after mounting on formvar coated copper grids. Screening of the sections was done on Phillips transmission microscope (Model EM 300). Observations were made on three main types of cells from mesophyll, bundle sheath and parenchymatous tissue, which were the main living cells of the leaf for metabolic functions.

The chloroplast diameter and volume were measured from a number of chloroplasts which were cut in meridional plane in serial sectioning in each genotype. The measurements were made in mesophyll chloroplasts (MC). The volume of the chloroplast was equal to $\frac{2}{3}\pi r^2 B$ where $r = \frac{A^2 + 4B^2}{A^2 B}$ (A was the diameter of the chloroplast, and B the maximum height in the meridional section). The average grana density in the chloroplast was equal to $1/3 \frac{A^2 + 4B^2}{A^2 B} \times n^2 \times m$ (m is number of grana along A and n along B). To characterize the cytoplasm, polyribosomal density was measured per cubic micron volume and was equal to $N \times \frac{10000}{x}$ per μ^3 where x was the magnification and N number of polyribosomes in one cm square. The average of three stages was used for general comparison of cytoplasms.

RESULTS AND DISCUSSION

The polyribosomal density was highest in S1 at D2 and D3 stages but highest in S2 at D1 (Table 1). The average grana density was highest in S2 and comparatively much lower in S1 and S3 while grana diameter and thylakoid/grana were higher in S1 and S3 but low

Table 1. Ultrastructural differences in the leaves of S1, S2 and S3 cytoplasms of pearl millet in sterile (A) and fertile (B) background at D1, D2 and D3 stages of growth (AV = average)

Character		S1			S2			S3		
		A	B	AV	A	B	AV	A	B	AV
Polyribosomal density/μ^3	D1	2	5		14	17		3	20	
	D2	14	10	7.83	4	7	9.16	13	6	8.33
	D3	12	14		4	9		2	6	
Grana density/MC	D1	136	111		129	100		161	125	
	D2	150	159	110.7	198	91	143.0	37	83	74.3
	D3	49	59		130	210		17	23	
Chloroplast diameter (μ)	D1	4.2	4.7		5.5	5.2		4.5	5.3	
	D2	2.7	3.7	3.5	3.4	4.2	4.2	3.4	3.4	3.7
	D3	3.3	2.6		3.0	4.0		3.7	2.1	
Chloroplast volume (μ)	D1	15.7	21.0		22.6	28.8		30.3	34.2	
	D2	4.1	11.4	10.6	8.8	17.8	16.5	9.8	8.4	15.9
	D3	7.1	4.1		5.7	15.0		10.5	2.2	
Grana diameter (μ)	D1	0.36	0.40		0.17	0.22		0.34	0.25	
	D2	0.30	0.40	0.36	0.26	0.17	0.21	—	0.33	0.32
	D3	—	0.33		0.23	—		0.38	0.29	
Thylakoid/grana	D1	10	15		8	14		16	14	
	D2	12	11	9.2	4	8	8.6	8	9	10.5
	D3	—	9		9	—		8	8	
Starch in MC*		–	+		–	–		–	+	
Starch in BC*		+	++		+	++		+++	+++	
EDM in ribosomal matrix*		–	+		+	++		–	+	
EDM in PM*		–	–		–	+		–	–	
EDM in bigger size in general cytoplasm*		+	++		–	–		+	–	
Fret length*		Long	Long		Short	Short		Long	Long	
Stroma space*		Medium	Large		Small	Small		Small	Large	
Shape of PM*		Thin Straight	Thin Straight		Thick Convoluted	Thick Straight		Thin Straight	Thin Straight	

– Not present/seen, + present in traces or small, ++ medium, and +++ high/large/more.

*Average of three stages.

in S2. Thus, the consideration of these cytoplasmic traits, associated with most important aspects of protein synthesis and light harvest/starch synthesis, has distinctly differentiated S2 from S1 and S3 cytoplasms. The data from 1977 to 1980 from All India Coordinated Millet Improvement Project Report [5] has revealed S1 and S3 as high yielding but more susceptible

to downy mildew than S2 which was low yielder and almost immune. The yield superiority of S1 and S3 may be related to better enzyme synthetic capacity, specially, during later stages because of high polyribosomal density and better light harvest due to higher grana diameter and thylakoid/grana than in S_2. Thus, structural superiority as far as productivity is concerned is established. This also indicated structural diversity for productivity and resistance and perhaps one of the reasons for general negative association between yield per se and resistance. Higher polyribosomal density at young leaf stage in S2 perhaps indicated early metabolic potentials for biochemical synthesis to resist downy mildew attack and establishment which generally takes place during early growth. Lack of such potential in S1 and S3 made them vulnerable to the pathogen.

Similarly larger fret connection and larger stroma space and low grana density in S3 followed by S1 and S2 in order indicated the possibility of faster and more efficient light absorption in S2 but more efficient dark reaction in S3 and S1, again pointing towards structural superiority for higher productivity in S3 and S1. Chloroplast volume and diameter were higher in S2 than in S3 and S1. The higher chloroplast volume and diameter may not be beneficial, but higher grana density is certainly an attribute for better energy capture. Therefore, the low yielding potential of S2 is correlated with the lacuna at dark reaction level or the efficient energy capture is diverted towards the defence mechanism making it almost immune to downy mildew but low yielder. The better productivity of S3 and S1 over S2 is also indicated by their slightly better starch accumulation in bundle sheath chloroplasts (BC).

The starch accumulation in traces in MC is seen in S1 and S3 but not in S2. In S1 cytoplasm the extent of starch accumulation in MC varied in different near isogenic lines (NIL) for resistance/immunity vs susceptibility to downy mildew [6]. The starch accumulation in MC (a C_3 character) of pearl millet which otherwise should not have taken place as it is a C_4 plant [7] indicated it to be due to a major cytoplasmic effect or because of nucleo-cytoplasmic interaction. The environmental stresses have been shown to induce in a single species both the C_3 and C_4 CO_2 fixation thus making the difference between C_3 and C_4 plants less distinct than considered earlier [8]. It may be argued that like the environmental stresses, in our material, either cytoplasm or nucleocytoplasmic interaction might have stimulated C_3 CO_2 fixation to a little extent in the presence of resistance gene specially in S1 cytoplasm. However, synthesis of starch in MC of C_4 plant is an indication of ribulose bisphosphate activity in it which along with the relationship between starch generation in MC and resistance need further analysis of precise biochemical nature. The observations of additional starch synthesis in the MC of S1 and S3 might be one of the factors for higher productivity of these in comparison to S2.

Mitochondria per cell did not show differences between cytoplasms whereas chloroplast/cell, myelin formation and pinocytosis were slightly more in S3 than in S2 but no categorical trend was observed.

Electron-dense material (EDM), presumably phenolics [9] was more in ribosomal matrix and near PM in S2 than in S1 and S3. However, EDM of bigger size in general cytoplasm was more in S1 and S3 but absent in S2 suggesting that phenolics present in general cytoplasm might have undergone some structural or functional changes becoming less useful for disease resistance than the newly synthesized phenolics in ribosomal matrix and near PM. The S2 cytoplasm also showed higher nuclear associations with other organelles which might be considered a desirable trait from disease resistance view point, where at the time of infection coordinated efforts to frustrate the pathogen can be speculated.

Thus, the present study has revealed that each of the cytoplasm is superior to the others in some aspects and inferior in others. Specifically the potentials of S2 cytoplasm which is highly resistant to downy mildew have been brought out and by using suitable nuclear genotype there is every possibility of getting inbreds and hybrids in S2 as well as in S3 cytoplasms having yield potential to the tune of the prevalent genotypes in S1 cytoplasm. This will help in diversifying the cytoplasmic base coping up with the genetic vulnerability of the crop to devastating downy mildew. Bringing together the chloroplasts of S2 having higher potential for light reaction and that of S_3 and S_1 having higher potential for dark reaction by protoplast fusion is another alternative by which resistance potential of S2 cytoplasm can be exploited.

Sterile and fertile status has also made the single cytoplasm to differ in all growth stages (Table 1). Large differences between A and B lines in S3 for polyribosomal density at D1 and in S2 for grana density at D2 may not be ruled out to be due to sampling variation. A lines in all the three cytoplasms except S1 and S3 at D2 were inferior to B lines in all three growth stages for polyribosomal density. While for grana density, at D1 A lines were superior to B lines in all the three cytoplasms; at D2 A line was superior only in S2 and at D3 all A lines were inferior to B lines. For some other structural characters also B lines, in general, showed higher values than A lines. Thus, sterile lines have shown a tendency to be inferior at structural level yet the superiority of B lines cannot be ruled out to be due to the superiority at functional level which to some extent has also been shown by the characters, based on functional capacity, like starch and EDM generation (Table 1) in this study.

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