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CYTOPLASMIC ULTRASTRUCTURE AND DOWNY MILDEW RESISTANCE IN PEARL MILLET

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ABSTRACT

In pearl millet, cytoplasmic diversity was studied using electron microscopy at three growth stages of male sterile and fertile lines which represented susceptible control, Tifton 23, and five other downy mildew resistant/immune lines. Structural superiority of the resistant lines over susceptible control was evident, particularly for the following attributes: polyribosomal density, granum density, mode of phenolic generation, mechanism for cytoplasmic compartmentalisation, organization of grana and stroma in chloroplast and shape of plasma membrane. This has been discussed as a possible criterion for selecting lines resistant to downy mildew.

Key words: Pearl millet, downy mildew, disease resistance, male sterile, ultrastructure.

The available screening techniques for downy mildew are laborious, time consuming and highly dependent on environmental factors as the ability of a genotype to tolerate or resist the pathogen in various phases of its growth is expected to be due to its superiority at functional level. The present investigation aims to find out cytoplasmic diversity between the pearl millet lines susceptible to downy mildew (*Sclerospora graminicola* S&H) and a series of other near-isogenic resistant lines derived from it.

MATERIALS AND METHODS

The leaf material was collected from the male sterile (A) and fertile (B) counterparts of the downy mildew susceptible pearl millet line Tifton 23, its highly resistant backcross derivatives, and mutants 5054, 5141 and 5071 as well as lines L 111 and 126D2, which are almost immune to downy mildew. The experimental methods were described earlier [1].

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RESULTS AND DISCUSSION

The comparison of cytoplasm with respect to sterile and fertile status showed that the fertile lines were superior to sterile ones in polyribosomal density but slightly inferior in granum density at young leaf stage only. The comparisons given below generally pertain to the genotypic values averaged over A and B lines at three stages of growth.

The polyribosomal density was maximum in the mutant line 5071, followed by 126D2, L 111; much less in 5141 and 5054; and lowest in Tifton 23 (Table 1; Fig. 1a, b, e, f). Granum density was also very high in the line 5071, followed by 5054, L 111, 126D2, moderately low in 5141 and very low in Tifton 23. Both types of density showed a general reduction from young leaf to boot leaf stage, and were higher in the resistant lines than in the susceptible parent Tifton 23. In general, granum diameter and thylakoid number/granum were also higher in the resistant near-isogenic lines than the susceptible ones. These two cytoplasmic features are important for diverse protein synthesis and light absorption, respectively.

Table 1. Ultrastructural differences in leaves of different pearl millet lines averaged over A and B at three growth stages

Character	Tifton 23	5054	5141	5071	L 111	126D2
Polyribosomal density/μ^3	6.80	7.83	9.83	15.67	14.67	15.50
Granum density/MC	40.17	134.00	85.80	136.50	121.67	109.80
Chloroplast diameter (μ)	3.95	3.80	4.02	3.95	3.50	3.25
Chloroplast volume (μ)	15.78	12.72	14.87	15.68	11.68	7.75
Granum diameter (μ)	0.23	0.28	0.24	0.30	0.35	0.32
Thylakoids/granum	11.20	8.17	12.80	14.00	12.60	13.30
Starch in MC	—	+	+	+	+	+
Starch in BC	++	+++	+++	++	+++	+++
EDM in ribosomal matrix	+	++	++	++	+++	+++
EDM in PM	—	++	++	+++	+++	+++
EDM of bigger size in general cytoplasm	++	+	+	+	+++	+++
Endoplasmic reticulum	+	+	++	++	+++	+++
Ribosomes around MC	+	++	++	++	+++	+++
Fret length	Medium	Short	Medium	Medium	Short	Short
Stroma space	Small to medium	Small to large	Small to large	Small to large	Large	Large
Shape of PM	Thin straight	Thick straight	Thin convoluted	Thick convoluted	Thick convoluted	Thick convoluted

Note. – Absent or not seen, + presence in traces or small, ++ medium, +++ high/large/more.

Chloroplast volume was lower in all the resistant lines than in Tifton 23, while chloroplast diameter did not reveal any specific correlation with resistance or susceptibility.

The rate of starch accumulation in the bundle sheath chloroplasts was highest in the line 5054, followed by lines 5141, 126D2, L 111, 5071 (Fig. 1e, f), and lowest in the parent variety Tifton 23, which also showed a general increase in starch accumulation from young leaf to boot leaf stage. Faster rate of starch synthesis indicates superior light as well as dark reactions in these chloroplasts. Lower and higher rate of starch synthesis could be associated with the susceptible and resistant lines, increasing the quantum of food reserves in the latter, or with their ability to synthesize starch quickly to cope up with the metabolic imbalances due to pathogen attack.

Starch content in the mesophyll chloroplasts (MC) was higher in the immune lines, 126D2 and L 111, slightly lower or equal in other resistant lines, and completely absent in the variety Tifton 23 (Fig. 1c, d). Pearl millet being a C_4 plant lacks the CO_2 fixing enzyme, ribulose biphosphate carboxylase in MC, which is a C_3 character [2], and therefore, not expected to synthesize starch in MC. The presence of starch in MC of almost all the resistant lines and its absence in cv. Tifton 23 points towards the presence and activity of this enzyme in MC of the resistant lines. However, this needs confirmation by further biochemical analysis. Environmental stresses have been reported to induce both the C_3 and C_4 CO_2 fixation in a single species, thus, making the difference between C_3 and C_4 plants less distinct than reported [3]. It can, however, be argued that like the environmental stresses, in our material, either cytoplasm or nucleocytoplasmic interactions may have stimulated C_3 CO_2 fixation to a little extent in the presence of the resistance gene. Whatever be the mechanism of starch generation in MC of the resistant lines, this single trait seems to cause distinction between the downy mildew susceptible and resistant lines.

Thylakoid number per granum in MC was higher in the resistant/immune lines (except 5054) and also showed smaller fret connections and more stroma space. This arrangement imparted a specific distinct shape to the cross-section of MC of the immune lines, L 111 and 126D2, clearly distinguishing them from others [4]. High thylakoid number with smaller fret connections provides large area for immediate dark reaction after getting reduction potentials ($NADPH_2$) from the well organized grana. The total number of chloroplasts and mitochondria per cell were also higher in the resistant/immune lines than in the susceptible ones. Thus, numerical and structural superiority of the resistant lines over susceptible ones is evident from the photosynthetic and respiratory processes, which may play a crucial role in the maintenance of metabolic balance in the host plant during infection.

The ribosomes around MC and electron dense material (EDM) in the plasma membrane were highest in the immune lines, L 111 and 126D2, decreased in the lines 5071, 5141 and 5054, and was lowest in cv. Tifton 23. Most probably, EDM is synthesized from phenolic compounds [5], which can fight infection, in general, or at the site of host–pathogen

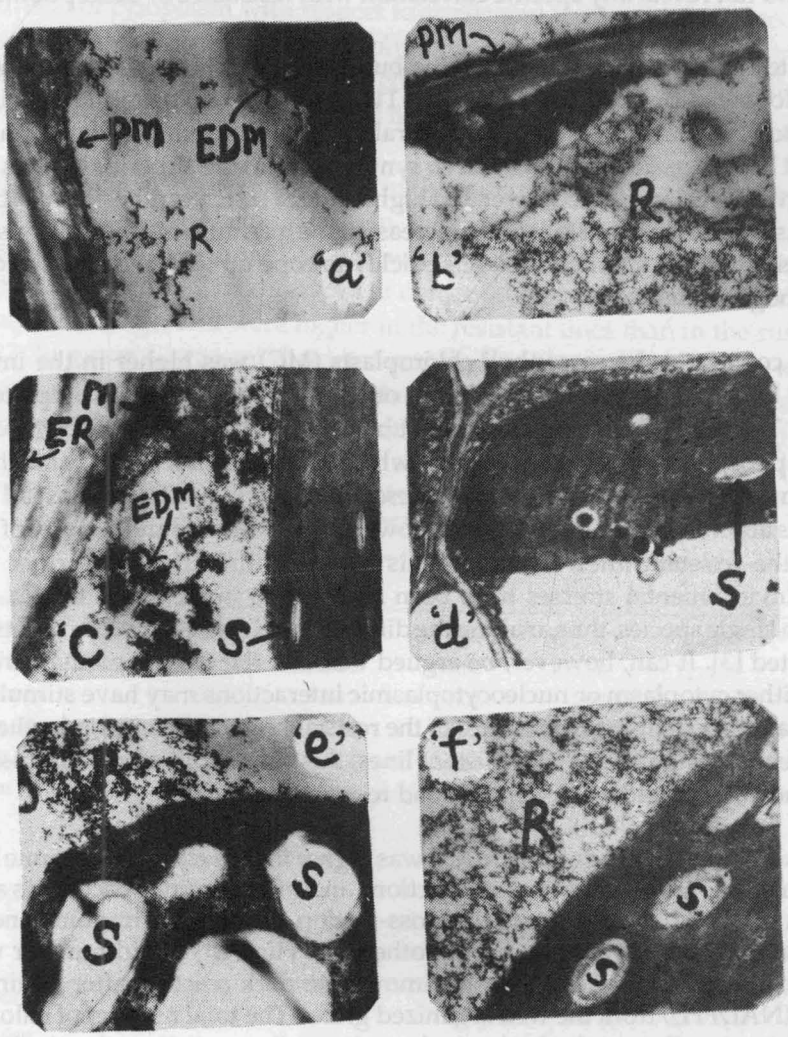

Fig. 1. Cytological ultrastructures in the resistant and susceptible genotypes of pearl millet. a) Tifton 23B, a susceptible line showing thin plasma membrane (PM), less ribosomes, and electron dense material (EDM). b) 126D2A, an immune line showing thick PM and more ribosomes/polyribosomes (R). c) 5071B, a resistant line showing endoplasmic reticulum (ER), EDM in ribosomal matrix, mitochondria (M) and starch (S) in mesophyll chloroplast. d) L 111A, an immune line showing starch generation (S) in MC. e) 5054A, a resistant line showing more ribosomes and polyribosomes (R), and starch in chloroplast. f) 126D2B, an immune line showing more ribosomes and polyribosomes (R), and starch in chloroplast.

interaction. Its presence in larger quantities in the ribosomal matrix (synthetic apparatus) and around the plasma membrane (living barrier) points towards the existence of a quick and strong defence mechanism in the resistant lines (Fig. 1c). However, the general distribution of EDM in the cytoplasm was equal to or slightly more and consisted of bigger bodies in cv. Tifton 23 than in some of the resistant lines (Fig. 1a). It is therefore suggested that the phenolic compounds present all over the cytoplasm might have undergone some structural or functional changes, becoming less useful in disease resistance than those synthesized locally in the ribosomal matrix or around the plasma membrane in the resistant lines.

Rough endoplasmic reticulum (ER), myelin and pinocytosis were generally more in the immune/resistant lines. In cv. Tifton 23, pinocytosis was not observed while some myelin was present, which decreased with age. The plasma membrane was thick and convoluted in the immune lines L 111 and 126D2 (Fig. 1b); thick and straight or thick and convoluted in 5071, thick and straight in 5054, and thin and convoluted in 5141 (all resistant); and thin and straight in the susceptible variety Tifton 23 (Fig. 1a). Thick or thin, convoluted membranes may exert better physical resistance than thin and straight membranes. Higher myelination of the resistant lines is helpful in compartmentalization of the cytoplasm, especially during infection, isolating the pathogen from the remaining cytoplasm and protecting the bulk of cytoplasm from toxins produced at the site of host–pathogen interaction. Pinocytosis generally occurs in the actively metabolizing cells where transportation against the plasma membrane is involved [6]. It controls ionic concentration within the cell which, in turn, controls the osmoregularity and pH which are so vital for the metabolism of the host cell and its survival. In the resistant lines, it acquires special significance for protection against pathogen attack, as the cells would be structurally better organized to cope up with the imbalances in ionic concentration. Similarly, the ribosomes present on smooth ER, making it rough, can be considered desirable from the viewpoint of quick transport of synthates than the smooth ER where the ribosomes synthesize synthates at some distance.

The functional relationship between nucleus and chloroplast, mitochondria, microbodies and some other organelles, which were more in the resistant lines, indicates the possibility of their coordinated functioning which may give a selection advantage during pathogen attack, as was also suggested by Stavely et al. [7].

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