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Ultrastructural Organelle Associations in Pearl Millet

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On the basis of cytoplasmic ultrastructural characterization of 24 genotypes of pearl millet it was concluded that various cytoplasmic organelles tend to associate with each other or the nucleus in the cytoplasm specially in resistant and infected lines and resistant hybrids indicating a coordinated functioning for efficient defence from any would be pathogen attack or for showing better vigour *per se*. Associations have been speculated to be one of the basic biological phenomena of living organisms for making collective efforts in the field of any activity where it is not possible individually.

Key Words: Pearl millet, Nucleocytoplasmic interactions, Ultrastructure, Electron microscopy, Downy mildew resistance

Introduction

Isonuclear lines having nuclear genotype L 110 of A1, A2 and A3 cytoplasms under sterile (A) and fertile (B) background in pearl millet (*Pennisetum americanum* L. Leeke) has been developed (Athwal et al. 1976). Similarly a large number of near isogenic lines for downy mildew resistance and A1 cytoplasm both in sterile and fertile background by mutation and backcrossing have been developed from susceptible line Tifton 23 A, alongwith some commercial hybrids in one of the resistant lines 5141 A for exploiting hybrid vigour in this crop in India (Murty et al. 1983, Thakur & Murty 1984). All these genotypes provided material to look for cytoplasmic differences under (I) isonuclear (II) near isogenic for resistance in A1 cytoplasm and (III) hybrids in A1 cytoplasm in line 5141 A backgrounds. Observing cytoplasmic organelles with respect to sterility and fertility status of cytoplasm is also provided. Though nucleus exerts control over metabolism through transcription, the cytoplasm is the centre of all metabolic activities where genetic code gets translated and thus is crucial to be screened for shape, structure or dimensions and organization of the various vital organelles. In the present piece of work an attempt has been made to characterize cytoplasm in relation to the organization of various organelles with respect to each other and the nucleus.

Material and Methods

The material under study consisted of cytoplasmic male sterile (A) and fertile (B) lines of: (a) downy mildew susceptible genotype ——— Tifton 23; (b) resistant and immune derivatives of Tifton 23 ——— 5054, 5141, 5071, L 111 and 126 D2; (c) isonuclear cytoplasmic lines ——— L 110 S1, L 110 S2 and L 110 S3; and (d) four field

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resistant hybrids ——— Bj 104 (5141 A × J 104), BK 560 (5141 A × K 560), BD 111 (5141 × D 111) and BM 46 (5141 A × M 46). The leaves of these 22 genotypes were fixed for electron microscopic (EM) studies at 30 (D_1), 40 (D_2) and 68 (D_3) days old stage. Two more genotypes HB_3 and NHB_3 were added and total 24 genotypes, grown in pots in growth chamber, were artificially soil inoculated by downy mildew pathogen. The infected leaves of Tifton 23 B, 5071 A, HB_3 and NHB_3 for which infection was got were also fixed and screened under EM.

The standard procedures of fixation, dehydration, resin block formation, sectioning and staining of the cut sections for EM studies were adopted (Hayat 1972). Ultrathin sectionings were done with LKB ultra microtome with 500-600 Å thickness. Screening of the sections was done on Phillips transmission electron microscope (Model EM 300) at 40 KV accelerating voltage. In each line at every stage about 20 to 30 observations were made on photographs. Some of the detailed ultrastructural studies have already been reported (Thakur & Murty 1992, Thakur et al. 1992a, b). In the present paper, the discussions on the association of ultrastructural organelles with one another and the nucleus in particular have been taken up.

Results and Discussion

Since the A and B lines are similar except the sterility or fertility causing factors in the cytoplasm and discernible differences for organelle associations between sterile and fertile lines of a single genotype were not observed, the A and B genotypes have been clubbed as one for the sake of making general comparisons of organelle associations. However, sterile cytoplasm has been found to be invariably and slightly inferior to fertile one both at ultrastructural and biochemical level (Murty et al. 1983).

The lines Tifton 23A, susceptible to downy mildew and progenitor of all other resistant isogenic lines and hybrids, showed side to side association of one mesophyll chloroplast (MC) with other and that of one bundle sheath chloroplast (BC) with other. Some times chloroplast of one cell associated from end to end with that of adjoining cells also. Nucleus with moderate activity was seen engulfing MC. In the later growth stage nucleus was seen lying isolated. In general organelles associations or aggregations were poorly observed in this susceptible line.

Most of the other lines showed comparatively higher frequency of associations of nucleus with moderate to high activity with MC and BC. Similarly either simple aggregation or end to end association of MCs and BCs was also frequent in all genotypes excluding Tifton 23A. Associations of MC of one cell with that of other cell with wall in between were also seen frequently. The association of MC or BC with microbody was there in all the lines except L 110 Sl and L 110 S3. Cytoplasm acquiring such large dimensions under EM the fortuitous missing of an observation of an infrequent association due to sampling error could not, however, be ruled out. Association of nucleus, MC and mitochondria were seen in 5071, L 111, 126 D_2, L 110 S_3 in healthy state and in Tifton 23B during disease manifestation.

Apart from this, association or aggregated presence of myelinations, vacuoles and mitochondria near cell wall; ribosomes, polyribosomes and electron dense material (EDM) near chloroplast or in the general cytoplasm; EDM in the ribosomal matrix, near plasma membrane (PM), BC, rough endoplasmic reticulum (ER); BC, rough ER, myeline, vesicles, vacuoles; ER, high ribosome, granular material between cell wall and chloroplast were also observed in the cytoplasm. Association of pinocytosis, vacuoles and nucleus, and chloroplast with

pronounced swellings was also seen, EDM was found to be deposited on or near vacuoles, PM and cell wall specially in infected material. It is perhaps incorrect to call such combination of characteristics as associations but rather these are certainly coordinated structural and functional attributes of a vigorous cytoplasm. The association specially of bigger organelles like nucleus, MC, BC and microbody etc. were exhibited to be more during 40 days old stage where maximum external manifestation of downy mildew takes place in pearl millet. In general, highest frequency of all associations was seen in lines L 111 and 126 D_2 which are highly resistant/immune to downy mildew disease.

Association of active nucleus with MC and BC at cytoplasmic level could be the gross manifestation of subtle molecular processes at DNA level of nucleocytoplasmic interactions for instance like that of the complementation between small and large subunit transcript of CO_2^- binding protein, ribulose biphosphate carboxylase/oxygenase (Rubisco). Pearl millet is a C_4 plant and it is well known that in these plants MC harvest CO_2 with the help of PEP carboxylase and this harvested intermediate is then transported to BC where Rubisco use the CO_2 for starch synthesis (Sane 1989). Thus, alternately association between MC or BC might facilitate faster trans-location by physical association. The association of pronounced swellings with MC also perhaps indicated towards a mechanism helping the MC to translocate intermediates of photosynthates from it to the BC. Associations could therefore be physical characterization of metabolic interactions at biochemical level.

Association of microbody could help provide enzyme quickly and mitrochondria could provide efficient respiration at the site of actual metabolism in healthy plant or during infection. Respiration rate increase during infection is an universally well known phenomenon. Observations of these and some other associations in higher frequencies in resistant and hybrid genotypes than suspectible indicated the better organizational potential of cytoplasmic organelles in them for co-adapted functioning in healthy stage to fight pathogen or other attacks. The functioning of plant cell organelles in a co-adapted manner in infected plants for directing the metabolism for its own benefit leading to the failure of pathogen to establish its nutritional relationship with the host has been postulated by Stavely et al. (1969). A constellation of superior ultrastructural traits in the resistant lines than susceptible progenitor have been reported (Thakur et al. 1992b). This indicated a strong cytoplasmic ultrastructural base in addition to associations in near isogenic resistant lines to its susceptible progenitor and might be the manifestation of pleiotropic effects of resistance gene(s).

The associations of EDM supposed to be phenolics/phytoalexins/ligmins (Mueller & Beckman 1976, Mould et al. 1991) with PM and cell wall also indicated resistance potential in healthy plant. Their amplified synthesis in the infected susceptible and resistant lines as has been reported by Thakur & Murty (1992) further pointed towards the potential of plant to frustrate the pathogen to establish nutritional relationship with host to check its further spread and is in line with Stavely et al. (1969). The attempt of the genotype to confine the phytoalexins/phenolics (EDM) towards thick and convoluted PM and cell wall by forming bigger vacuoles in the healthy cell near infected one and surrounding the host pathogen from all sides by EDM (Thakur & Murty 1992) indicated a hyper-sensitive type of defence mechanism specially in infected plants. Similarly presence of ER, vesicles, membraneous structures, pinocytosis,

ribosomes and polyribosomes etc. seen more frequent in resistant lines than susceptible are all beneficial cytoplasmic traits helping better transport, compartmentalization of cytoplasm for isolating specific reaction, efficient enzyme synthesis, etc. and is a part of the inherent cytoplasmic strategy for achieving resistance or vigour or may be any other biological feat not possible without coordinated cell activity.

Since all the resistant genotypes have been bred and selected for resistance and high yield, the exhibition of maximum associations at 40 days old stages which also correspond with maximum external manifestation of downy mildew, could not be fortuitous but an exhibition of inherent cytoplasmic potential of genotype for coadapted functioning probably rendered by resistant genes. The observation of higher associations and better organised cytoplasm in L 111 and 126 D_2 (immunity to downy mildew) than others further supported this paradigm.

Thus, the postulation of Stavely et al. (1969) for co-adopted functioning during pathogen attack could be modified to be as "the hypothesis of coordinated structure and functioning strategy in healthy plant" which might be for resistance to pathogen or any other feat giving vigour and productivity and selection advantage during growth and development. At the time of attack or for achieving any other feat during life span of plant, these already existing strategies might be induced to get the success at the focus of action/interaction. The ultrastructural cytoplasmic organelle associations could be considered analogous of basic biological properties and characteristics of highest evolved living organisms like mammals where organisms associate for overcoming would be danger or show collectivity at the time of danger to fulfil a feat not possible individually. In plant system this could also be the inherent tendency at cytoplasmic level of particularly competitive genotypes.

Emperically such associations might become one of the several criteria of selection in selecting for disease resistant or superior cytoplasm in cytoplasmic male sterile line for improving yield potential *per se* or for hybrid vigour.

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