Chemical Composition of Cells and Tissue of the Body of Living Organism : A Histochemical Study

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Introduction :

Karihaloo and Malilc, (2006) conducted a research on "Seed Epidermis Development and Histochemistry in Solanum melongena L. and S. violaceum Ort. In this research, structure, development and histochemistry of the seed epidermis were studied in Solanum melongena L. and S. violaceum Ort. using light and scanning electron microscopy. The epidermal cells at the endosperm mother cell stage of ovule development had thickened outer periclinal walls, consisting of two layers, a thin inner layer, and a thick outer layer. The latter stained positively for pectic substances and became further thickened, during the course of seed development; more so observed in S. melongena. The inner layer of the outer periclinal wall was also thickened by the depositions of cellulose but remained comparatively thin. The development of the inner periclinal and anticlinal walls took place by the uneven deposition of concentric layers. These secondary wall thickenings, appeared as pyramids in transverse section stained for cellulose, lignin and pectin, respectively. Further uneven secondary thickenings near the outer part of the anticlinal walls, resulted in the formation of projections, which were hair- or ribbon-like in appearance. In S. melongena, these projections progressed only for a short distance from the anticlinal walls. In S. violaceum, on the other hand, they grew much longer forming striations (a stripe or stripes of contrasting colour) inside the outer periclinal wall. In S. melongena, partial removal of the outer periclinal wall by enzyme etching, exposed to the surface view which showed a beaded appearance of the cell boundaries. Complete erosion of the outer periclinal wall revealed the hair-like projections of the underlying anticlinal walls. In S. violaceum, enzyme treatment exposed the striations, which formed bridge-like structures over the curves in the anticlinal wal1s.

Histochemistry is the branch of science that deals with the chemical composition of the cells and tissues of the body of living organism. It is the study of chemical components of cellular and subcellular tissues that are often studied with the aid of a microscope. Histochemistry enables to localize various chemicals present in tissues. The aim of microscopic histochemistry is in the localization, identification of substances and enzyme activities within cells and tissues.[1]

Zhi-qiang et al., (2009) conducted a research on "Relationship between development period and the starch dyeing of eggplant microspore". The relationship between the dyed starch of six varieties of eggplant microspores with different development period was studied. The results showed that the rate of dyed starch of microspores increased in the whole plant, with the difference value of the base of the sepal split length enlarged, however, the variations were significant in different varieties. The rate of dyed starch had a significant peak at inchoation (just beginning to form and therefore not clear or developed) at uni-nucleate stage. The rate of dyed starch of the other five varieties increased with microspore developmental period in the whole plant. The rate of starch dyeing increased slowly before uni-nucleate stage, nevertheless the rate of starch dyeing was accelerated after uni-nucleate stage.

Hartmann and Wydra (2009) studied the "Resistance induction by silicon against bacterial diseases of tomato, eggplant, cucumber and geranium". To contribute to the development of new integrated practices for the control of bacterial diseases, root and/or foliar application of silicon to eggplant,

geranium, tomato and cucumber were investigated. In eggplant and geranium, wilt incidence caused by Ralstonia solanacearum was reduced in silicon-treated plants, and initially delayed by two days. Bacterial numbers were significantly reduced in stems of eggplant and geranium. Immuno-histochemical studies of possible molecular mechanisms of silicon-mediated resistance on cell wall level in eggplant and geranium showed a strong yellow autofluorescence of the xylem parenchyma and vessel walls, indicating the production of phenolic substances in inoculated plants without silicon application, but not in plants treated with silicon. In geranium, inoculation resulted in an increased staining for (l-5)- α-L-arabinan side chains of rhamnogalacturonan-I in cell walls, which was less in inoculated plants without silicon application. Foliar- and/or root-application with silicon to tomato genotypes with different levels of resistance, and to cucumber, inoculated with Pseudomonas syringae pv. tomato and Pseudomonas syringae pv. Lachrymans, causing bacterial speck in tomato and angular leaf spot in cucumber, respectively. These however, resulted in slightly reduced bacterial speck in moderately resistant tomato genotypes. In cucumber, a weak retardation in the initial development of angular leaf spot was observed. A reduction in bacterial populations was observed in tomato, but not in cucumber. In tomato, the enzyme activity of guaiacol peroxidase increased after inoculation, but no effect due to silicon treatment was observed, while in cucumber, the enzyme activity also increased after inoculation, and was higher in silicon-treated plants. A major role of the genetically determined resistance of a genotype was decisive, thus, silicon-induced resistance can most effectively be triggered in genotypes, exhibiting a moderate resistance against a pathogen.

Yoko Horiuchi et al., (2003) conducted "Evolutional study on acetyicholine expression" for the histochemical changes taking place in the host tissues on account of infection. Acetylcholine is a well-known neuro-transmitter in the cholinergic nervous systems of vertebrates and insects; however, there is only indirect evidence for its presence in lower invertebrates, such as plants and fungi. This study investigated the expression of Acetylcholine in invertebrates (sea squirt, sea urchin, trepang, squid, abalone, nereis, sea anemone, coral and sponge), plants (arabidopsis, eggplant, bamboo shoot, cedar, hinoki, pine, podcarp, fern, horsetail and moss), fungi (yeast and mushroom) and bacteria by assaying Acetylcholine content and its synthesis, focusing on the presence of two synthetic enzymes, choline acetyltransferase (ChAT) and carnitine acetyltransferase (CarAT). Using a specific radioimmunoassay, Acetylcholine was detected in all the samples tested. The levels varied considerably, however, with the upper portion of bamboo shoots having the highest content (2.9 μmol/g). Moreover, the activity in most samples from the animal kingdom, as well as in bamboo shoots and in the stems of the shiitake mushroom, were found sensitive to both ChAT and CarAT inhibitors. Levels of Acetylcholine synthesis were lower in the samples from other plants, fungi and bacteria but were insensitive to ChAT and CarAT inhibitors. These findings demonstrate the presence of Acetylcholine and Acetylcholine-synthesizing activity in evolutionally primitive life as well as in more complex multicellular organisms. In the context of the recent discovery of non-neuronal acetylcholine in various mammalian species, these findings suggest that acetylcholine been expressed in organisms from the beginning of life, functioning as a local mediator as well as a neurotransmitter.

Observation of Localization of Various Metabolism :

There are a number of histochemical studies conducted by earlier research workers on healthy and infected eggplants and other similar plants, however sparse studies have been carried out on Sclerotinia blight (Sclerotinia minor) and Verticillium Wilt (Verticillium dahliae) infected eggplant samples. Hence, the present study is undertaken to observe the localization of various metabolites e.g. starch, cellulose, tannin, lignin, lipids, protein, polysaccharides and enzyme activity, etc. in healthy and infected brinjal plant samples.

Histo-chemical Analysis of Brinjal Plants :

In the present investigation, uniform methodology was used. Fresh, normal and diseased material was brought from the field, washed throughly to remove the dust particles. The hand sections were cut using razor and used immediately for histochemical test and photomicrograph.

Starch was localized by IKI reaction method suggested by Johnson (1940).

Preparation of IKI solution : 2.0 gm of KI was dissolved in 100 ml distilled water and then 0.2 gm of 12 was added to it.

Procedure : Fresh hand cut sections were placed in IKJ solution for 1 minute and then mounted in the same solution.

Observation and Results : We found that starch granules were blue to black in colour. In normal axis of brinjal, starch granules were observed in the cortical region only. The starch granules did not appear in the vascular and pith region. On the contrary, in the hypertrophied tissues of the same age, the starch granules were found scattered in the cortex stele pith. The fimgal oospores did not contain starch granules and were densely collected in the center in the normal axis of the cortex, in comparison to the hypertrophied axis. Starch is found in upper and lower epidermis, mesophyll cell, cortical cells, and pith parenchyma, respectively. It is the principal ergastic substance of the protoplast. Similar results were also observed by many researchers earlier. Webstar (1970) reported the presence of starch grains and lipid in tomato and cucumber. Orion and Bronner (2003) showed the presence of starch droplets in nematode galls. Starch grains present in the cortex of nematode root galls and their absence near giant cells. Shah and Raju (2007) also reported the absence of starch grains near the larval cavity.

(Plate-l2, A = healthy; B and C = diseased plants)

Cellulose test in the present study was done by IKI-H_2SO_4 method (Johnson, 1940).

Procedure : Fresh hand cut sections were kept in IM solution for 10 minutes and mounted on a glass slide. Through the slides of cover slip a drop of 65% H2S04 acid was slowly allowed to diffuse and sections were observed as such under the microscope.

Observation and Results : In was observed that cellulose stained blue to black. Epidermis and cortex were intensively stained blue, showing the presence of cellulose in the normal stem. In the hypertrophied axis, the color reaction was quicker and intense, indicating excess of cellulose deposition in the diseased tissue. The fisngal oospores blue embedded in the cortex stained deep blue and immediately turned black. (Plate-l3, A = healthy; B and C = diseased plants)

Lignin was localized by phloroglucinol HC1 test (Johnson, 1940; Singh, 1987).

Preparation of Stain :1.0 gm of phloroglucinol was dissolved in 100 ml of 15% ethanol.

Procedure : Fresh hand cut sections of normal and diseased axis were placed in phioroglucinol solution, a drop of 20% HC1 was added to the stain and sections were removed, mounted in glycerin and observed immediately.

Observations : Lignified tissues appeared red in colour. Lignin was observed in vascular tissues in both normal and diseased plant. The vascular region in the normal axis stained brick red, showing excess of lignin deposition in the secondary tissues, while in the diseased tissues lignification was confmed to the isolated vascular structures only. Fungal oospores also stained dark brown, showing

the presence of lignified tissues in them. (Plate-14, A = healthy; B and C = diseased plants).

Proteins were localized by the amido black method (Weime, 1959).

Preparation of amido black dye : Amido black was prepared by adding 0.5 gm of amido black to the solution containing 5.0 gm of mercurous chloride (HgC12) and 5.0 ml of glacial acetic acid in 100 ml distilled water. The stain was filtered and used.

Procedure : Fresh hand cut sections of normal and diseased axis were stained by amido black dye for 2-3 minutes and subsequently washed in 20% acetic acid for 5 minutes and later in distilled water. The sections were mounted temporally in glycerin and photo micrographed.

Observation : Protein stained blue to black, large amount of protein was found in the cortical and phloem cells of the normal axis. Epidermis vascular cylinder and pith did not show presence of protein.

However, in the diseased axis, phloem did not stain. The outer warty wall of the oospores stained deep blue in colour, showing the intense deposition of the protein in and around the site of fungal oospores. Xylem of the disease also did not take stain of protein. Proteins are scattered cells of cortex, mesophyll cells and pith. (Plate-15, A = healthy; B and C = diseased plants).

Tannins were localized by Lugol's Iodine method (Haridass and Suresh Kumar, 1986).

Preparation of Lugol's Iodine solution : Lugol's iodine solution was prepared by adding IKI solution (4g I_2 + 6g KI + 100 ml distilled water).

Procedure : Fresh hand cut sections of normal and diseased axis were treated in Logol's solution for few minutes. To this a drop of dilute NH_4oh solution was added. The sections were mounted in glycerin.

Conclusion :

Tannins appeared brown in colour. In the normal axis, vascular cylinder took a positive stain for tannin, xylem stained dark in colour showing high intensity of tannins in these cells. The tannins were observed in a few cortical cells only.

However, in the diseased axis the xylem elements stained showing tannins but colour intensity was lesser in comparison to the normal axis, showing poor presence of tanniferous cells. In the cortical region, high tannins intensity was evident around the fungal oospores. Pith also contained tanniferous cells in the diseased axis.

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