RESEARCHERID
THOMSON REUTERS

[Pandey * *et al.*, 6(7): July, 2017]
IC™ Value: 3.00

ISSN: 2277-9655
Impact Factor: 4.116
CODEN: IJESS7

IJESRT

INTERNATIONAL JOURNAL OF ENGINEERING SCIENCES & RESEARCH TECHNOLOGY

HISTOPATHOLOGICAL CHANGES IN PAPAYA DUE TO PAPAYA RING SPOT VIRUS ISOLATE IN TERAI REGION OF EASTERN UTTAR PRADESH

Ravindra Kumar Pandey*

*P.G. Department of Botany, M.L.K.(P.G.)College ,Balrampur (U.P.)-271201
(Affiliated to Siddharth University Kapilvastu, Siddharth Nagar-(U.P.)

DOI: 10.5281/zenodo.829745

ABSTRACT

Papaya Ring Spot Virus induces various histopathological changes in Carica papaya L. The morphological symptoms induced are ring spot, distortion and mottling symptoms on foliage, stunting in stem, fragile roots and concentric ring and deteriorated fruit. The anatomical studies of 90 days old symptomatic leaf stem and root sample revealed extensive deformation of parenchyma tissues in leaf, poor differentiation of vascular tissue without disintegration, reduced size of xylem cells in roots. The root tissues also showed increased lignifications and reduced protoxylem. Hyperplasia in cells externally visible as ring spots was observed. Hypertrophy in cells due to poor differentiation of secondary tissues caused weak and fragile stem and roots in host. The disease was very widespread in the study region causing 71-86% yield losses (Khurana 1968).

KEYWORDS: Papaya ring spot virus, Histopathological, palisade tissue.

I. INTRODUCTION

The Carica papaya,L. belongs to family Caricaceae.In India it is grown particularly in the state including Andhra Pradesh,Tamilnadu,Assam,Bihar,Maharashtra,U.P.,Gujrat,Punjab ,West Bengal,M P Karnatka and other parts of the country.The fruit is grown for its whole some medicinal qualities and hence,has ahigh economic value however,widespread occurance of virus incidence in this region, is major constraint for commercial cultivation of this crop restating it to kitchen garden only(Singh et.al, 2005). The virus induces chlorotic mottling,ringspot and distortion sympoms on foliage, stems,ftuits and even on roots of the host. The disease is naturally transmitted by aphids, with varied digree of transmission rates in a non persistat manner, particularly,Myzus persicae and Aphids gossypi which are most efficient vectors in the study reason (Glasa et. al 2000) .The distortion of foliose, stem and root has a direct impact on productivity of the plants affecting carbohydrate assimilation and hence yield loss, 70-90 % and sometimes even 95% .The present study was undertaken to reveals the histopathological manifestation of virus at cellular and tissue level to examine the host pathogen relation.

II. MATERIALS AND METHODS

Pure culture of the virus was maintained under insect proof chamber in the Botany Department, M.L. K.P.G.College, Balrampur is used as inoculum. The papaya seedlings were raised from the seeds of different varieties of Papaya taken from NBPGR New Delhi.

Healthy seedlings were raised in earthen pots of 30 cm. culture pots (with 2 seedlings per pot) in two sets (of five pots each) in the insect proof chamber of Botany Department of the college. One of the sets was mechanically inoculated with standard inoculum (SI) of papaya ring spot virus (PRSV) at three leaf stage (15 days old plants) prepared from virus sample and other set was maintained as healthy control. The plants were observed for symptoms up to 90 days.

For histopathological studies samples of stem leaf and roots were taken after 90 days from diseased and healthy plants. The plant samples were fixed in formalin acetic acid (F.A.A) solution consisting of 50ml of 95% ethyl alcohol, 5ml of glacial acetic acid, 10 ml of 40% formalin and 35 ml distilled water for 48 hours. Samples were dehydrated in graded ethanol-butanol series and xylene (Willey, 1971], infiltrated and embedded in paraffin at 56-58 0C using paper boat method. Cross-sections, 12 µm thick, were cut using a rotary microtome adhesive

ISSN: 2277-9655
Impact Factor: 4.116
CODEN: IJESS7

with Haupt's adhesive. The sections were stained using safranine fast green [Johnson ,1940] and iron-haematoxylin mounted in Canada balsam and then examined under light microscopy.

III. RESULTS AND DISCUSSION

The symptoms were typically ring spot type, ranging from mild to severe distortion of foliage, lamina was so reduced that sometimes only veins were visible, however; in some samples foliage showed mild mottling visible as chlorosis. Deformed fruits with rings pots were found. Plants showed crowning of top leaves and denuded appearance due to defoliation, reduced fruit setting and also severe stunting in some areas.

Histopathology of the symptomatic samples revealed that, leaf is the most severely affected part of the host. The bright field micrograph of infected leaf showed reduced leaf thickness. The palisade parenchyma cells below the upper epidermis show a loss in columnar appearance with reduced intercellular spaces extending towards abaxial epidermis. The epidermal and mesophyll cells appeared almost distinguishable. The palisade cells are also variously deformed and almost indistinguishable from spongy cells. Reduced number of chloroplasts appearing as fused masses is seen. Abundant air spaces are also visible in the spongy layers. The cells appear irregular in outline. In diseased leaves, the chloroplast become rounded and clumped together in the cell. The chloroplast become cup shaped, with the opening of the cup generally facing the cell wall. Islands of tissue in the mosaic showing various shades of green and yellow contain different strains of virus which affect the chloroplast. In distinct ways, in dark green islands of tissue, which contain very little virus, chloroplast appear normal.

A reduction in vascular elements with subsequent poor differentiation of xylem, phloem and parenchymatous elements appearing as a proliferating cell mass is visible. The cross-section shows a reduction in diameter of xylem vessels, phloem area as well as the thickness of the leaf blade. A few laticifers are also seen which appear intact and normal.Reduced mesophyll cells and degeneration of chloroplasts suggests that virus infection induces gradual chlorosis, thus affecting normal photosynthesis. The thickening of veins and veinlets may be due to enlargement of bundle sheath parenchyma, but a reduction in size of vascular elements suggests a reduced translocation efficiency of infected host plant resulting in disturbed metabolism and hence, an overall reduction in yield of the plant.

Changes in the vascular tissues of leaf were observed at reduction in size and number but later on became necrotic. The sheath cells of vascular bundles necrose following degeneration nearby mesophyll. Changes in the xylem vessel walls are evident in areas where the surrounding bundle sheath and mesophyll are necrotic. The walls of such vessels are stained much darker than those of normal vessels. This dark staining effect is first evident at corners of the vessels, subsequently extending the entire wall. Simultaneously degeneration is general throughout the bundle. Similar results were obtained by Porter, 1954; Roberts, 1989; Raychaudhary, 1977; Kunkalikar, 2007; Shukla, 2011. In the stem Xylem cells appear to be more compact compared to such cells in healthy stem showing a slight reduction in size of cells externally visible as reduction in girth of infected stem.The infected root also showed no significant damage however, the protoxylem cells are reduced in number and cells also show lignifications.

The overall finding revealed that, leaf is the most severely affected part of the host. (Table 1) Papaya ring spot virus (PRSV) isolate in the study region causes deformation in palisade cells with broad intercellular spaces, appearing almost spherical and indistinguishable from the spongy cells, reduction in number of chloroplasts, reduced xylem elements in infected leaf. These observations are in confirmation to the findings of earlier reports of hyperplasia in light green and yellow areas and poor chlorophylls. An association of hypertrophy of mesophyll cells and chlorophyll destruction in bulging affected parts of host leaf tissues has been earlier reported in virus yellows. A reduction in the size and number of chloroplasts in the yellow streak of sugarcane disease affected with chlorotic streak virus has also been observed [Esau, 1956).Deficiency in chloroplast accompanied by hyperplasia and hypertrophy of palisade cells is reported in stripe disease of narcissus(Caldwell,1938;Singh,2002).Thus histopathology may help in studying host pathogen relationship that ultimately may help in disease management in near future.

IV. CONCLUSIONS

The overall finding revealed that, leaf is the most severely affected part of the host. The Papay Ring Spot Virus isolate in the study region causes deformation in palisade cells with broad intercellular spaces, appearing almost

ISSN: 2277-9655
Impact Factor: 4.116
CODEN: IJESS7

spherical and indistinguishable from the spongy cells, reduction in number of chloroplasts, reduced xylem elements in infected leaf. Changes in the vascular tissues of leaf were observed at reduction in size and number but later on became necrotic. The sheath cells of vascular bundles necrosed following degeneration nearby mesophyll. Changes in the xylem vessel walls are evident in areas where the surrounding bundle sheath and mesophyll are necrotic.

V. ACKNOWLEDGEMENTS

The author is thankful to Principal M. L. K. P. G. College Balrampur and Head Deptt of Botany for providing basic facilities to perform the present study.

Table-1 Histopathological observation on papaya leaf tissues infected with PRSV

Measurements(μ)	Healthy(μ)	Infected(μ)
Average of Mid vein thickness	1317	732
Average of Blade thickness	189	161
Average of Palisade tissue thickness	87	59
Average of Spongy tissue thickness	71	54
Average of Vascular bundles length	233	270
Average of Vascular bundles width	197	157
Average of Number of xylem vessels/bundle	75	65
Average of Xylem vessel diameter	35	20

(Data in the table are statistically verified and an average of five repetitions)

Table No.2 Effect of P.R.S.V.on number of chloroplasts in palisade cells of Caica papaya (L.)Leaf.

Repetitions	Number of Chloroplasts in each palisade cell		X (A-B)	X^2
	Healthy (A)	Diseased (B)		
01	27	22	05	25
02	30	20	08	64
03	28	17	11	121
04	33	21	12	144
05	35	20	15	225
06	28	24	04	16
07	26	22	04	16
08	24	18	06	36
09	29	19	10	100
10	28	19	09	81

$\Sigma X = 84$	$\Sigma X^2 = 828$

t- Calculated value – 7.530

Significant at 1 % and 5% levels respectively. The observed value of 't' is 7.530 which is greater than the table value of 't' for 9 degree of freedom at 5% and 1% level of significance i.e. 2.262 and 3.250 respectively. The

ISSN: 2277-9655
Impact Factor: 4.116
CODEN: IJESS7

number of chloroplast in palisade cells of infected bottlegourd plant is reduced in comparison to healthy ones. These observations are significant when analysed statistically.

Table No.03 Effect of PRSVon length of palisade cells in Caica papaya (L.)leaf.

Repetitions	Length of Palisade cells in (μ)		X (A-B)	X^2
	Healthy (A)	Diseased (B)		
01	38.87	35.76	3.11	9.67
02	41.98	34.21	7.77	60.37
03	45.09	32.65	12.44	154.75
04	43.54	34.00	9.54	91.01
05	46.65	31.10	15.55	241.80
06	45.09	29.54	15.55	241.80
07	48.00	32.65	15.35	235.62
08	49.76	37.32	12.44	154.75
09	46.65	29.54	17.11	292.75
10	48.00	38.87	9.13	83.35

$\Sigma X = 117.13$	$\Sigma X^2 = 1565.87$

t- Calculated value – 8.310

Significant at 1 % and 5% levels respectively. The observed value of 't' is 8.310 which is greater than the table value of 't' for 9 degree of freedom at 5% and 1% significance i.e. 2.262 and 3.250 respectively. The length of palisade cells in infected bottlegourd plant is reduced in comparison to healthy ones. These observations are significant when analysed statistically.

VI. REFERENCES

[1] Caldwell, J. and James, A.L.:Annals Appl. Botany, 25(2): 244-253 (1938).
[2] Clark, M.F. and Barr Joseph, M.: In: Methods in Virology 51: 85,AP, N4Y(1984).
[3] Esau,K.:American J. Botany, 43: 739-748 (1956).
[4] Glasa, M; Subr, Z; Ciampor, F; and Kudela, O (2000). Some properties of a Beet mosaic virus isolate from western Slovakia. Biologia-Bratislava.55(1):85-89.
[5] Johnson, D.A.: Plant Microtechnique.McGrawHill Book Co. Inc., 523 (1940).
[6] Kunkalikar, S., Byadgi, A.S., Kulkarni, V.R.,Krishnareddy, M., and Prabhakar, A.S.N.: Indian J. Virology, 18(1): 33-35 (2007).
[7] Porter, C.A. (1954). Histopathological &Cytological changes induced in Plant by CMV (Marmor cucumeris H)contrin. Boyce Tompsoninst, 17: 43-71
[8] Raychaudhary, S.P. and Nariani, T. K.:Viruses and Mycoplasma Diseases of Plants in India. Oxford IBHPublishing Company, NewDelhi, 160-167 (1977).
[9] Roberts, I. M(1989). Indian cassava mosaic virus: ultrastructure of infected cells. Journal-of-General-Virology. 1989, 70: 10, 2729-2739.
[10] Singh, V.: Studies on Different Strains of Papaya Ringspot Virus with Reference to Identification, Histopathology and Varietal Susceptibility. Ph.D. Thesis, D.D.U. Gorakhpur University,Gorakhpur. India. (2002)
[11] Singh, V., Rao, G.P. and Shukla, K.: Phytopathology, 58: 212-216(2005).
[12] Singh, V. and Shukla, K.:Ann. Plan. Protec.Sci. 19: 142-146(2011).
[13] Willey, R.I.: Microtechniques: A laboratoryGuide. MacMillan Publishing Co. Inc.,NewYork (1971).

CITE AN ARTICLE

Pandey, Ravindra Kumar. "HISTOPATHOLOGICAL CHANGES IN PAPAYA DUE TO PAPAYA RING SPOT VIRUS ISOLATE IN TERAI REGION OF EASTERN UTTAR PRADESH." *INTERNATIONAL JOURNAL OF ENGINEERING SCIENCES & RESEARCH TECHNOLOGY* 6.7 (2017): 486-89. Web. 15 July 2017.