Histopathological Studies on Urdbean, *Vigna mungo* Infected by Urdbean Leaf Crinkle Disease

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Abstract

The anatomical changes in stem, petiole and different leaves of urdbean leaf crinkle infected plant parts were highly conspicuous and were increase in number of layers and size of parenchyma cells and epidermal cells. The transverse section of infected stem of urdbean showed well developed cortex layer which was three times thicker than that of healthy leaf. The epidermal cells of infected tissue were significantly bigger than in healthy leaf. The number of vascular bundles increased in infected stem. The number of rows of xylem vessels and number of vessels per row were almost double in infected stem. The diameter of xylem vessels (metaxylem and protoxylem) increased significantly over healthy vessels. There was significant increase in number of layers of parenchyma cells and cambial cells and diameter of phloem parenchyma in diseased plant compared to healthy stem.

Keywords: Urdbean, *Vigna mungo*, urdbean leaf crinkle, histopathology, virus

Introduction

Urdbean (*Vigna mungo* (L.) Hepper) is an important pulse crop, grown all over India during summer and winter. Urdbean becomes a victim of a large number of diseases caused by both fungi and viruses. Among the virus diseases, Urdbean leaf crinkle virus (ULCV) is considered to be the most serious one causing considerable damage to the crop depending on season and variety cultivated (Reddy *et al.*,2005). In India the disease was first reported by Chohan and Kalia (1967) from Punjab under the name 'curly top'. Since viruses lack metabolic mechanism of their own, they depend on the invaded host cells to supply material and energy for their biosynthesis. This causes disturbances in the physiology and anatomy of infected plants. Patel *et al.*, (1999) reported that increase in leaf area and number of stomata in diseased plants increased. To understand the ULCV induced anatomical changes within diseased plant, histopathological studies are essential, and these changes are discussed in this paper.

Materials and methods

The histopathological studies on urdbean infected by urd bean leaf crinkle disease were carried out at Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore across alternate years in five phases up to 2002. The mean data are interpreted and discussed in this paper.

Thirty days after inoculation, free hand sections of stem and petiole of infected plants were taken and transferred immediately into water, then into 95% ethanol for 30 to 60 minutes. The sections were transferred to 1% solution of safranine in 50% ethanol for 12-24 hours (Chamberlin, 1932). The excess safranine was removed by transferring the section into 50% ethanol. A small drop of HCl was added. Then the sections were washed with water thoroughly for five minutes and stained with Delafield's haemotoxylin stain for ten minutes. The excess stain was removed by extensive washing with water for 5-10 minutes. If the sections were deep purple, one drop of HCl was added and washed for 5-10 seconds. The acid was washed out with water for 20-30 minutes. The sections were similarly transferred to 50, 95 and 100% ethanol and finally xylol allowing five minutes in each solvent. The sections were placed in a drop of glycerin on a glass slide and observed under compound microscope. The width of cortex layer, number of layers of parenchyma cells, diameter of parenchyma cells, length and breadth of epidermal cells,

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number of layers of cambial cells and elements of vascular bundles were recorded in healthy and infected sections. Similarly free hand thin sections of leaf from infected and healthy plants were taken and transferred immediately into water. They were placed on a clean glass slide with a drop of water, covered with cover slip and observed under compound microscope. For each type of plant part ten sections were observed.

Results and discussion

Histopathology of stem and petiole

The stem epidermal cells of infected plants were significantly bigger than cells in healthy plants. There was no significant increase in number of layers of parenchyma cells. But the diameter of parenchyma cells increased four times as that of healthy cells. The number of layers of cambial cells and number of vascular bundles also increased significantly over healthy plants. The number of rows of xylem vessels and number of vessels per row were almost double in infected stem compared to healthy plant. The diameter of xylem vessels (metaxylem and protoxylem) also increased significantly over healthy. There was significant increase in number of layers of phloem parenchyma cells and diameter of cell in infected tissue. The width of cortex layer of infected tissue was three times that of healthy plants (Table 1). The epidermal cells of petiole of infected plant enlarged length wise. There was significant increase in number of layers and also diameter of parenchyma cells over healthy plants. The number of vascular bundles was same in healthy and infected petiole, but the number of rows of xylem vessels and vessels per row significantly increased in infected petiole. The infected xylem vessels (metaxylem and protoxylem) enlarged significantly over healthy plant. The number of layers of phloem parenchyma was more in infected plants, the cell size and width of cortex layers increased significantly in infected plants (Table 1).

Histopathology of enlarged, normal and small size leaves

The enlarged leaf of diseased plant was 13.6% thicker than the healthy leaf. The epidermal cells of infected plants had increased size. The palisade cell layer and cell size also increased significantly over healthy plant. The spongy cell layer and diameter of spongy cell increased. The thickness of mid vein of infected plant was double that of healthy plant and the epidermal cell of mid vein of infected leaf was larger in size than in healthy plant. The number of rows of xylem vessels and number of vessels per row and their cell size also increased significantly when compared with healthy tissue of mid vein (Table 2, Fig. 1a).

The normal size of infected leaf was thicker than the corresponding healthy leaf (Table 2) but the epidermal cells were enlarged. The thickness of palisade layer and spongy cell layer was more than healthy. The number of palisade cells per millimeter was more in infected tissue whereas spongy cells decreased significantly compared to healthy

Table 1. Histopathology of healthy and infected stem, petiole (mean of ten sections)

	Stem			Petiole		
Cells	Healthy	Infected	CD (P=0.01)	Healthy	Infected	CD (P=0.01)
Epidermal cell (l x b) (μ)	27 x 16	39 x 28	6.2 x 1.2	21 x 17	26 x 18	1.48 x NS
No. of layers of parenchyma cells	4.5	4.9	NS	5.0	5.6	0.4
Diameter of parenchyma cells (μ)	18	84	4.4	29.0	36.0	4.9
No. of layers of cambial cells	2	3.5	0.3	-	-	-
No. of vascular bundles	14.4	15.2	0.4	5	5	NS
No. of rows of xylem vessels	1.5	2.7	0.4	3.2	5.8	0.5
No. of xylem vessels per row	3	5.5	0.6	3.2	3.9	0.5
Diameter of metaxylem (μ)	38	64	3.0	38	40	1.2
Diameter of protoxylem (μ)	12	24	2.3	20	21	0.7
No. of layers of phloem parenchyma	3.5	6	0.6	3.5	5.3	0.6
Diameter of parenchyma (μ)	71	120	5.5	-	-	-
Width of cortex layer (μ)	133	471	7.0	113	151	7.6

NS = Not significant

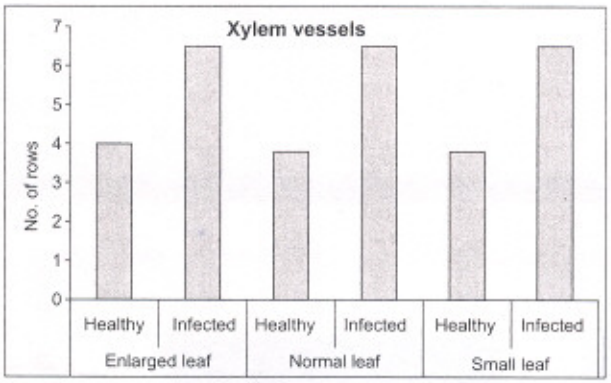

Figure 1a. Histopathological changes in in xylem vessels of urdbean leaves

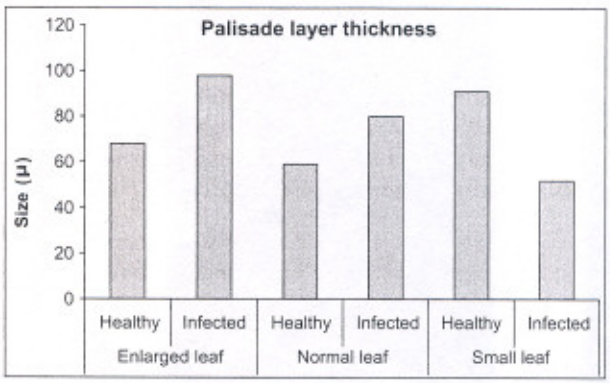

Figure 1b. Histopathological changes in palisade layer thickness of urdbean leaves

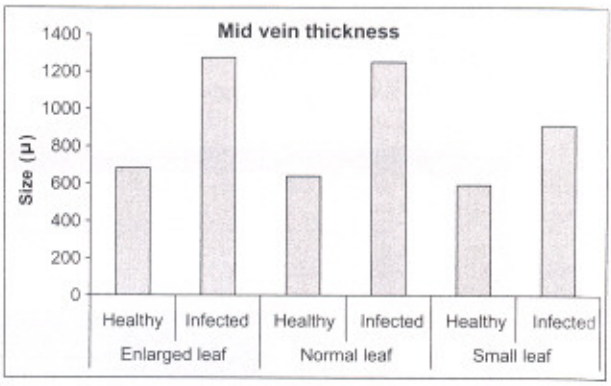

Figure 1c. Histopathological changes in mid vein thickness of urdbean leaes

leaves. But the diameter of both the cells increased significantly in infected leaf tissue. The diameter of [illegible]m vessels (metaxylem and protoxylem) increased significantly over healthy leaf tissue of mid vein. The small size leaf of infected plant was 24.1% thinner than the healthy leaf. The epidermal cells of infected plants had decreased size. The palisade cell layer thickness decreased significantly over healthy plants, but cell size increased over healthy plants (Fig. 1b). The spongy cell layer thickness decreased significantly over healthy plant but cell size increased over healthy plants. The thickness of mid vein of infected plant was double that of healthy plant (Fig. 1c) and the epidermal cell of mid vein of infected leaf was larger in size than in healthy plant. The number of rows of xylem vessels, vessels

Table 2. Histopathology of healthy and infected leaves (enlarged, normal and small size) (mean of ten sections)

	Leaf (enlarged)			Leaf (normal size)			Leaf (small size)		
Cells	Healthy	Infected	CD (P=0.01)	Healthy	Infected	CD (P=0.01)	Healthy	Infected	CD (P=[illegible]1)
Leaf thickness (µ)	168	191	6.0	172	196	4.5	199	151	9.7
Epidermis of leaf (l x b) (µ)	17 x 14	21 x 17	0.9 x 0.9	17 x 13	22 x 17	0.7 x 0.8	17 x 21	12 x 13	0.8 x 12
Diameter of palisade cells (µ)	18	21	0.8	16	17	0.8	16	19	0.8
Thickness of palisade cells layer (µ)	68	98	7.2	59	80	5.1	91	52	5.1
Spongy cells layer (µ)	60	66	3.5	48	56	3.5	42	40	1.1
Diameter of spongy cells (µ)	21 x 20	25 x 21	1.1 x 0.9	21 x 20	26 x 21	0.9 x 0.9	20 x 22	32 x 22	0.4 x NS
No. of rows of xylem vessels	4	6.5	0.4	3.	6.5	0.6	3.	6.5	0.6
No. of xylem vessels per row	3	3.5	0.4	3	4	0.5	3	4	0.5
Diameter of mid vein metaxylem (µ)	21	26	1.1	20	25	0.9	19	24	1.3
Diameter of mid vein of protoxylem(µ)	8	11	0.8	8	10	0.1	8	10	0.1
Mid vein thickness (µ)	684	1274	5.	640	1250	7.2	591	911	7.6
Epidermal cells of mid vein(l x b) (µ)	21 x 20	25 x 21	1.1 x 0.9	21 x 20	26 x 21	0.9 x 0.9	20 x 22	32 x 22	0.4 x NS

l = length; b = breadth; NS = Not significant

per row and their cell size also increased significantly when compared with healthy tissues of mid vein (Table 2).

The palisade and spongy cells in infected leaves were larger in size than in healthy leaves. The leaf blade became thicker than healthy in enlarged and normal sized leaf, because of great increase in the number and size of different cells. The reduction in thickness of small sized leaf is due to less development of palisade and spongy cell layers and also reduction in the size of epidermal cells compared to healthy (Table 2). In all types of infected leaves, mid vein vascular bundles, parenchyma cells, xylem and phloem vessels developed more than in healthy plants. In another disease, producing similar symptoms on leaf namely sugarbeet curly top, Stevens (1983) observed abnormal phloem development with hyperplasia of phloem parenchyma. It is well known that viruses induce anatomical changes in diseased plants. Poor development of parenchyma cells, vascular bundles, epidermal cells was also seen in tobacco leaves infected by TMV (Esau, 1967). Brar and Ratual (1990) reported urdbean leaf crinkle virus induced biochemical chages in urdbean. This study revealed that increase in amino acid with decrease in protein and chlorophyll content in infected young plants. Some viruses have been shown to lower auxin concentrations (Matthews, 1981). Another intriguing possibility is that both virus and growth substances influence leaf anatomy through an effect on nucleic acid metabolism.

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