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Short Note

Histopathology of Bt Cotton Roots Infected with *Rotylenchulus reniformis*

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Histopathological studies on *Rotylenchulus reniformis* infected three Bt hybrids *viz.*, Bunny Bt, Rasi Bt, and Jagannath fusion Bt in comparison with their respective non Bt (Refugia) were carried out. *R. reniformis* caused heavy damage to cortical, endodermis, pericycle, phloem cells in addition to extensive hypertrophy, dissolution and breakdown of cell wall in non Bt cotton. Whereas in Bt the cell damage caused due to was not severe compared to non Bt cotton. The compact arrangement of cortical and phloem cells and reduced area of phloem vessels in Bt cotton might have reduced severity of *R. reniformis* damage in Bt cotton.

Key words: Bt hybrid, Cotton, roots, reniform nematode damage, compact cell arrangement.

Bacillus thuringiensis originally evolved for the management of boll worm was reported to be effective against several plant parasitic nematodes in Bt crops like tomato, potato, arabidopsis, and alfalfa *etc.* (Urwin *et al.,* 1998; Urwin *et al.,* 2001; Samac and Smigockis, 2003; Li *et al.,* 2007). However the work carried out on histopathology of Bt cotton infested with plant parasitic nematode is very meagre. Hence the present investigation was made to study the histological changes in Bt and non Bt cottons due to reniform nematode, *Rotylenchulus reniformis* to understand the host parasitic relationship and to know the mechanism of resistance in Bt cotton.

Materials and Methods

Bt and non Bt hybrids *viz*., Bunny Bt (BGI – *Cry* 1Ac gene), Rasi Bt (BGII- *Cry* 1Ac and *Cry* 2Ab) and Jagannath Bt (Fusion- *Cry* 1Ab and *Cry* 1Ac) with their respective non Bt (Refugia) were raised in earthen pots (1 kg) containing steam sterilized pot mixture. The infective stage juveniles / pre adult stage of *R. reniformis* obtained from pure culture of *R. reniformis* maintained on castor (Sivakumar and Seshadri, 1971) were inoculated @ 2000 juveniles/ pot near the root zone at 15 days after sowing (DAS).

The experiment was terminated 15 days after inoculation. The infected plants were uprooted with intact root system and the roots portion was gently cut into small bits of 1cm length and fixed in FAA for 24 h. Then the roots were dehydrated through tertiary butyl alcohol series followed by infiltration and embedded in moulds of paraffin wax with 52- 55º C melting point. After hardening, paraffin moulds were trimmed and fixed on the holder of rotary microtome and sectioned at a thickness of 10 µm. The sections were stained in Safranin and Fast Green stain (Johanson, 1940) and mounted in DPX mountant. Mounted slides were left flat to dry for 24h at room temperature.

The microtomic sections were observed under a binocular research microscope and the changes in cellular structures due to infestation of *R. reniformis* in Bt and non Bt cotton were observed.

Results and Discussion

Damage caused by *R. reniformis* in Bt cotton root cells were less compared to non Bt cotton in the present study. After gaining entry through epidermis, the nematode penetrates intercellularly as well as intracellularly through the cortex, endodermis and pericycle and reached the phloem where they feed (Rebois *et al.,* 1975). Root section of non Bt cotton showed very loosely packed cortical cells with more number of scattered xylem vessels compared to Bt cotton. The cells modified due to nematode feeding, push the pericycle and endodermis towards the periphery of the cell. As a result these layers lose their identity and collapse. Further due to nematode infection, cavities of various sizes in the cortex region and heavy damage to the endodermis and pericycle regions which is the feeding site for *R. reniformis* were observed. Hypertrophy, collapsing and dissolution of cell wall as well as granulation in the cells adjacent to the feeding site were visible at the site of feeding. The modified cells have thick wall and a granular protoplasm, which stained deep pink with safranin (Fig 1).

Whereas the Bt cotton infested with *R. reniformis* showed less number of xylem vessels with thick wall and reduction in area occupied by phloem.

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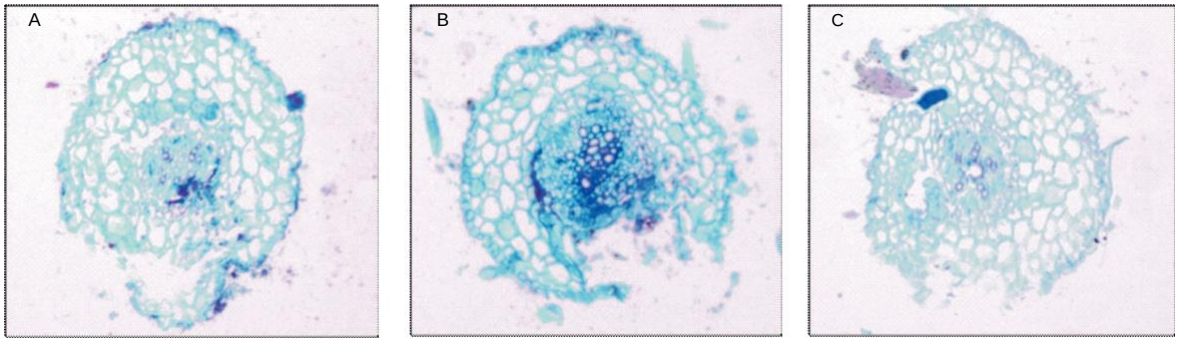

Fig. 1. Microtome section of non Bt cotton roots inoculated with *R. reniformis*

A-Bunny non Bt cotton root showing head region of *R. reniformis* embedded deep in the phloem region. Scattered xylem vessels and heavily damaged phloem vessels. **B** –Rasi non Bt cotton root section showing hypertrophied cells with granular protoplasm, breakdown of cortical, epidermal and pericycle cells and large cavity indicating damage due to nematode. **C** – jagannath non Bt cotton root section showing portion of *R. reniformis* head region deep in the cortex region. Cavities in the cortex and damage to endodermis and pericycle region.

Cortical cells, xylem and phloem were compactly arranged with thick cell wall compared to non Bt (fig 2).

The reniform nematode, is reported to be typical feeder of cortex and phloem cells on cotton (Birchfield, 1962). The histopathology of feeding has been studied in detail by Rebois *et al*. (1970) in soybean wherein the authors reported that modified cell formation was associated with feeding in pericycle tissues adjacent to protoxylem pole. The phloem feeding habit of *R. reniformis* resulted in hypertrophy, hyperplasia, thickening of cell wall,

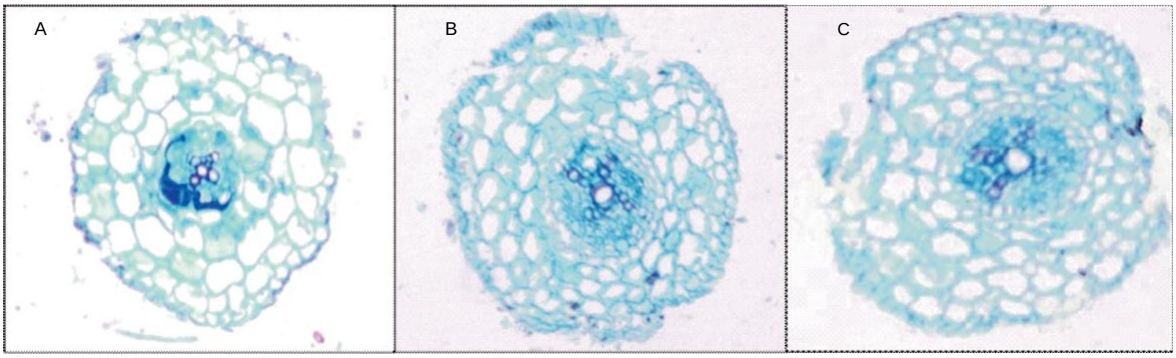

Fig. 2. Microtome section of Bt cotton root inoculated with *R. reniformis*

A – Bunny Bt cotton root section showing compact xylem and compact, thick walled cortical cells. Less number of xylem and phloem vessels. **B** – Rasi Bt cotton root section showing less number of xylem vessels. Phloem vessels are thick walled and compact with much reduced phloem area. **C –** Jagannath Fusion Bt cotton root section showing compactness in the arrangement of epidermal and cortical cells

granulation of protoplasm as reported by Wallace (1963), Endo (1971) and Sivakumar and Seshadri (1972). The present investigation confirms the above findings as nematode embeds its head deep in to the cortex damaging the cortical cells, pericycle and phloem cells. Large cavities were seen in the cortex with hypertrophied and hyperplasial cells at the feeding site.

The cross sections of Bt cotton roots clearly showed compactness in the arrangement of epidermal and cortical cells which were thick walled. Xylem vessels were few in number and the area occupied by the phloem cells also less compared to non Bt. This might be the possible reason for less preference of Bt cotton by *R. reniformis* compared to non Bt with regard to root cellular structure. So far no work on the influence of *R. reniformis* to Bt and non Bt cotton has been carried out. Hence detailed study on the ultrastructural changes induced by *R. reniformis* in Bt and non Bt cotton roots needs to be intensified to relate the nematode infestation with plant anatomical characters of Bt/ non Bt cottons.

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