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Leaf sheath histochemistry and stylet path in relation to brown planthopper resistance in rice

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ABSTRACT

Among the four rice varieties examined, the leaf sheath of the resistant variety, Ptb33 and the moderately resistant variety, ASD 16 had thin septa of cells between air chambers when compared to susceptible varieties namely TN 1 and Basmati 370. The cross section of the leaf sheaths of the susceptible plants viz., Basmati 370 and TN 1 revealed that they had wider air chambers and vascular bundles with lower lignification when compared to moderately resistant and resistant varieties, viz., ASD 16 and Ptb 33 respectively. Sclerenchymatous cap and extra parenchymatous sheath were found over the vascular bundles in Ptb 33, which was not found in other three varieties. The BPH stylet sheath probing pattern on rice varieties by microtome sectioning revealed significant variation in the stylet path. The stylet path was branched in Ptb 33, whereas it was unbranched, wavy, thinner and single tube-like in susceptible rice varieties like TN 1 and Basmati 370.

Key words: *rice, leaf sheath, brown planthopper, feeding, stylet path, histology*

The brown planthopper (BPH) *Nilaparvata lugens* (Stal.) is one of the major insect pests of rice frequently causing widespread destruction of the crop and heavy yield loss (Shi e*t al.*, 2003; Park *et al.*, 2007). BPH, the major sucking pest, upon arrival, explores the surface of a potential host plant by repeatedly tapping the tip of the labium against the plant surface to find the suitable site of feeding. Various physical factors in plants such as solidness of stems and thickening of tissues affect the utilization of a plant as a host by phytophagous insects (Panda and Khush, 1995). Labial tapping allows BPH to differentiate the smooth epidermis over the vascular bundles from the less suitable inter-veinal epidermis, which is rough and covered by waxy scales. After locating a suitable probing site, the labium is firmly oppressed to the plant surface and stylets are inserted to form a stylet sheath (Sogawa, 1973; Backus, 1985). These stylet sheaths of BPH may be branched or unbranched. The production of stylet sheaths has often been used as a quantitative measure of homopteran feeding (Bowling 1979, Marion-Poll *et al*., 1987, Bing *et al*., 1991). The research reported herein investigated the possible presence of impediments to BPH stylet penetration and access to phloem in selected rice varieties.

MATERIALS AND METHODS

The basal stem portion (1.5 cm above the collar region) from the 45-day-old healthy potted plants of four rice varieties *viz*., BAS 370, TN 1, ASD 16 and Ptb 33 were used for the study representing susceptible, highly susceptible, moderately resistant and highly resistant BPH variety, respectively (Elaiyabharathi, 2005). Five plants were maintained for each variety where each plant represented each replication. For the stylet pathway study, one cm length of the basal region of the 45-day-old rice plants was confined using a sachet as described by Elaiyabharathi (2005) and two newly emerged female BPH insects were released for each plant and allowed for 24 h feeding. After 24 h, the BPH exposed region was cut and used for studying the insect stylet pathway.

The cut stem portions were fixed in FAA solution (5ml. of 40% formaline + 5ml. of glacial acetic acid+90ml. of 70% ethanol) initially. After 24 h of

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fixing, the specimens were dehydrated with graded series of tertiary butyl alcohol (TBA) (Sass, 1940). Infiltration of the specimens was carried out by gradual addition of paraffin wax (melting point 58-60 °C) until TBA solution attained super-saturation. Then, the specimens were cast into paraffin wax for microtomy sectioning. The paraffin embedded specimens were sectioned with the help of a rotary microtome. The thickness of the sections made ranged between 10 and12 µm. Dewaxing was done by following customary procedure (Johansen, 1940). The sections were stained with toluidine blue (O'Brien *et al*., 1964) and photomicrographs were taken using Nikon Labphot 2 microscopic unit.

RESULTS AND DISCUSSION

Being a polychromatic stain, staining of microtome sections with Toluidene dye resulted in clear appearance of tissues and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells and violet to the mucilage.

In the very susceptible variety, Basmati 370, the leaf sheath recorded a horizontal row of wide circular air chambers separated by three or four cell thick septa (100-120 µm thickness). The vascular bundles were relatively small and collateral in nature. Metaxylem elements were 30- 40 µm thick. The leaf sheath of the susceptible variety, TN 1 was noticed with wide air chambers separated by two-cell thick septa of 80 – 100 µm thickness. Vascular bundles were large with circular metaxylem elements, whose walls were less lignified and about 40 µm wide.

In the moderately resistant variety ASD16, the leaf sheath had turgid cell layers with straight and well preserved one or two cells thick septa (70-80 µm thickness). The epidermal cells had echinate cuticular layers. Vascular bundles were large, metaxylem walls were thick, lignified and were 40-50 µm wide. Moreover in the resistant variety Ptb33, the leaf sheath had larger, rectangular air chambers separated by thin septa (50 µm thickness) of one or two cells wide. Vascular bundles were large and had sclerenchymatous cap and parenchymatous sheath. Metaxylem elements were elliptical with thick lignified walls and were 20-30 µm wide (Table 1 and Fig. 1).

Ptb 33 had sclerenchymatous cap and extra parenchymatous sheath over its vascular bundles which were not apparent in other three varieties examined. These observations indicated that these extra sclerenchymatous and parenchymatous bands in the stem of resistant genotypes could offer mechanical resistance to the insects for its stylet penetration (Wallace *et al*., 1974; Peraiah *et al*., 1979; Alagar, 2005). Similarly, the cross section of the leaf sheaths of the susceptible plants *viz*., Basmati 370 and TN 1 revealed that they had wider air chambers and larger vascular bundles with lower lignification when compared to moderately resistant and resistant varieties, *viz*., ASD 16 and Ptb 33, respectively. Tissue hardness and stems with small cross sectional area have been associated with a restriction in the penetration of stylet and feeding. The highly lignified tissues of *Medicago* clones were associated with resistance to the potato leafhopper *Empoasca fabae* (Harris) (Brewer *et al*., 1976) which acted as a physical barrier offering resistance to sucking insects.

Table 1. Comparison of anatomical features and BPH stylet path among selected rice cultivars.

Variety	Septal thickness (µm)	Vascular bundles	Metaxylem elements	Stylet path	
				Nature	Thickness (µm)
Basmati370	100-120	Medium in size	Less lignified, ~30- 40 µm wide	Wavy, tube-like	~5
TN1	80-100	Small in size, collateral	Less lignified, ~ 40 µm wide	Wavy with dark inclusions	~5
ASD16	70-80	Larger in size	Lignified, ~40-50 µm wide	Undulated, dark, 2-3 paths found adjacent	~10
Ptb33	50-60	Large with sclerenchym-atous cap	More lignified, 20-30 µm wide	dark, dense, with more branches	~10

Note: (~ About)

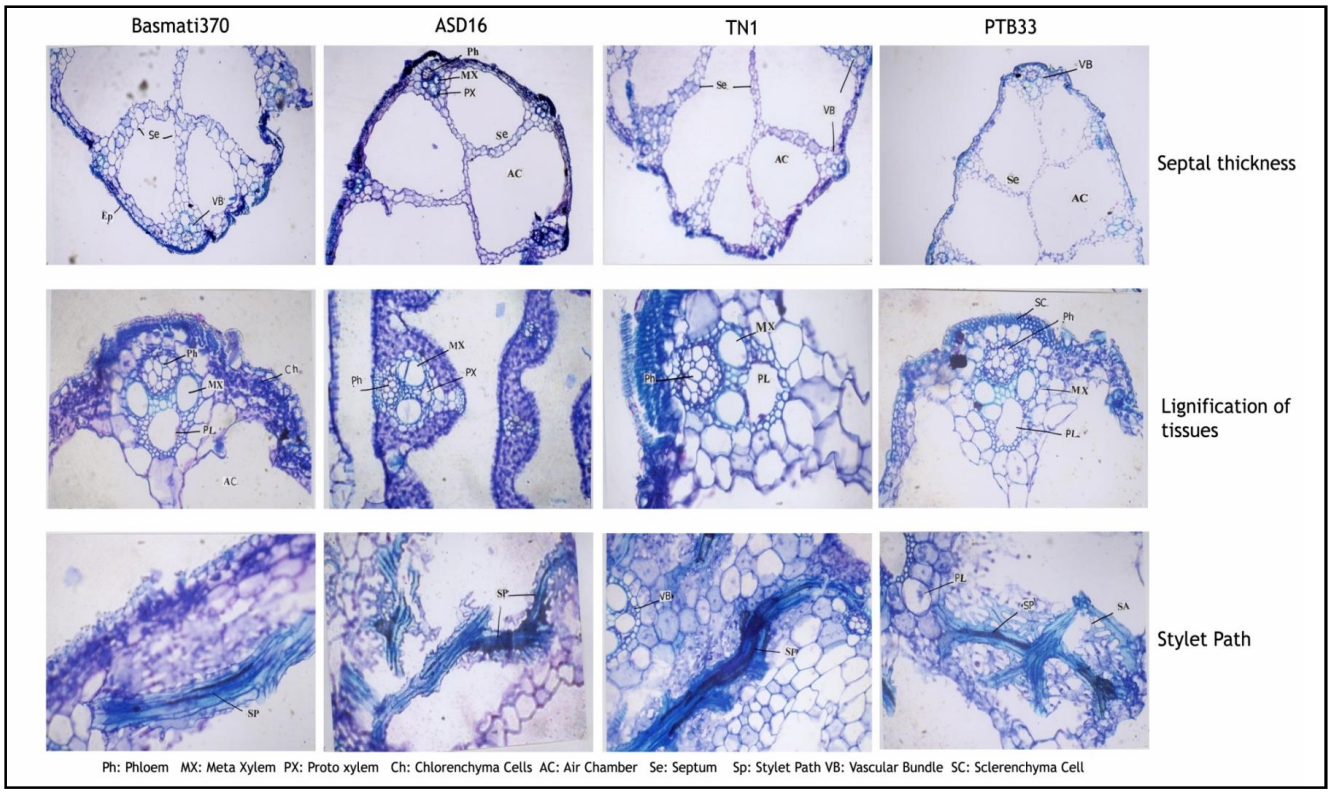

Fig. 1. Leaf sheath anatomy and BPH stylet path in selected rice cultivars.

The investigations on the stylet path revealed that in Basmati370, the stylet path traversed the air chambers through the septa and reached the vascular strand and was wavy with dark inclusions with about 5 µm thickness. In TN 1, the stylet path was wavy, tube-like, and thick without branches, traversed the air chambers through the septa, and reached the vascular strand. The path was 5 µm in thickness. However, in ASD 16, the stylet path was undulate and dark, and found in the vicinity of the vascular bundle. Two to three paths were seen adjacent to each other that were about 10 µm in thickness (Fig. 1).The stylet path was branched in the resistant variety, Ptb 33 and mostly aimed at the vascular bundles. The path was dark, dense, and about 10 µm in thickness. The stylet seems to have been inserted many times in search of the vascular tissues and hence noticed with branches (Fig. 1). It is probable that while searching for the vascular bundles; the stylets have been inserted repeatedly causing the apparent branched view of the stylet path (Sogawa, 1982). On the other hand, in TN I, Basmati 370 and ASD 16, the stylet path was wavy with no branches directly reaching the vascular strand, indicating their susceptibility. Feeding behaviour of BPH varies between resistant and susceptible accessions (Rangasamy, 2009) and stylet path vary accordingly. The nymphs selectively feed from phloem tissues when the host is susceptible and xylem feeding is found on resistant accessions (Khan and Saxena, 1984; Elaiyabharathi, 2005). According to Bing *et al*. (1991), higher rate of stomatal entry of stylets was due to easier penetration. Mauseth (1988) reported that the sclerenchyma cells have thickened secondary cell walls having certain amounts of cellulose, hemicelluloses, lignin etc., and the presence of lignin provides a stable protection to the vascular bundle. It can also be concluded that rather than total lignin, deposition of lignin in a few sclerenchyma cells with thick cell walls is important as a barrier to BPH. Wang *et al*. (2008) reported that the toughness of leaf sheath tissue was not an effective defense in the resistant B 5 rice because BPH probed into the thick segment of the outer leaf sheath.

Thus, it is evident that, the susceptible varieties have wide air chambers and thick septa between them compared to resistant varieties. Vascular bundles are without much lignification in susceptible varieties thus

permitting easy penetration of stylets. Stylet penetration into the leaf sheaths occurred more frequently in susceptible lines than in resistant lines, suggesting an impediment to BPH stylet penetration in resistant varieties. Sclerenchyma cap and extra parenchymatous cells around the vascular bundles and lignification may be one among the several features of the BPH resistance offering mechanical resistance to insects for stylet penetration.

REFERENCE

Alagar M 2005. Mechanisms of resistance in rice, *Oryza sativa* (L.) to brown planthopper, *Nilaparvata lugens* (Stal.). Ph.D. Thesis. (unpublished). Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore. 162p.

Backus E 1985. Anatomical and sensory mechanism of leafhoppers and planthoppers. In: *The leafhoppers and planthoppers*. (Nault, L.R. and J.G. Rodriguez, eds). pp. 163-194.

Bing JW, Novak MG, Obrycki JJ and Guthrie WD 1991. Stylet penetration and feeding sites of *Rhopalosiphum maidis* (Homoptera : Aphididae) on two growth stages of maize. Ann. Entomol. Soc. Am 84: 549-554.

Bowling CC 1979. The stylet sheath as an indicator of feeding activity of the rice stink bug. J. Econ. Entomol 72: 259-260.

Brewer GJ, Sorensen EL, Hober K and Kreitner GL 1976. Alfalfa stem anatomy and potato leafhopper (Homoptera: Cicadellidae) resistance. J. Econ. Entomol 79: 1249-1253.

Elaiyabharathi T 2005. Comparative analysis on techniques to screen for the quantitative resistance to brown planthopper, *Nilaparvata lugens* (Stal.) (Homoptera: Delphacidae) in rice (*Oryza sativa* L.). Ph.D. Thesis (Unpublished), Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore-3.

Johansen DA 1940. Plant Micro technique. Mcgraw-Hill Book Co Publications, New York, 523p.

Khan ZR and Saxena RC 1984. Techniques for demonstrating phloem or xylem feeding by leafhoppers (Homoptera: Cicadellidae) and planthoppers (Homoptera: Delphacidae) in rice plants. *J. Econ. Entomol* 77: 550-552.

Marion-Poll F, Della Giustina W and Mauchamp B 1987. Changes of electric patterns related to feeding in a mesophyll-feeding leafhopper. Entomol. Exp. Appl 39: 115-124.

Mauseth JD 1988. Plant anatomy. The Benjamin/Cummings Publish. Co, Menlo Park, California.

O'Brien TP, Feder N and Mc Cull ME 1964. Polychromatic staining of plant cell walls by toluidine blue-O. Protoplasma 59: 364-373.

Panda N and Khush GS 1995. Host plant resistance to insects. CAB International and IRRI, Manilla, Philippines, 431p.

Park DS, Lee SK, Lee JH, Song MY, Song SY, Kwak DY, Yeo US, Jeon NS, Park SK and Yi G 2007. The identification of candidate rice genes that confer resistance to the brown planthopper (*Nilaparvata lugens*) through representational difference analysis. Theor. Appl. Genet 115: 537–547.

Peraiah A, Sethi M and Roy JK 1979. Anatomical characters of rice stems in relation to brown planthopper resistance. Oryza 16: 73-74.

Rangasamy M 2009. Role of leaf sheath lignification and anatomy in resistance against southern chinch bug, *Blissus insularis* barber (Hemiptera: Blissidae) in St. Augustine grass lines. J. Econ. Entomol 102(1):432-439.

Sass JE 1940. *Elements of botanical microtechnique*. McGraw Hill Book Co,New York. 222p.

Shi Z, Ren X, Weng Q, Li X and He G 2003. Construction of a genomic library from a brown planthopper resistant line using a transformation-competent vector and identification of clones spanning the Qbph1 locus. Plant Sci 165 (4): 879-885.

Sogawa K 1973. *Feeding behaviour of the brown planthopper and brown planthopper resistance of indica rice Mudgo*. Bull No.4, lab Appl. Entomol., Faculty of Agric., Nagoya University, Chikusa, Nagoya, Japan, 151p.

Sogawa K 1982. The rice brown planthopper feeding physiology and host plant interactions. Annu. Rev. Entomol 27: 49-73.

Wallace LE, McNeal FH and Bery MA 1974. Resistance to both *Oulema melanopus* and *Cephus cinctus* in pubescent waved and solid stemmed wheat selections. J. Econ. Entomol 67: 105-107.

Wang Y, Ming Tang, Peiying Hao, Zhifan Yang, Lili Zhu and Guangcun He 2008. Penetration into rice tissues by brown planthopper and fine structure of the salivary sheaths. Entomol. Exp. Appl, 129 (3) : 295-307