

sourced the *cryIIAc* gene construct for its *Bt* brinjal from Monsanto. Mahyco has done the entire transformation, i.e. fitting the gene construct in the right place of the brinjal genome. Mahyco has submitted a patent application for this unique event, EE1 with the patent authority. This event EE1 was integrated into eight of the company's own brinjal hybrids like MHB 4, 9, 10, 80, 99, 11, 39 and 111. In addition, it has supplied the event EE1 to University of Agriculture Sciences (UAS), Dharwad for backcrossing with their several popular varieties, including Udupi gulla. According to another report, UAS, Dharwad gave seeds of its promising brinjal varieties, including Udupi gulla to Mahyco, which has backcrossed them into a transgenic product and presented the backcrossed seeds under a USAID-funded project devised by Agriculture Biotechnology Support Programme II (ABSP II) team members at Cornell University, USA. UAS, Dharwad will conduct field trials and distribute the disease-resistant seeds to farmers on cost basis. Farmers can use these varieties over succeeding generations, unlike Mahyco's hybrids that have to be bought for

every fresh sowing. Although this act apparently appears to be generous, there is a catch as is evident from the statement of the spokesperson of Mahyco that 'the question of royalty arises only if universities undertake commercial sales'. Thus what costs the farmers would have to pay for different varieties of *Bt* brinjal is yet unknown. Also there is the danger of indigenous Mattu gulla being contaminated with the *Bt* gene, once the commercial cultivation of Udupi gulla with backcrossed *Bt* gene begins in Mattu village.

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## Leaf anatomical basis of woolly aphid resistance in sugarcane

Among various abiotic and biotic stresses, sugarcane woolly aphid, *Ceratovacuna lanigera* Zehenter has recently become a serious biotic constraint, threatening sugarcane cultivation, particularly in peninsular India and causing significant loss in cane yield and sugar recovery. Host plant resistance is one of the important components of integrated pest management, which is environmentally safe and ecologically stable. Leaf anatomical characters and biochemical contents of leaf are known to play a role in attributing resistance against insects.

The present study was envisaged to find the possible mechanism and basis of resistance for the woolly aphid involving two resistant clones, SNK 192 and SNK 754 and two susceptible clones, CoC 671 and Co 92920, through histological studies. Microscopic measurements for leaf anatomical parameters were recorded as mean value based on ten leaf samples collected from each clone. Further, each

leaf sample was observed in five microscopic fields.

In the present study leaf thickness, measured as the distance between the lower and upper leaf epidermis, did not differ significantly between resistant and susceptible groups of clones (Table 1). Similarly, group mean distance between lower epidermis and phloem in resistant clones was more compared to susceptible clones, even though the group mean did not differ significantly. Hence it is likely that the presence of more number of cells and greater distance between the stomata of lower epidermis and phloem, make it difficult for the aphid to reach the phloem for gathering food in resistant clones. Similar observation was also reported in resistant cotton genotypes for sucking pest<sup>1</sup>.

The distance between large and small, large and medium and medium and small vascular bundles varied significantly between the resistant (Figures 1 a and 2 a)

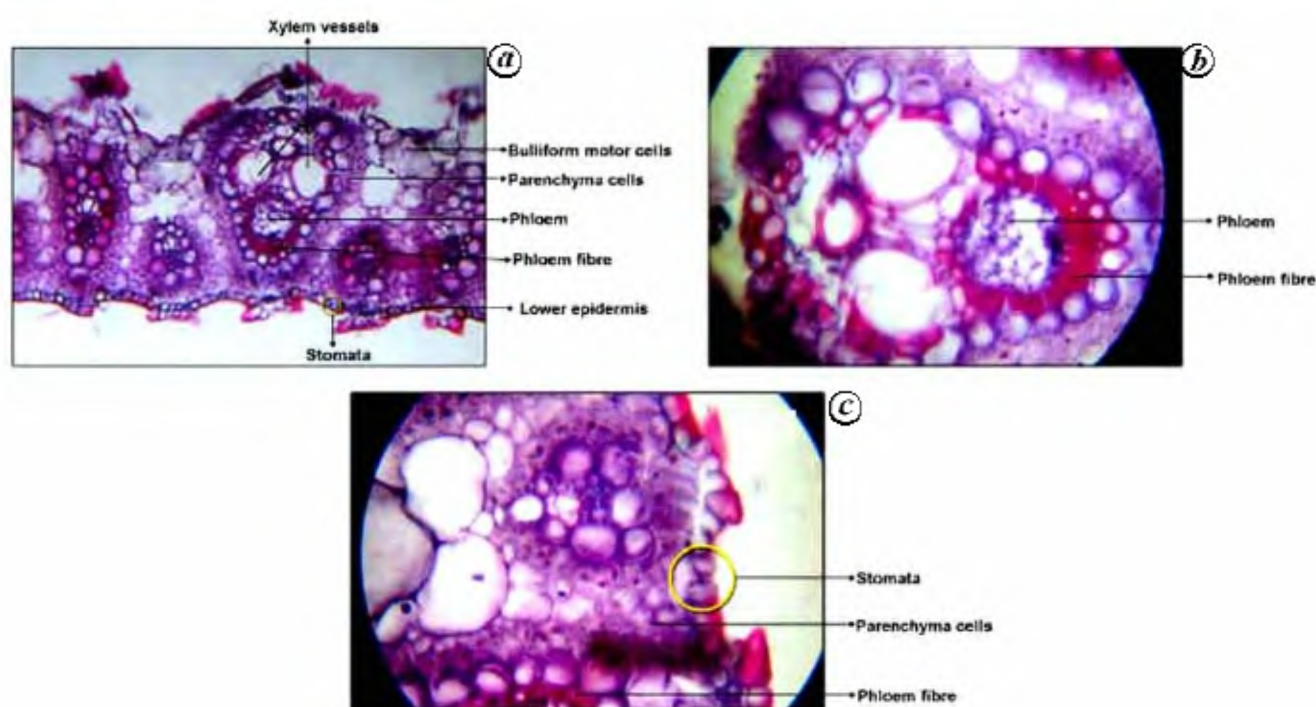
and susceptible clones (Figures 3 a and 4 a). This distance indicates the free space between vascular bundles. The mean distance was more in susceptible clones indicating the presence of more free space compared to resistant clones. The space between vascular bundles adds either toughness or succulence to the internal leaf anatomy of clones. Further, phloem width was significantly more in resistant clones compared to susceptible clones, indicating availability of greater space for the aphid for sucking.

Parenchyma cells are important cells of the leaf, wherein vital metabolic activities take place. Significant difference was observed in the width of parenchyma cells around the bundle sheath between resistant and susceptible clones. For successful tapping of food, the aphid has to pierce its stylet either intracellularly or intercellularly to reach the phloem. It has been reported<sup>2</sup> that most of aphid species penetrate through their stylet intercellularly

**Table 1.** Mean microscopic measurements (10×) on leaf anatomical features of sugarcane woolly aphid resistant and susceptible clones

Character	Range (mm)	Group mean (mm)		Calculated 't' value
		Resistant	Susceptible	
Thickness of leaf	0.651–0.871	0.757	0.720	0.946
Distance between lower epidermis and phloem (sucking distance)	0.110–0.117	0.116	0.114	1.349
Distance between large and small vascular bundles	0.045–0.078	0.055	0.059	2.244**
Distance between large and medium vascular bundles	0.157–0.239	0.187	0.230	5.859**
Distance between medium and small vascular bundles	0.096–0.184	0.118	0.124	14.944**
Width of large vascular bundle	0.241–0.296	0.281	0.272	1.319
Width of phloem	0.081–0.119	0.118	0.084	28.485**
Width of parenchyma cells	0.010–0.034	0.025	0.011	8.690**
Thickness of phloem fibre	0.017–0.024	0.021	0.017	18.311**

\*\*Significant at 1% level of probability.



**Figure 1.** *a*, Transverse section of leaf blade of resistant clone SNK 192 with large vascular bundle flanked by two small and medium ones (10×). *b*, Thick fibre around phloem (45×). *c*, Distance between stomata and phloem separated by more number of parenchyma cells (sucking distance; 45×).

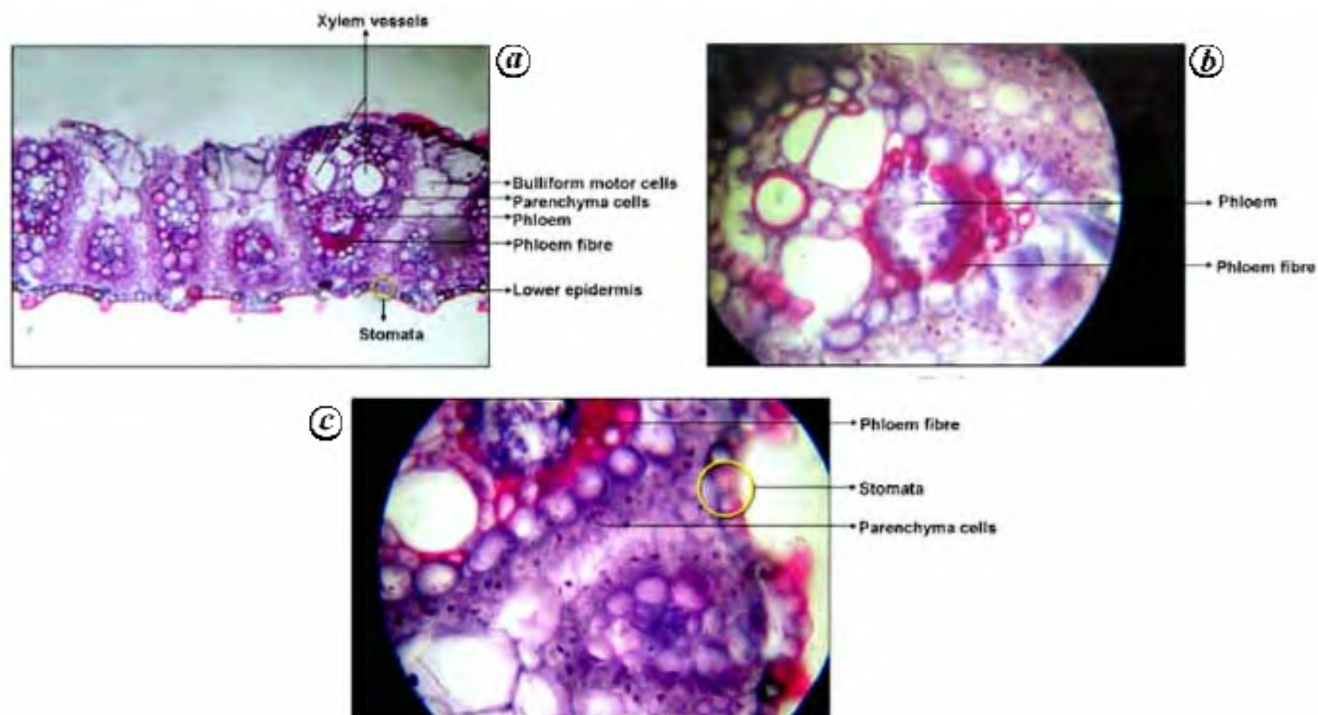
and secrete hydrolytic enzymes particularly pectinase which dissolves middle lamella of cell wall. Thus the presence of a thick layer of parenchyma cells may act as a barrier for aphid stylet penetration and during this process the aphid may well damage more number of cells in resistant clones leading to the triggering of host defence mechanism by producing toxic substances.

Phloem fibre is another important component of the vascular bundle, which provides mechanical strength against col-

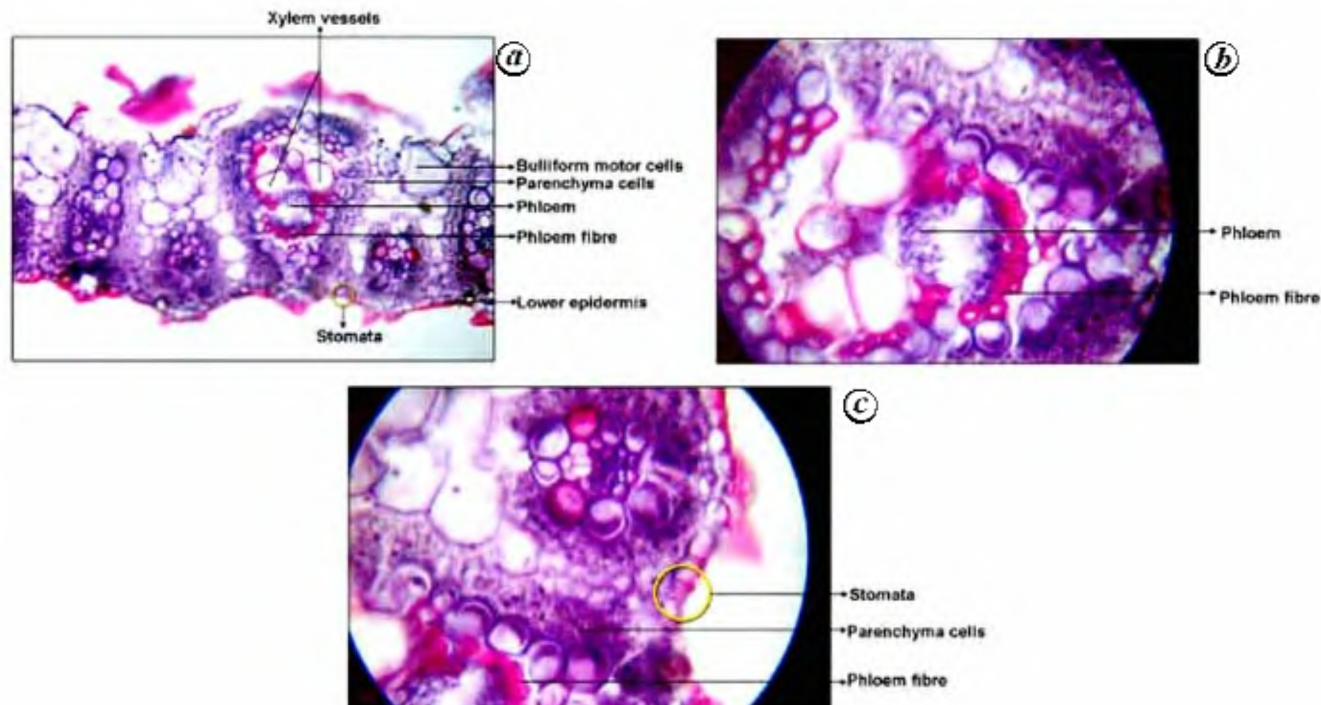
lapse and protects against invaders. In the present study resistant clones had significantly thicker phloem fibre (Figures 1 *b* and 2 *b*) compared to susceptible clones (Figures 3 *b* and 4 *b*). This may act as mechanical barrier for stylet penetration to suck the sap from phloem in resistant clones. Similar observations were also made by earlier workers<sup>3,4</sup>, wherein they noticed thickened phloem fibre around phloem tissue and vascular bundles in resistant clones of sugarcane against pyrilla pest.

Based on absorbance values of hydroxamic acid (Hx) content on these resistant clones<sup>5</sup> and field observations<sup>6</sup>, the resistance mechanism operating may be antibiosis or antixenosis (feeding deterrence or toxic effect) and basis of resistance may be biochemical, as Hx content was higher in resistant clones compared to susceptible clones, as indicated from mean per cent absorbance values, based on preliminary study.

In a field study<sup>6</sup>, released apterous nymphs could not colonize on resistant

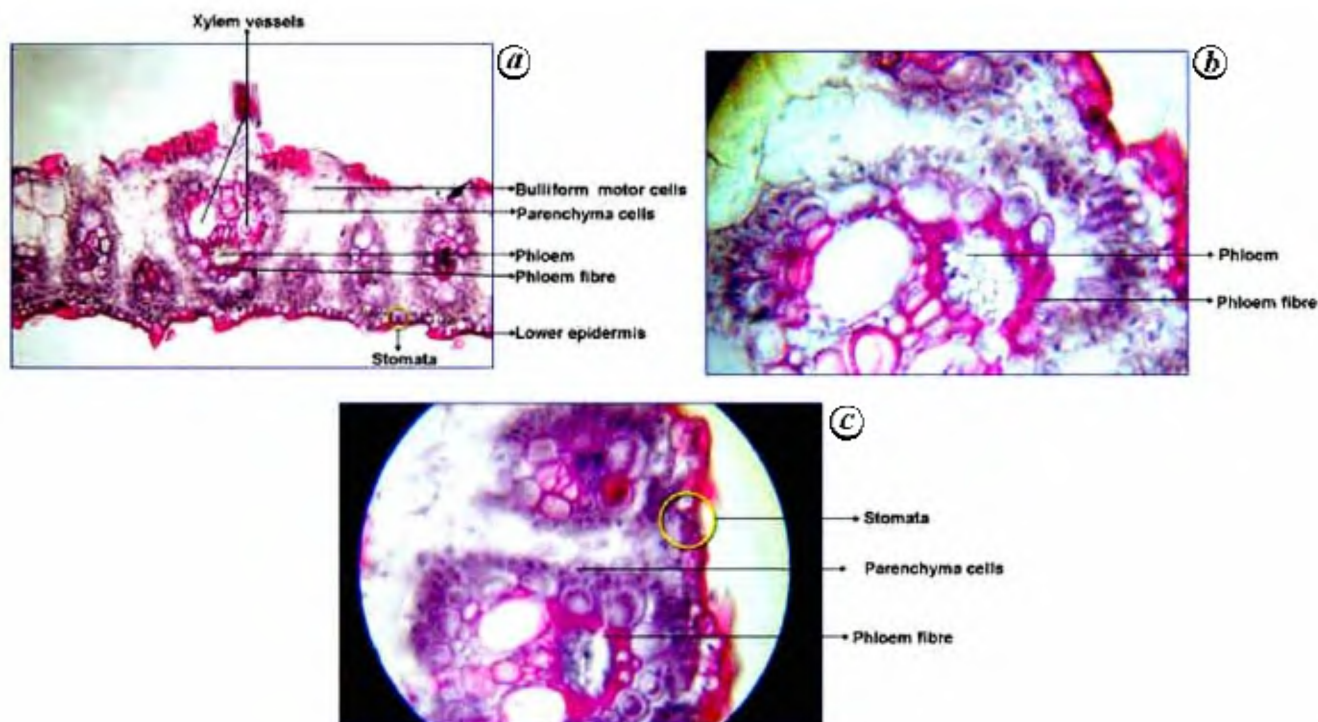


**Figure 2.** *a*, Transverse section of leaf blade of resistant clone SNK 754 with large vascular bundle flanked by two small and medium ones (10×). *b*, Thick fibre around phloem (45×). *c*, Distance between stomata and phloem separated by more number of parenchyma cells (sucking distance; 45×).



**Figure 3.** *a*, Transverse section of leaf blade of susceptible clone Co 92020 with large vascular bundle flanked by two small and medium ones (10×). *b*, Thin fibre around phloem (45×). *c*, Distance between stomata and phloem separated by less number of parenchyma cells (sucking distance; 45×).





**Figure 4.** *a*, Transverse section of leaf blade of susceptible clone CoC 671 with large vascular bundle flanked by two small and medium ones (10×). *b*, Thin fibre around phloem (45×). *c*, Distance between stomata and phloem separated by less number of parenchyma cells (sucking distance; 45×).

clones leading to complete mortality after 48–72 h of release, compared to susceptible clones which colonized immediately after 24 h of release. Further, aphids released on resistant clones did not secrete honeydew, indicating lowered or no ingestion of sap from phloem. The death of aphids might be due to toxic effect of sap of resistant clones or feeding deterrence leading to starvation death.

Similar results were reported by earlier workers<sup>7–9</sup>, wherein they observed higher amount of Hx in aphid-resistant wheat cultivars and the resistance was attributed to feeding deterrence due to toxic effects of phloem sap. However, feeding deterrence was present at the mesophyll tissue level<sup>9</sup> but not in the phloem. The higher amount of Hx in resistant clones could be due to inherent capacity to produce higher Hx or triggering of host defence system by more number of parenchyma cells penetrated by aphids in resistant clones.

Interestingly, histological observations in the present study also support the

views explained above. It is most likely that in resistant clones,  $\beta$ -glucosidase enzyme activity in mesophyll may be triggered which cleaves glucoside to aglucons which is a form of Hx acid acting as toxic compound or feeding deterrent to aphid probing<sup>8,10,11</sup>.

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