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LOCATION OF VASCULAR BUNDLE IN CROSS- SECTION STEM OF DIFFERENT RICE DIFFERENTIALS

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KEYWORDS

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ABSTRACT

The present investigation entitled "Location of vascular bundle in cross- section stem of different rice differentials"was carried out in the laboratory, Department of Entomology, College of Agriculture, IGKV, Raipur (C.G.) during 2013-2014.Anatomical investigations on PHSS lines were carried out to study the distance between the epidermis to phloem located in vascular system of rice stem. This observation have been taken from the internodal and nodal position both , to study the variation in cross section of stems of both positions in same variety. And also to study the variations among different varieties. The distance from vascular bundle to epidermis, varied from 57.65 μm to 100.38 μm in different gene differential. Maximum distance was observed in Ptb33 (100.38 μm). The same measurement was taken for the nodal section also and the distance from vascular bundle to epidermis, varied from 52.62 μm to 94.38 μm. Maximum distance was observed in genotype Ptb 33 (94.38 μm).Minimum distance was observed inTN1.

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INTRODUCTION

Taxonomically, rice is classified in the family *Poaceae*and subfamily *Oryzoideae*. There are 21 wild species and two cultivated species of *Oryza*. *Oryza sativa* has different ecotypes *viz.*, *indica*, *japonica* and *javanica* which represent specialized gene pools that make it possible to cultivate rice under diverse conditions. In crop improvement programme, to increase the productivity breeder needs to maintain a pool of diverse desirable donor parents (Joshi *et al.*, 2013). There are many constraints in rice production, insects, pests remain a constant problem in all the rice growing regions (Manikandan Narayanasamy *et al.*, 2014).

BPH is a phloem sap-feeding insect that mostly feeds on the leaf sheath of rice plants and ingests nutrients using its piercing mouthparts or stylet. The feeding process of BPH can be divided into two phases including (i) probing, which is performed in parenchymal tissues, and (ii) sucking, which is done after stylets insertion into vascular bundles (Sogawa, 1982). During feeding process, BPH salivary stylets penetrate rice plant tissues and form stylet or salivary sheaths to feed on photo assimilates translocation in the phloem sieve elements. In general, phloem sap-feeding insects secrete a watery saliva that is continuously secreted during feeding may interact with phloem proteins to prevent their coagulation (Tjallingii, 2006; Will and van Bel, 2006) or may also contain effectors that modulate plant defense responses (Hao *et al.*, 2008; Miles, 1999). The stylet bundle and salivary sheath are frequently found entirely within the wall of a plant cell and it can be seen that saliva may penetrate the cell wall into an adjacent cell. Therefore, BPH feeding behavior is very damaging to the host plant, even in those penetrations which do not result in prolonged uptake of phloem sap (Spiller, 1990).There may be chance of escape due to unreachability of neonates to proper feeding site in deeply situated vascular bundleof Ptb 33 but it is available superficially in TN 1.

Nitta *et al.* (2000) investigated the varietal differences in the number andcross area of large vascular bundles (LVs) at the neck internode in 18 rice cultivars. Total or phloem areas of L1 were larger in TI and SDI than in JP and JN. Sarwar and Prodhan, (2000) carried out anatomical investigations of 8 rice cultivars (*Oryza sativa* L.). Four from each of traditional (local) and modern (high yielding) cultivars were carried out to study the variation among cultivars. The number, position and arrangement of vascular bundles vary along the cultivars.

The removal of assimilates and reduction in photosynthetic rate of leaves by BPH feeding has the greatest effect on growth and yield on rice plant. Plant death can occur if the amount of energy supplied is less than that required for tissue maintenance (Watanabe and Kitagawa, 2000; Yuan *et al.*, 2005). In addition to feed on rice plant directly, BPH also causes indirect damage by transmitting viruses, which cause ragged and grassy stunt diseases (Heinrichs, 1979). One strategy for minimizing losses due to BPH is the utilization of BPH resistance genes. Consequently, breeding BPH resistant cultivar is an objective to stabilize the yield production of rice.

Free-hand sectioning of living plant tissues often provides an adequate method for rapid and inexpensive microscopicobservation of their internal structure. For anatomical investigation hand sectioning method was followed. Anatomical investigations on PHSS lines were carried out to study the distance between the epidermis to phloem located in vascular system of rice stem. This observation have been taken from the internodal and nodal position both, to studythe variation in cross section of stems of both positions in same variety. And also to study the variations among different varieties. The aim of this study was to further explore the interactions between the BPH insects feeding and rice plants in an attempt to elucidate the mechanisms involvedin rice resistance to the BPH.

MATERIALS AND METHODS

The experiment wascarried out in the laboratory, Department of Entomology, College of Agriculture, Indira Gandhi KrishiVishwavidyalaya, Raipur (C.G.) during 2013- 2014. PHSS (Planthopper Special Screening) entries provided by DRR under AICRIP programme. Twenty rice cultivars were used for this anatomical investigation (Table 1). The plant samples were collected at tillering stage (30-45 days after transplanting) from the 3rd node and internode (from the top) and fixed separately in Craf III and FFA(Johansen, 1940; Sass,1958). For anatomical investigation hand sectioning method was followed. All sections were cut using a new double-sided razor blade. First, a leveling cut was made for the purpose of forming a right angle with the axis of the stem to ensure that the sections made were cross sections. After each cutting, the cut section that collected on the blade were transferred to a drop of water on a slide and covered with a cover slip by lowering it an angle onto the drop of water containing the sections. The sections were mounted in 50% glycerine and were examined under trinoculor microscope, followed by these steps :- a) Turned the revolving nosepiece to engage 10X objective. b) placed the specimen on stage. c) turned the X-knob and Y-axis knob to move the specimen in light path. d) Adjusted the brightness with the light intensity knob. e) turned the coarse and fine adjustment knobs to bring the specimen into focus. f) Adjusted the interpupillary distance and dioptre. g) center the field iris diaphragm and adjusted the aperture iris diaphragm and field iris diaphragm. i) after readjusting focus, field and aperture iris diaphragm, observation is started.

Anatomical investigations on PHSS lines were carried out to study the distance between the epidermis to phloem located in vascular system of rice stem. This observation have been taken from the internodal and nodal position both , to study thevariation in cross section of stems of both positions in same variety and also to study the variations among different varieties.

The aim of this study was to further explore the interactions between the BPH insects feeding and rice plants in an attempt to elucidate the mechanisms involved in rice resistance to the BPH. The mouthparts of BPH, like other phloem feeding insects, consist of a stylet bundle which forms the piercing and sucking organ (Sogawa,1982; Seo *et al.*, 2009). BPH feeds on the plant by inserting the stylet bundle with an accompanying salivary sheath into the plant locating the phloem tissue and then regulating the ingestion of the pressurised plant sap (Sogawa,1982; Seo *et al.*, 2009).

The vascular tissue system itself is comprised of several different tissues with complex functions.

There is a description of it

Phloem tissue andthexylem tissue in the vascular tissue system.

Phloem Tissue

The phloem tissue is comprised of phloicfibers, sieve tube members and companion cells. The companion cells are extrodinarily distinct in the phloic tissue. The phloicfibers are found primarily above the phloem, although the outer edge of the rice stem is known to sometimes have a continuous ring of fiber cells just below the epidermis consisting of phloicfibersas well as "other" fibrous schlerenchyma tissue.

Table 1: List of differential varieties carrying different BPH resistant gene

PHSSEntries	R Gene	List of differential varieties Designation	Cross
1	bph2	ASD 7 (ACC 6303)	Karsamba Red
2	Bph3+Bph17	RathuHeenati (ACC 11730)	RathuHeenati
3	bph4	Babawee (ACC 8978)	-
4	?	Milyang 63	Tongil/IR 946-33-2-2-2//YR675-131-2
5		TN1	S.Check
6	Bph6	Swarnalatha (ACC 33964)	-
7	bph7	T 12 (ACC 56989)	-
8	bph8	Chinsaba (ACC 33016)	-
9	bph9	Pokkali	-
10		TN1	S.Check
11	Bph1+	IR 64	IR 5657-33-2-1/IR 2061-465-1-5-5
12	Bph18	IR 65482-7-216-1-2-B	IR 31917-45-3-2-2*3/*O.australiensis*
13	bph2	IR 36	IR1561-228//4*IR661-1-140-3-117/*O.nivara*///CR 94-13
14	?	MUT NS 1	-
15		TN1	S.Check
16	?	OM 4498	IR 64/OMCS 2000//IR 64
17	?	RP 2068-18-3-5	
18	bph2+Bph3+	Ptb33	
19	BPH20/21	IR71033-121-15	
20		TN1	S.Check

Table 2: Location of vascular bundle in cross section stems of twenty different rice differential varieties (Internodal Position)

PHSS entries	Designation	VB – E(μm)	P – E(μm)
1	ASD 7 (ACC 6303)	76.51	121.33
2	RathuHeenati (ACC11730)	93.96	111.56
3	Babawee(ACC 8978)	92	110.52
4	Milyang 63	76.39	112.85
5	TN1	74.06	114.94
6	Swarnalatha(ACC33964)	84.3	125.1
7	T 12 (ACC 56989)	79.77	127.36
8	Chinsaba (ACC 33016)	71.08	109.46
9	Pokkali	66.09	79.41
10	TN1	63.61	75.61
11	IR 64	80.87	123.54
12	IR 65482-7-216-1-2-B	77.13	108.66
13	IR 36	60.95	110.95
14	MUT NS 1	59.46	108.86
15	TN1	59.35	102.66
16	OM 4498	58.21	106.28
17	RP 2068-18-3-5	99.02	121.03
18	Ptb33	100.38	112.47
19	IR71033-121-15	58.14	95.57
20	TN1	57.65	67.53

(VB = Vascular bundle, E = Epidermis, P = Phloem, μm = micrometre)

Table 3: Location of vascular bundle in cross section stems of twenty different rice differential varieties (Nodal Position)

PHSS Entries	Designation	VB – E (μm)	P – E (μm)
1	ASD 7 (ACC 6303)	79.21	127.47
2	RathuHeenati (ACC 11730)	93.23	134.44
3	Babawee(ACC 8978)	92.34	131.38
4	Milyang 63	76.66	123.62
5	TN1	72.08	122.57
6	Swarnalatha(ACC 33964)	90.92	130.44
7	T 12 (ACC 56989)	88.06	129.55
8	Chinsaba (ACC 33016)	70.88	121.67
9	Pokkali	68.26	121.2
10	TN1	67.96	119.73
11	IR 64	89.24	130.12
12	IR 65482-7-216-1-2-B	87.91	129.42
13	IR 36	64.93	118.09
14	MUT NS 1	64.59	118.01
15	TN1	60.7	115.76
16	OM 4498	60.14	114.51
17	RP 2068-18-3-5	93.26	137.92
18	Ptb33	94.38	146.68
19	IR71033-121-15	59.99	108.89
20	TN1	52.62	100.57

(VB = Vascular bundle , E = Epidermis , P = Phloem , μm = micrometre)

Xylem Tissue

The xylem tissue is comprised of xylary fibers, protozylem vessel members, metaxylem vessel members and xylem parenchyma cells. The primary function of the vessel members is to transport water and solutes, such as minerals and ions.

Variations in cross- section of stems

The internal anatomy of the nodal region of the stem of rice plant differs from that of internodal position . The diaphragm and the air channel can be described as part of the arenchymous center. The cross sections of the stem in both the nodal region, just below the panicle and through the panicle, the anatomy of the stem changes, as vascular bundle distribution changes, as there is the absence or presence of an aerenchymous center and as the crosssections look the least or the most like cross-section through an internodal region.

RESULTS AND DISCUSSION

Anatomical variation with respect to vascular bundle location varies among the rice differentials. After cutting the cross sections of these rice differentials, distance between the i) vascular bundle and epidermis, ii) phloem and epidermis, was taken under trinocular microscope. The purpose of this observation is to gain the knowledge about their anatomical differences with respect to vascular bundle location ininternodal and nodal position of rice stem .

Internodal position

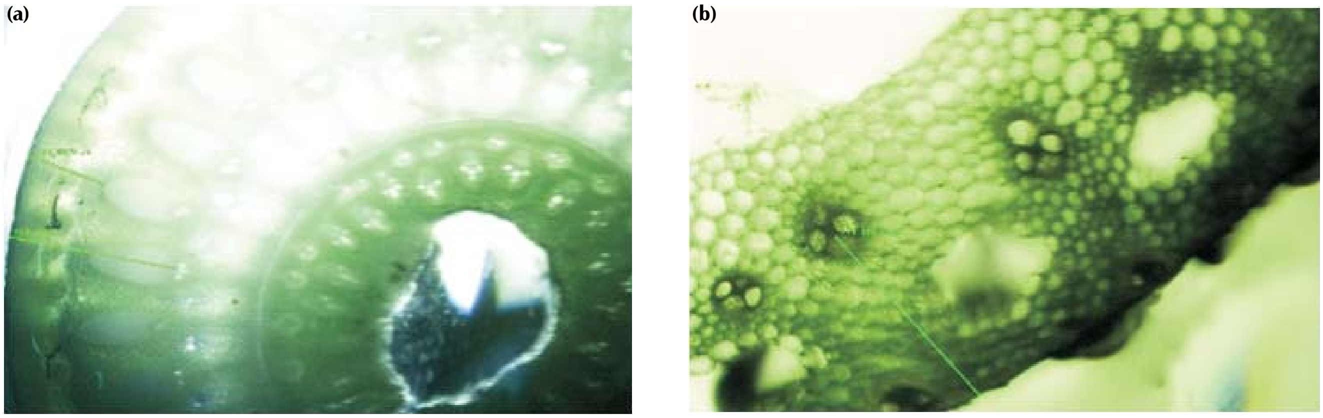

Figure 1: (a) Nodal position of rice stem and (b) Internodal position of rice position

Among the internodal position observations,the set of gene differentials exhibited average value of distance from vascular bundle to epidermis, varied from 57.65 μm to 100.38 μm. Maximum distance was observed in Ptb33 (100.38 μm). Followed by RP 2068-18-3-5 (99.02 μm), RathuHeenati (ACC 11730) (93.96 μm), Babawee (ACC 8978) (92 μm), Swarnalatha (ACC 33964) (84.3μm) and IR 64 (80.87μm). (Table 2)

Minimum distance was observed in PHSS 20 *i.e.* TN1 (57.65μm), followed by IR71033 -121-15 (58.14 μm), OM 4498 (58.21 μm), MUT NS 1 (59.46 μm), IR 36 (60.95 μm), Pokkali (66.09 μm), Chinsaba (ACC 33016) (71.08 μm), Milyang 63 (76.39 μm), ASD 7(ACC 6303) (76.51 μm), IR 65482-7-216-1-2-B (77.13 μm) and T12 (ACC 56989) (79.77 μm). (Table .2)

Along with these, distance from phloem to epidermis was also measured. Maximum distance was observed in variety T 12 (ACC 56989) (127.36 μm),followed by Swarnalatha (ACC 33964) (125.1 μm), IR 64 (123.54 μm), ASD 7 (ACC 6303) (121.33 μm), RP 2068-18-3-5 (121.03 μm). Minimum distance was observed in PHSS 20 ie.TN1 (67.53 μm), followed by Pokkali (79.41 μm), IR 71033-121-15 (95.57 μm), OM 4498 (106.28 μm) , IR 65482-7-216-1-2-B (108.66 μm), MUT NS 1 (108.86 μm), Chinsaba (ACC 33016) (109.46 μm), Babawee (ACC 11730) (110.52 μm), IR 36 (110.95 μm), RathuHeenati(ACC 11730) (111.56 μm) , Ptb 33 (112.47 μm) and Milyang 63 (112.85 μm) (Table 2)

Nodal position

Among the nodal position the set of gene differentials exhibited average value of distance from vascular bundle to epidermis, varied from 52.62 μm to 94.38 μm. Among the distance between vascular bundle and epidermis, maximum distance was observed in genotype Ptb 33 (94.38 μm), followed by RP 2068-18-3-5 (93.26 μm), RathuHeenati (ACC 11730) (93.23 μm), Babawee(ACC 8978) (92.34 μm) and Swarnalatha (ACC 33964) (90.92 μm).

Minimum distance was observed in PHSS20 ie. TN1 (52.62 μm) , followed by IR 71033-121-15 (59.99 μm), and OM 4498 (60.14 μm) (Table no.4.3).The distance between phloem (located inside vascular bundle) and epidermis was also observed under trinocular microscope. Maximum distance was observed in Ptb 33 (146.68 μm), followed by RP 2068-18-3-5 (137.92 μm), RathuHeenati (ACC 11730) (134.44 μm), Babawee (ACC 8978) (131.38 μm), Swarnalatha (ACC 33964) (130.44 μm) and IR 64 (130.12 μm). (Table. 3) .Minimum distance was observed in PHSS20 ie.TN1 (100.57 μm) followed by IR71033-121-15 (108.89μm), OM 4498 (114.51 μm), and MUT NS 1 (118.01 μm). Anatomical studies observed among the set of rice differentials have depicted in (Table. 3).

Eames and McDaniels, 1947, Sharman (1942) and Esau (1953), makes a general comment that 'the complex arrangement of vascular bundle (in grass haulm) is related to the variable orientations of the different traces of the same leaf . The results obtained are little bit similar to the works done by Nitta *et al.*, (2000) , who investigated the varietal differences, in the number and cross area of large vascular bundles (LVs) at the neck internode in 18 rice cultivars.

Brown planthopper (BPH), *Nilaparvata lugens* (Stal), is one of the major phloem feeder which causes serious damage to rice plant. They injure the plants through direct sucking plant sap from phloem, there may be chance of escape due to unreachability of neonates to proper feeding site in deeply situated vascular bundle of Ptb33 but it is available superficially in TN 1.

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