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Study of the Histological Basis for Lodging Tolerance in Rice (*Oryza sativa* L.)

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ABSTRACT

The present study entitled "study of the histological basis for lodging tolerance in rice (Oryza sativa L.)" was conducted to find out histological basis of lodging nature of rice by studying relevant parameters in three different groups of rice germplasm viz., lodging susceptible varieties (Swarna, BPT-5204, Tellahamsa and RNR-15048); lodging tolerant varieties (MTU-1112, MTU-1121, MTU-1166 and MTU-1001); and stable strong culm mutant lines (SP-351, SP-353, SP-360 and SP-70). Histological studies revealed that in rice physical strength of the culm was highly correlated to the number of vascular bundles of cross sectional area of the basal culm (3rd internode) as well as the thickness of dermal sclerenchyma. It should be noted that pattern of distribution of vascular bundles didn't differ in the varieties / lines under the present study. Therefore, if time and facilities permits, these two histological parameters should be considered while measuring lodging nature of rice culms.

Key words: *Number of vascular bundles, distribution pattern of vascular bundles and thickness of epi /hypodermal lignified tissues in rice culm.*

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple food crops of Asia, Africa, and South America, and serves as a primary source of food for more than half of the world population[6]. It is the main source of the 35-60% dietary calories consumed by more than 3 billion people[4]. It is considered as the world's most diverse crop and is probably the most versatile crop. It is grown below sea level in Kerala, India, at more than 3000 m elevation in the Himalayas, and at sea level in the deltas of the Asian rivers. It can be found from 53^0 North in Northeastern China to 35^0 South in New South Wales, Australia[9]. Total world rice production was about 740.9 miilion tonnes with an area of 160.6 million hectares and in India rice production was about 106.65 million tonnes from 44 million hectares with a productivity of 2462 kg/ha (FAO STAT, 2015).

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Due to the exponential rate of population growth, it is estimated that a 40% increase in rice yield is needed by 2030 to fulfill the growing demand without affecting the resource base[6]. It is observed that genotypes having higher solid pith area have higher lodging resistance capability which possess thicker sclerenchyma layer with, sclerenchyma cell layers. Number of vascular bundles is positively correlated with lodging resistance lines because vascular bundles contribute to mechanical strength[5].

MATERIALS AND METHODS:

An experiment was carried out in the experimental field of ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad and the laboratory of the Department of Crop Physiology, College of Agriculture, Rajendranagar, and National bureau of plant genetic resources Hyderabad during *kharif*, 2014-2015. 12varieties or lines of rice were taken for the present study to understand the histological basis of lodging tendency in rice three different groups of rice germplasm viz., lodging susceptible varieties (Swarna, BPT-5204, Tellahamsa and RNR-15048); lodging tolerant varieties (MTU-1112, MTU-1121, MTU-1166 and MTU-1001); and stable strong culm mutant lines (SP-351, SP-353, SP-360 and SP-70). In the present experimental studies the histological parameters Number of vascular bundles in the cross sectional area of culm, distribution pattern of vascular bundles and thickness of epi / hypodermal lignified tissues were studied.

Preparation of permanent mounted slides of rice culms:

Transverse sections of third internode of rice culm were made, at 12 µm thickness, with rotary microtome with steel blades using the paraffin embedding procedure as described by Johansen (1940). Reagents as well as detailed procedure used were given below.

Reagents

i) Formalin-Aceto-Alcohol (FAA): 50% ethyl alcohol, glacial acetic acid and formaldehyde were mixed in the volumetric ratio of 18: 1: 1, to prepare FAA, which was used for killing and fixing the tissue samples.

ii) Alcohol series: Dehydration of tissue samples was done by the following series of solutioqns of water, ethyl alcohol and tertiary butyl alcohol.

Distilled water (mL)	95% ethyl alcohol (mL)	Tertiary butyl alcohol (mL)	100% ethyl alcohol (mL)	App. total % alcohol in final solution
50	40	10	--	**50**
30	50	20	--	**70**
15	50	35	--	**85**
--	45	55	--	**95**
--	--	75	25	**100**

iii) Toluidine Blue stain: 0.05 % (w/v) solution of the polychromatic stain 'toluidine blue'in ethanol was prepared, which was used to stain the sections.

iv) Mayer's adhesive: To mount the paraffin sections on to the slides, Mayer's adhesive was used which was prepared by thoroughly mixing the white of one fresh egg to about equal quantity of glycerol and 1 g sodium salicylate. The mixture was filtered through sterile cheese cloth.

Procedure:

Internodal samples (third internode from base) of length of 0.5 to 0.75 cm length were cut with a sharp blade (Fig. 3.3), which were immediately killed and fixed in FAA solution for a minimum period of 24 hours or till the samples get cleared. Then the samples were dehydrated by immersing in alcohol series solutions of increasing strength in the order of 50%, 70%, 85%, 95%, 100% alcohol, with at least 1 hour duration at each step, followed by

immersion in 2 changes of tertiary butyl alcohol again for at least one hour at each step. Then, these dehydrated tissue samples were infiltrated with paraffin in an oven maintained at 60-80 ^{0}C temperature. Infiltration with paraffin again was done gradually to prevent damage to the tissue by following changes of infiltrating solution viz., 1:1 mixture of Tertiary butyl alcohol and light paraffin oil, paraffin topped with 1:1 mixture of tertiary butyl alcohol and light paraffin oil followed by at least two changes of pure paraffin. Then the samples were infiltrated with and embedded in an embedding mixture of paraffin and bee wax (3:1 w/w) in paper boats. Infiltrated samples were then neatly trimmed to cuboid shape, attached to the sample holder of rotary microtome with sufficient paraffin wax at the base and were microtomed at 12 micro meter thickness, to get the ribbon of serial sections of the sample. These ribbons were then 3cut into small pieces, which were then mounted on to the slides smeared with a thin layer of Mayer's adhesive. These mounted slides were flooded with water and the paraffin ribbons were straightened on a warming plate maintained at 45 ^{0}C. After straightening, excess water was drained from the slides, which were then observed under microscope to select the slides with better sections. The selected slides were then left overnight on the warming plate maintained at 45 ^{0}C to make the sections adhere to the slides. Slides with adhered paraffin sections were deparaffinised by immersing in xylene, till all the paraffin dissolved leaving only the sections on the slide. Later the slides along with adhered sections were immersed in 1:1 mixture of xylene and absolute ethyl alcohol for about one hour. Slides with sections were flooded for about 5 minutes with 0.05% Toluidine blue solution to stain the sections. After staining sections were mounted using DPX mountant, duly covered with a cover slip . Later the slides were dried in an oven at 70^{0}C for 2-3 days, additional mountant was scraped off the slide, and slides were wiped clean to remove smudges of mountant over the cover slip aswellas slide with a lint free cloth wetted with xylene.

Microphotography:

Mounted sections on the slides were used for micro-photography, at 100x magnification with a digital camera mounted, binocular, compound microscope (OLYMPUS - CX41).

Measurement of tissue thickness in microscopic sections:

Calibration of the 'ocular micro-meter' : A 'stage micrometer'(with 10 µM spaced equidistant lines) was kept on the stage, at the required magnification level (100 x) and an 'ocular micro meter' was placed into the micrometer mounting frame of a NCWHK 10X eye piece in such a way that the micrometer lines are facing downwards. Then the micrometer fitted eye piece was rotated till the lines of the ocular micrometer are parallel with those of the stage micrometer. Lines on the left edges of the two micrometers were matched by moving the stage micrometer so that the lines of the ocular micrometer are superimposed over those of the stage micrometer. Then the number of ocular micrometer divisions that fall within a given number of stage micrometer divisions was checked at three different scale locations, and the average size of a division of the 'ocular micrometer' was calculated at that specific magnification.

Measurement: Mounted rice culm sections, were observed at 100 x magnification, with a calibrated ocular micrometer fitted eye piece, to measure the thickness of the epi / hypo dermal lignified tissue. To measure the thickness, ocular fitted eyepiece was rotated in such a way that a line of the scale was just touching stem surface like a tangent. No. of divisions encompassing the peripheral lignified tissue were counted at five different locations of the section, and the average thickness of the lignified tissue was computed.

Number and distribution pattern of vascular bundles in the stems

Mounted rice culm sections were observed under low power magnification (40x magnification), to know the number and

distribution pattern of vascular bundles in basal part of rice culm.

Scanning electron micrography

The basal third internodes'of rice culms were cut into 5 mm pieces and fixed in 2.5% glutaraldehyde in phosphate buffer for 2 hours at 40 ^{0}C.At the end of the incubation, the samples were washed with phosphate buffer post fixed with2% osmium tetroxide for 4 hours and dehydrated using a graded series of ethanol .The dehydrated samples were dried with critical-point liquid carbon dioxide as a transition fluid. The dried materials were adhered on to aluminium specimen mounts with double-stick adhesive tape .The samples were later coated gold-palladium in an automated sputter coater (JEOL IFC-1600) and examined with a scanning electron microscope (JEOL-JSM 5600) as per the standardized protocols at RUSKA lab, college of veterinary Science, Rajendranagar, Hyderabad. Due to paucity of funds only the mutant lines under the present study, were taken up for scanning electron micrography.

RESULTS AND DISCUSSION

Total number of vascular bundles present in the cross sectional area of the rice culms were presented in the (table.1). These results show that the rice varieties / lines under the present study differed in the number of vascular bundles of the culm. Lowest number of vascular bundles per cross sectional area was observed in BPT-5204 (18), whereas the mutant line SP-360 showed the highest i.e., 27 vascular bundles. It is also evident that except BPT-5204 (18) and RNR-15048

Table 1: Histological parameters of rice culm (Mean of 5 samples)

Variety / line	At 50% Flowering stage		At full ripening stage	
	Number of vascular bundles	Thickness of epi-hypo dermal sclerenchyma	Number of vascular bundles	Thickness of epi / hypodermal sclerenchyma
SWARNA	20.0	10.0	20	14.0
BPT5204	18.0	8.0	18.0	10.0
TELLAHAMSA	20.0	12.0	20.0	15.0
RNR15048	23.0	9.0	23.0	12.0
MTU1112	22.0	11.0	22.0	14.0
MTU1121	21.0	18.0	21.0	20.0
MTU1166	21.0	10.0	21.0	15.0
MTU1001	20.0	10.0	20.0	13.0
SP351	25.0	21.0	25.0	25.0
SP353	26.0	23.0	26.0	26.0
SP360	27.0	28.0	27.0	32.0
SP70	26.0	26.0	26.0	28.0

(23) all the other released varieties of rice showed not much difference in vascular bundle number in culm. Thickness of the epi/hypodermal sclerenchyma tissue layer in rice culms of all the rice varieties / lines of the current study are also presented in the table, which showed that the varieties / lines of rice differed in this character as well. Out of all the rice varieties / lines BPT-5204 showed lowest sclerenchyma thickness both at 50% flowering and full ripening stages, while the mutant line SP-360 showed highest sclerenchyma thickness both at 50% flowering and full ripening stages. From these results (table1), it was also evident that lignified layer thickness in rice culms increased with time between 50% flowering stage and full ripening stages.

Micro-photographs of mounted slides of 10µM thick sections of basal (3rd internode) of all the rice varieties / lines under the current study are presented in the fig. 1, 2, 3, 4, 5 and 6, both at 50% flowering and full ripening stages.

Further, scanning electron micrographs of mutant lines of rice at full ripening stage are presented in the fig.7. These figures show that the distribution pattern of vascular bundles in all the rice varieties / lines under the current study is similar i.e., vascular bundles are arranged in two concentric circles in the rice culm, out of which vascular bundles lying on the circumference of inner circle are larger than those lying on the circumference of outer circle. Further, in all the varieties / lines outer, smaller vascular bundles are separated by aerenchyma cavities, apparently formed by lysogenic process (Figures.1 to 7). Correlation between the number of vascular bundles in the culm and physical strength of culm and between the thickness of epi/hypodermal sclerenchyma and physical strength of culm, at both 50% flowering and full ripening stages was worked out and presented in the (table 2). These results showed that physical strength of rice culms is highly significantly correlated to both number of vascular bundles of culm and thickness of epi/hypodermal sclerenchyma of culm.

Table 2: Correlation between histological parameters and physical strength of the culm in rice

Sl. No.	Histological parameters of culm	Correlation coefficient with physical strength of culm (r)	
		50% flowering stage	Full ripening stage
1.	Number of vascular bundles	0.954**	0.909**
2.	Thickness of epi/hypodermal sclerenchyma	0.953**	0.922**

** - Significant at 1% level.

Discussion on histological parameters Dependence of physical strength of the rice culms on the number of vascular bundles[1,2,7] and on the fusion of outer vascular bundles with dermal sclerenchyma which can be equated to the thickness of epi/hypodermal lignified tissue, were reported earlier and the present findings either in terms of differences in histological parameters in varieties differing in lodging nature or in terms of physical strength dependence on histological parameters support them[8].Ramaiah and Mudaliar It should be pointed out that the distribution pattern of vascular bundles in rice culms was similar in all the three groups of rice varieties / lines studied, implying that this parameter didn't play a role in the lodging nature of rice in these varieties[3]. However, it was reported by Chuanren *et al.* that fusion of outer vascular bundles with epidermal sclerenchyma was observed in lodging tolerant rice varieties, which was not observed in any rice variety / line studied in the present work.

Histological studies revealed that in rice physical strength of the culm was highly correlated to the number of vascular bundles of cross sectional area of the basal culm (3rd internode) as well as the thickness of dermal sclerenchyma. It should be noted that pattern of distribution of vascular bundles didn't differ in the varieties / lines under the present study. Therefore, if time and facilities permits, these two histological parameters should be considered while measuring lodging nature of rice culms[10] *et al* . There is a lot of scope to improve the lodging tolerance studies in rice. The present study didn't look into the effect of weight of the panicle on lodging nature and also collective resistance of the adjacent plants to external lodging force like wind or water currents. In the histological studies, it should be pointed out that time and infrastructural facilities are a great limitation. If they permit, arrangement of vascular bundles with respect to each other in the cross sectional area of rice culm could become another interesting study, ex. Intervascular bundle distance. Further distance between the outer and smaller vascular bundles and epidermis could be an interesting study, since there were reports of fusion of outer vascular bundles with epidermal sclerenchyma in lodging tolerant strains.

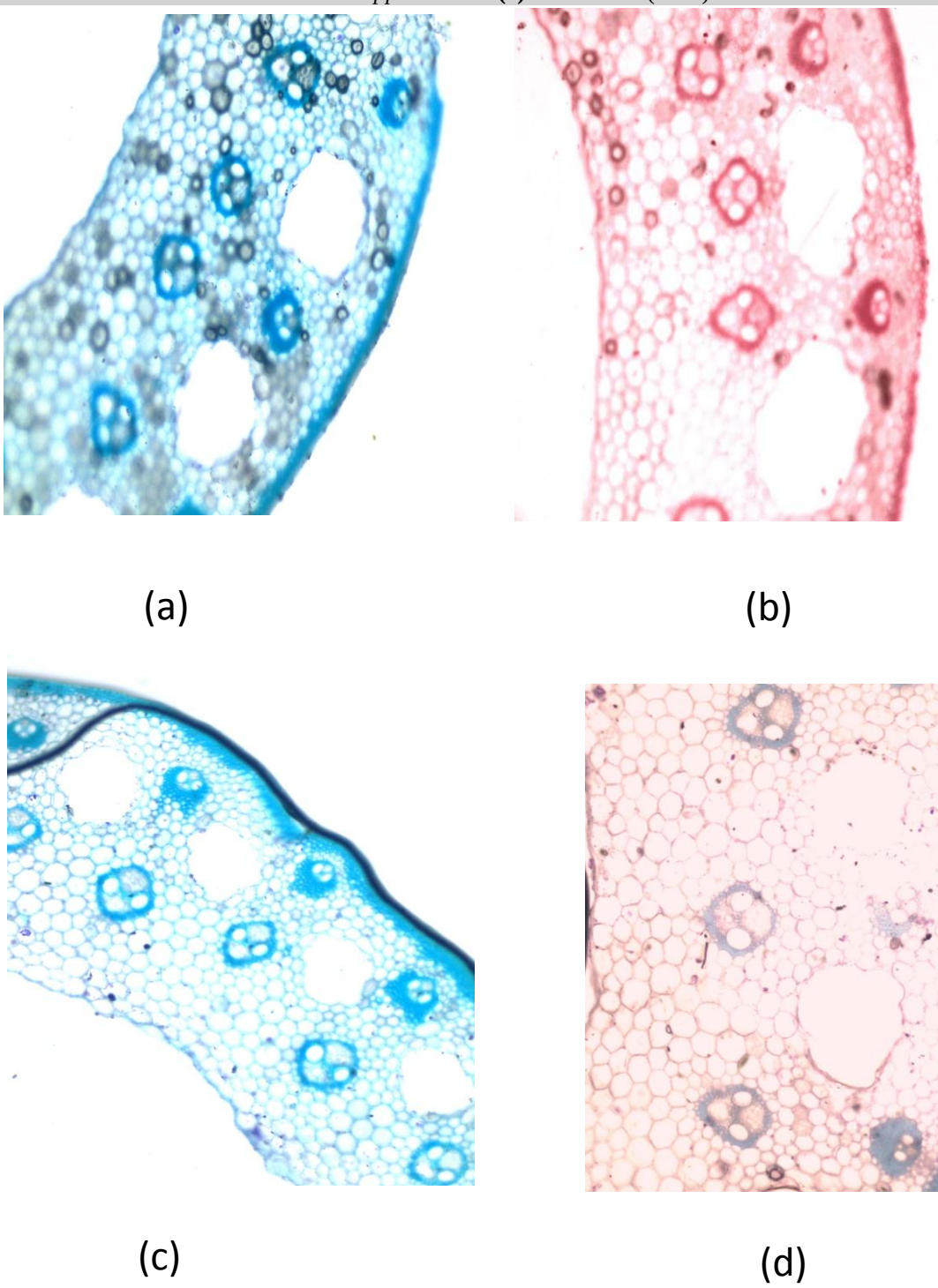

Fig. 1 Transverse sections of basal internode (3rd internode) of rice culm (100x)
(a) Tellahamsa at 50% flowering stage
(b) Tellahamsa at full ripening stage
(c) RNR 15048 at 50% flowering stage
(d) RNR 15048 at full ripening stage

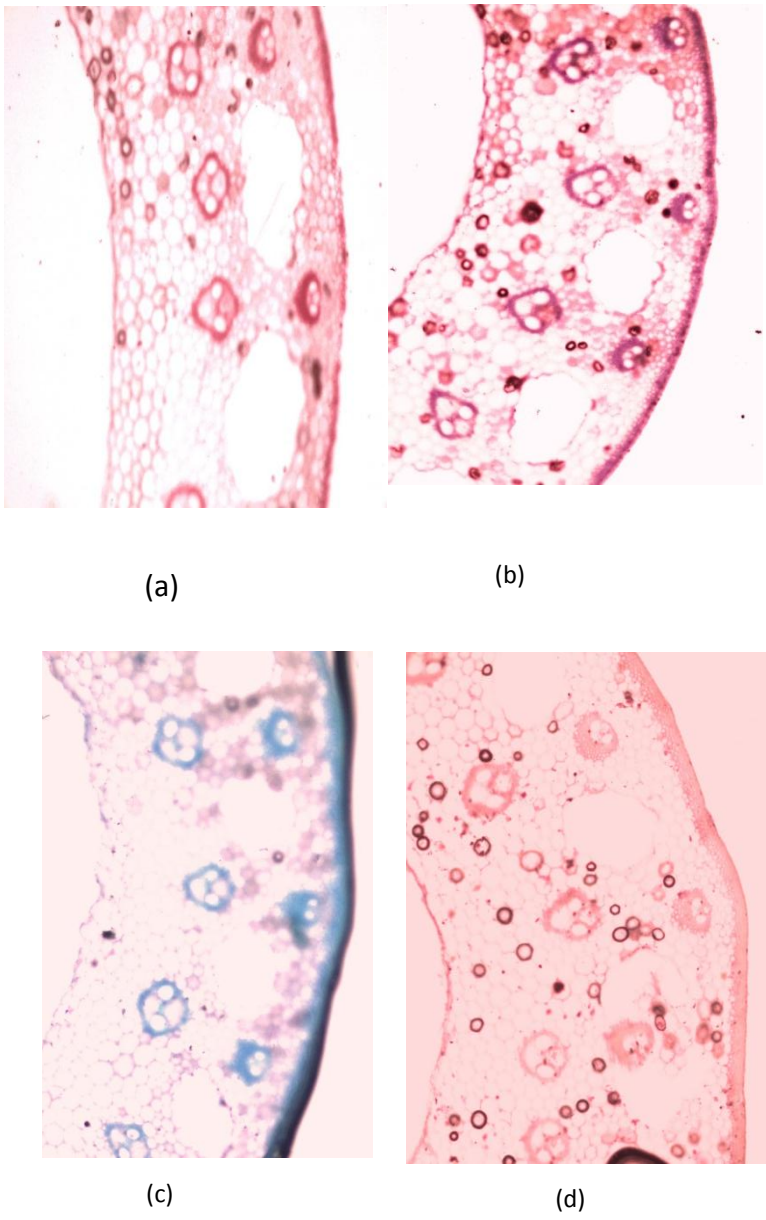

Fig .2 Transverse sections of basal internode (3rd internode) of rice culm (100x)

(a) Swarna at 50% flowering stage
(b) Swarna at full ripening stage
(c) BPT 5204 at 50% flowering stage

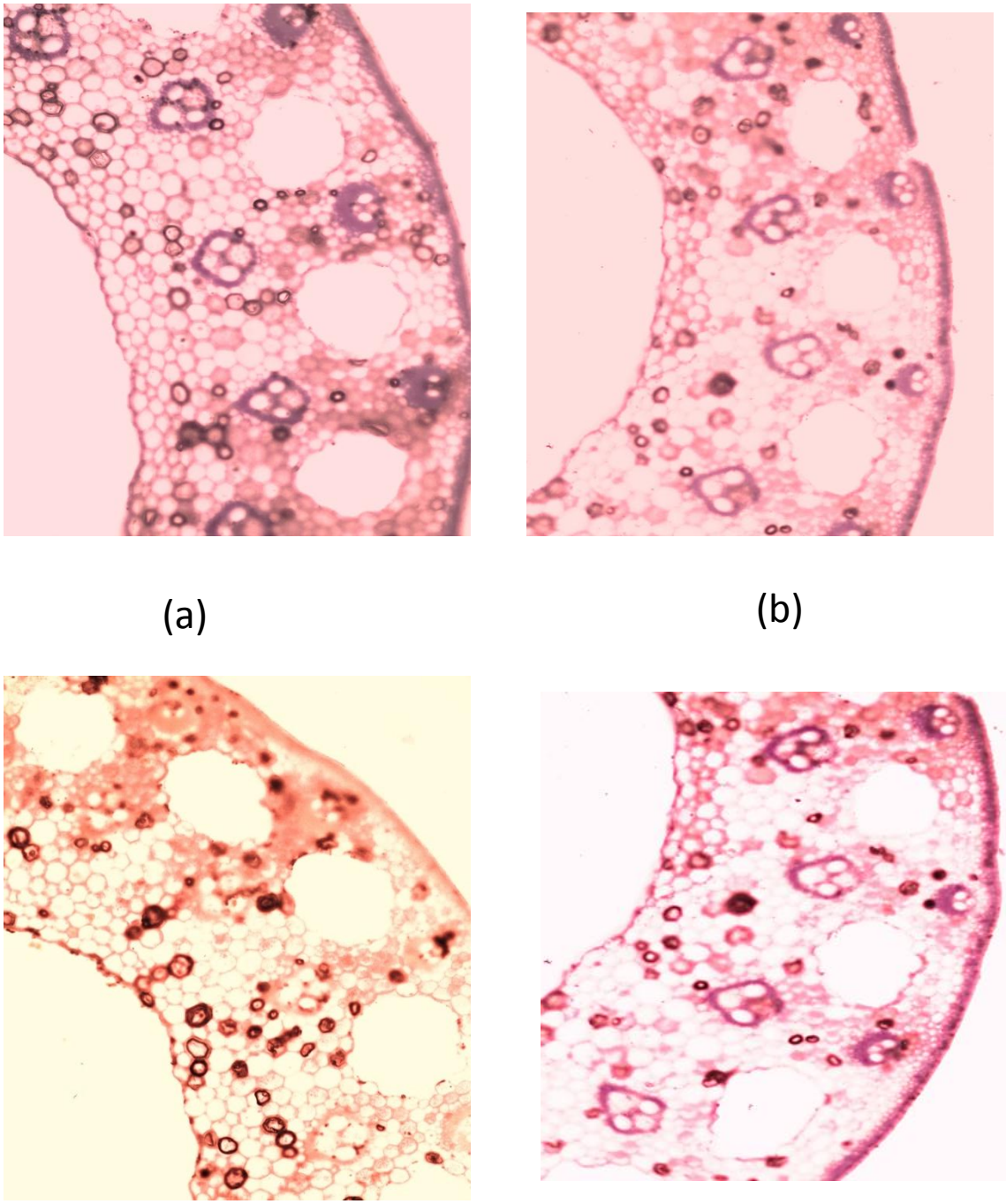

Fig. 3 Transverse sections of basal internode (3rd internode) of rice culm (100x)
(a) MTU-1112 at 50% flowering stage
(b) MTU-1112 at full ripening stage
(c) MTU-1121 at 50% flowering stage
(d) MTU-1121 at full ripening stage

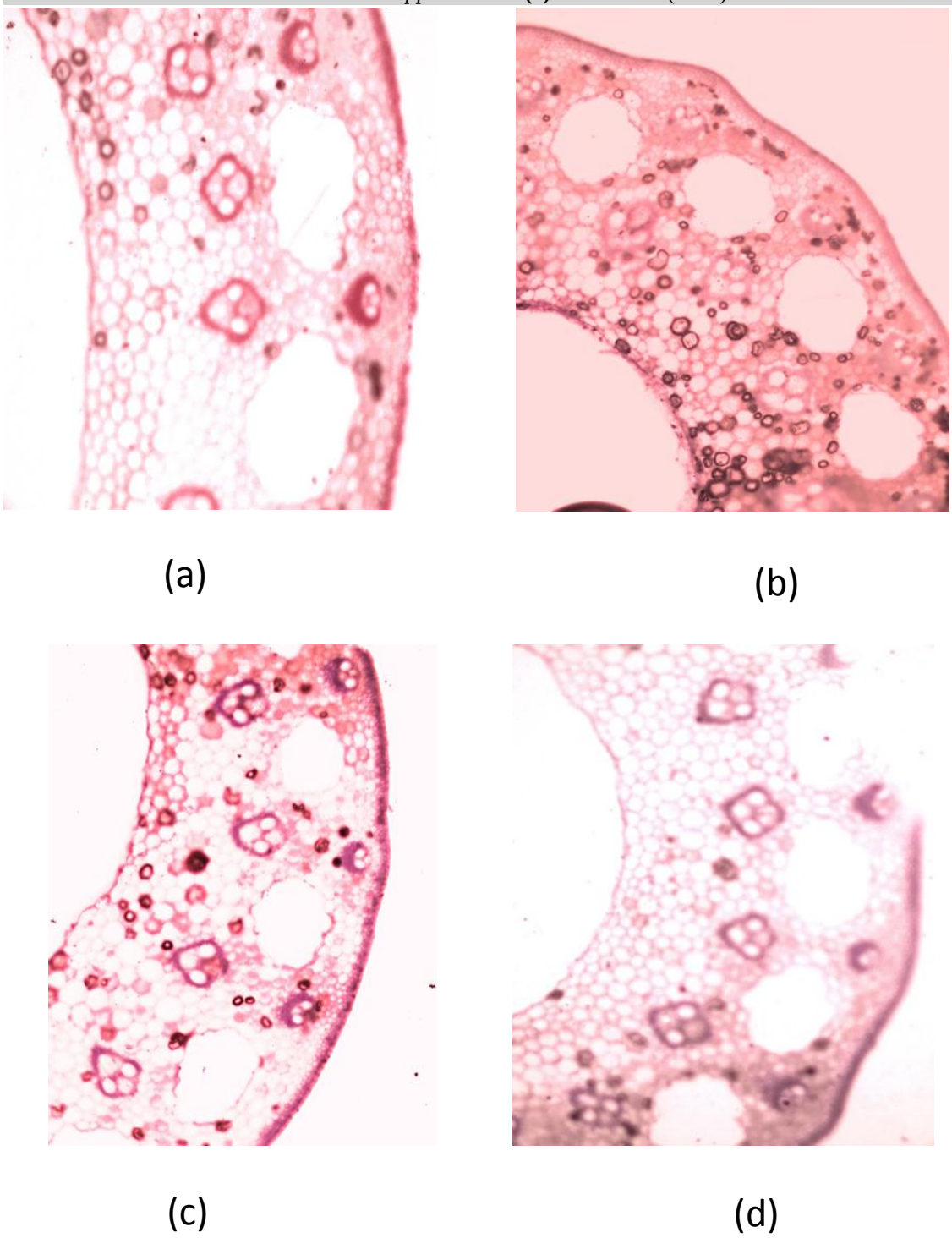

Fig. 4 Transverse sections of basal internode (3rd internode) of rice culm (100x)
(a) MTU-1166 at 50% flowering stage
(b) MTU-1166 at full ripening stage
(c) MTU-1001at 50% flowering stage
(d) MTU-1001at full ripening stage

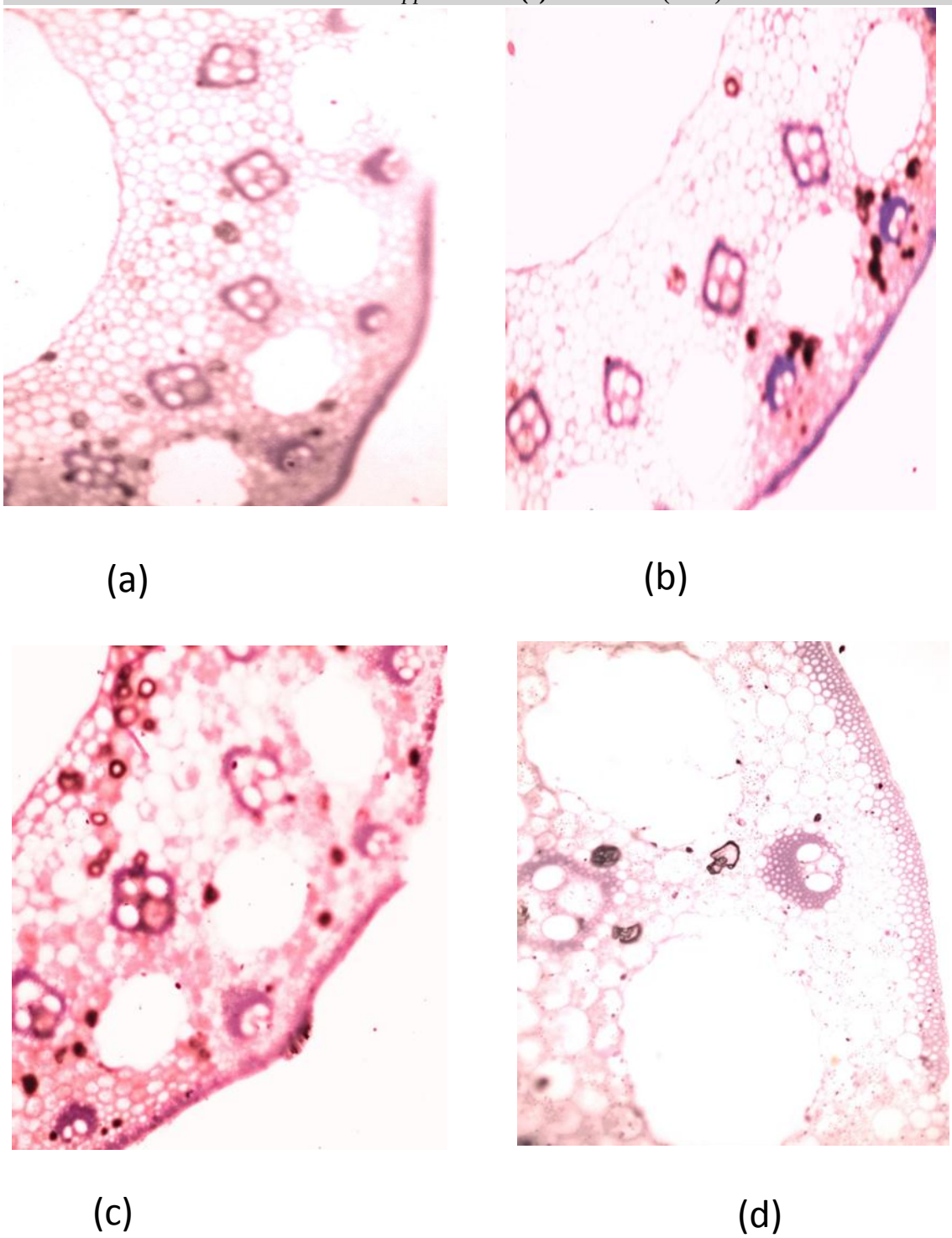

Fig. 5 Transverse sections of basal internode (3rd internode) of rice culm (100x) (a) SP-351 at 50% flowering stage
(b) SP-351 at full ripening stage
(c) SP-353 at 50% flowering stage
(d) SP-353 at full ripening stage

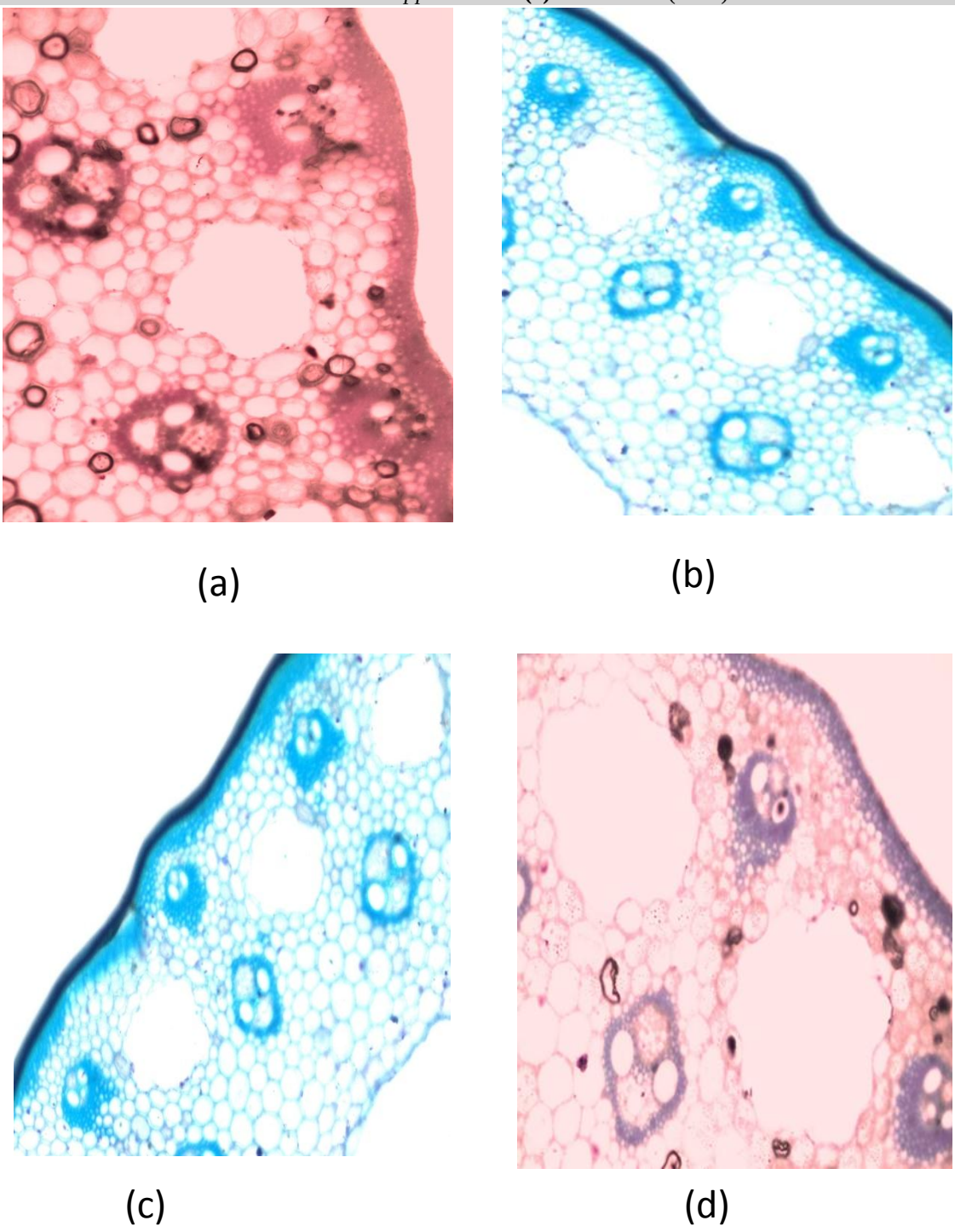

Fig. 6 Transverse sections of basal internode (3rd internode) of rice culm (100x)
(a) SP-360 at 50% flowering stage
(b) SP-360 at full ripening stage
(c) SP-70 at 50% flowering stage
(d) SP-70 at full ripening stage

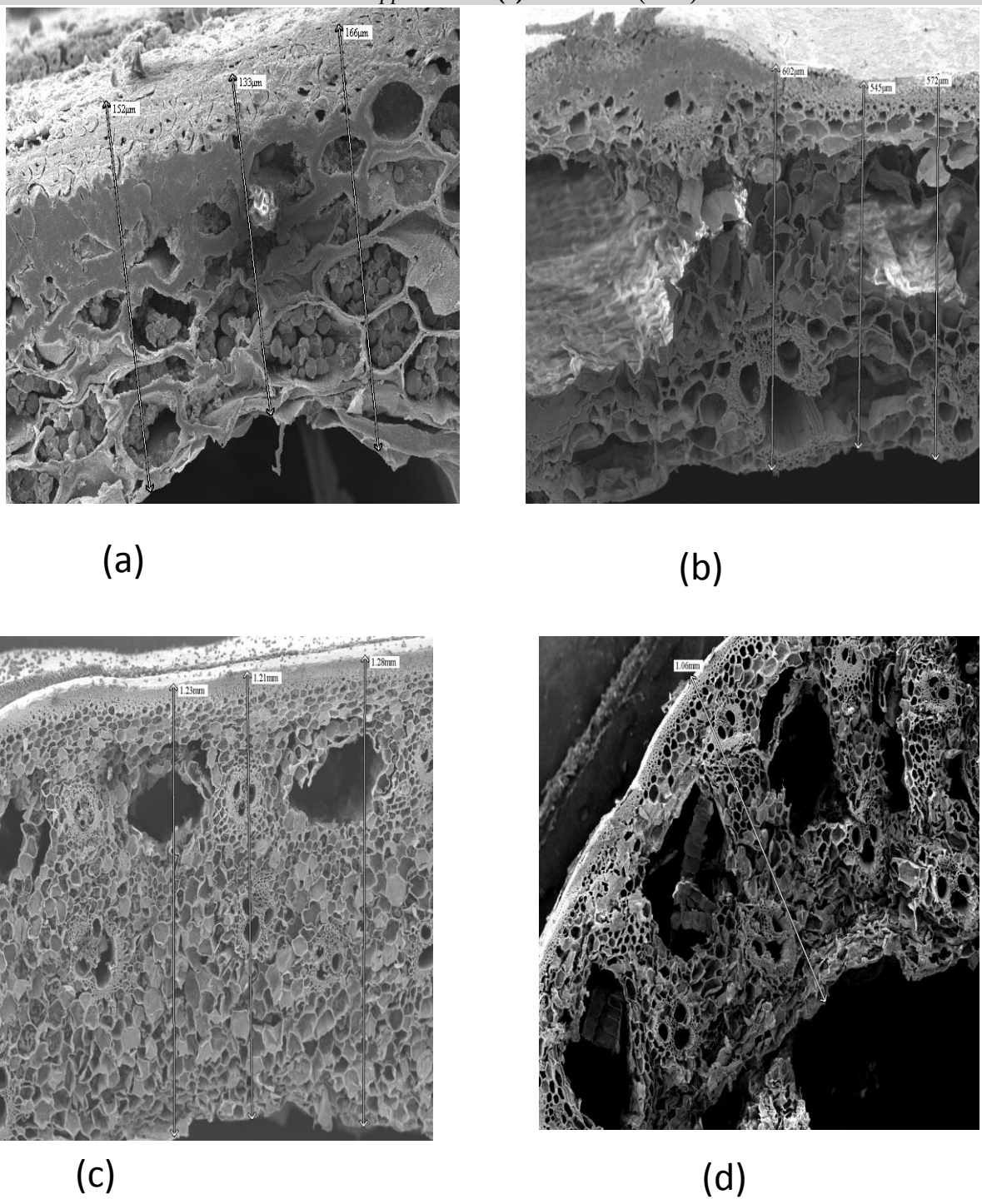

Fig. 7 Scanning electron micrographs of transverse sections of basal internode (3rd internode) of rice culm at full ripening stage

(a) SP-351 (1000x)
(b) SP-353 (700x)
(c) SP-360 (400x)
(d) SP-70 (700x)

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