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RESEARCH ARTICLE

HISTOLOGICAL STUDIES ON RICE PLANT IN ASSOCIATION WITH CYANOBACTERIA, *Oscillatoria foreaui* FREMY

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ABSTRACT

The abundance of cyanobacteria in paddy fields and the consequent sustenance of the nitrogen status of rice fields is an important aspect of economic relevance. Aerenchyma is a constitutive feature in rice, and has thus served as one of the two model plants (the other being corn) for studies on the formation of irregular lysigenic aerenchyma among the monocot, formed as a result of the breaking down of cortical cells. An increase in the proportion of aerenchyma is dependent on the rice cultivar, and the developmental stage of the plant. In the present study, the significant result is with reference to the increased diameter of vessels in all organs of plants associated with cyanobacteria.

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INTRODUCTION

Cyanobacteria (blue-green algae) are diazotrophic prokaryotes with oxygen evolving photosynthetic system. Cyanobacteria are cosmopolitan in distribution. The effective use of cyanobacteria in the rice fields due to their ability to fix nitrogen has been an aspect of much interest (Singh, 1961; Venkataraman, 1981; Kannaiyan, 1985). Cyanobacteria are almost exclusively free-living and are ideally suited to an independent existence. Nevertheless, a few species form specific associations with various plant groups such as algae, fungi, bryophytes, pteridophytes, gymnosperms and angiosperms (Fogg *et al*., 1973). The establishment of novel associations between higher plants and a variety of nitrogen fixing microorganisms is one of the present day approaches. Creation of artificial N_2-fixing associations involving free-living cyanobacteria and crop plants has been found to be feasible (Gantar *et al*., 1991; Gantar, 2000; Svircev *et al*., 1997; Rai and Bergman, *et al*., 2002). Toledo *et al*. (1995) have shown that *Avicennia* seedlings colonized artificially promote the growth of the seedlings. The effect of aluminium on the morphology and cell structure of rice roots have been studied by Alvarez *et al*., (2012). The formation of air spaces is not restricted in rice to the roots alone. The cortical gas lacunae extend up from the root-shoot zone and into the shoots (leaves, leaf sheaths and tillers) to maintain efficient bi-directional longitudinal transport of gases between the roots and the shoots. Intensively vacuolated cells were observed in the cortex and the epidermis in longitudinal sections of Al stressed roots (Alvarez *et al*., 2012). The present study is an attempt to study the effect of associating cyanobacteria, *Oscillatoria foreaui* on the rice plant *Oryza sativa* L. *var*. ADT-43 on the changes in the anatomical features of rice plant.

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MATERIALS AND METHODS

Culture medium and culture conditions

The culture chosen for the study (*Oscillatoria foreaui* Fremy) was obtained from the culture collections of CAS in Botany, University of Madras, Chennai. A number of isolates were screened for their ability to form association with the root system of rice plant, *Oryza sativa* L. ADT-43.

Description of the culture

Oscillatoria foreaui Fremy

Trichomes single forming a spongy thallus, without sheath, motile showing typical oscillatory movements. Terminal cells blunt. Trichomes 2.5µm broad, cells longer and end cells rounded. Culture was grown and maintained in BG_{11} medium (Rippka *et al*., 1979). Culture was maintained at 27±1°C under fluorescent illumination of 30 to 40 $\mu Em^{-2}s^{-1.}$ Light was provided by 3 warm white fluorescent tube lights. The culture chamber was fitted with Sangmo Weston Ltd., S650 313F model automatic timer suitable to facilitate alternating light and dark phases as and when required.

Anatomical studies on seedlings of rice plant (*Oryza sativa* L.)

Specimen of the roots of cyanobacteria associated seedlings and those without any association were cut, properly trimmed and immediately fixed in FAA (Formalin: Acetic acid: Alcohol in the ration 1:1:8) in the field itself. Fixing was done for a minimum period of 6hrs and the materials were washed in distilled water. Anatomical studies on the underground culm and leaf of non-associated and cyanobacteria associated seedlings were carried out for *O. sativa* var. ADT-43. The specimen was passed through graded series of tertiary butyl alcohol (TBA) as given by Sass (1957). The samples were kept overnight in each grade and were finally given two changes in pure (100%) TBA. Following dehydration, paraffin infiltration (paraffin wax congealing point 58 – 60°C) was carried out. Initially thin strips of wax was added and kept in hot air oven at low temperature. The temperature was gradually increased and the TBA was allowed to evaporate. Then 3 changes of pure wax were given. After super saturation, specimen was left in molten wax for few days for complete infiltration of tissues and later blocks were prepared. Serial sections were prepared to the thickness of 8-12µm with rotary microtome. Two types of staining were adopted for all sections. Tannic acid-Ferric chloride + Safranin combination was tried (Foster, 1934). Toluidine blue was also followed in parallel to safranin procedure (O'Brien *et al*., 1967). Toluidine blue was found to be more satisfactory for the present study because of metachromatic property of the stain. Sections were mounted in DPX for permanent preparation. During observation, parameters such as vessel diameter, diameter of protoxylem lacuna and size of the vascular strands were studied. For menstruation of cell dimensions, minimum 10 readings were taken and the average values were considered for comparison. General description of structure and organization was also made for the organ studied. Photomicrographs were taken with NIKON LAB PHOT 2 in different magnifications.

RESULTS

Anatomical features of *O.sativa* in the presence and absence of cyanobacteria

Structure of leaf of *O.sativa* L. *var*. ADT-43 without associating cyanobacteria

The laminar part measures 70µm and the midrib measures 285µm in thickness. The lamina consists of an adaxial epidermis with thick cuticle bearing prominent conical projections. The epidermal cells are either oblong or cubical (Plate 1b). At regular interval occurs highly dilated, thin-walled bulliform cell system, a characteristic feature of certain members of Poaceae. The abaxial epidermis is more or less similar to the adaxial epidermis. The central bulliform cells measure 22.5x30µm. The mesophyll tissue is homogeneous with 7 or 8 tiers of compact chlorenchymatous cells. The vascular system of the lamina comprises of several parallel vascular strands of uniform size. Each vascular strand has an adaxial cluster of xylem elements and abaxial patch of phloem elements. A single layer of spherical bundle sheath cells encloses the vascular bundle. There are sclerenchymatous bundle sheath extensions on both adaxial and abaxial parts of the vascular bundle (Plate 1b). The midrib is shallowly obtriangular in cross-sectional view with the narrow angle being abaxial. There are two vascular bundles placed along central median part of the midrib, one at the adaxial side, the other at the abaxial side (Plate 1a). The strands are ensheathed by a single layer of sclerenchymatous cells. The bundles are collateral with large metaxylem elements and protoxylem lacuna. The bundle measure 100µm in diameter; the metaxylem elements are 35µm in diameter. There are 2 wide air chambers placed laterally on either side of the median part of the midrib. The air chambers measure 75µm in diameter. The total thickness of the midrib in vertical plane is 285µm (Table 1). The ground tissue of the midrib consists of compact thin-walled chlorenchymatous cells.

Structure of leaf of *O.sativa* L. *var*. ADT-43 associated with *Oscillatoria foreaui*

The structure and dimensions of leaf of plants associated with cyanobacteria exhibit certain striking differences (Table 1). The laminar part of the associated plant is 90µm thick. The bulliform cells are similar in general organization, but increase in total volume. The bulliform cells measure 40x45µm in size. The epidermal layers and mesophyll tissues do not show significant change. The vascular bundles of the lateral veins are slightly reduced in dimensions (Plate 1d). The midrib part of the leaf is 470µm in vertical plane. The air chambers are very much dilated measuring 280µm in diameter. The metaxylem elements of the vascular bundles are much dilated and measure 50µm in diameter. The ground tissues also are significantly hypertrophied. The vascular bundle of the midrib is 135µm in diameter.

Table 1. Anatomical features of non-associated and *Oscillatoria foreaui* associated *Oryza sativa* L. *var*. ADT-43

Anatomical features	Size variations (µm)	
	Associated	Non-associated
Leaf		
Thickness of lamina	90	70
Size of Bulliform cells	40x45	22.5x30
Diameter of vascular bundle	135	100
Midrib thickness	470	285
Diameter of air space	280	75
Diameter of metaxylem	50	35
Underground culm		
Diameter of vascular bundle	230	115
Vertical diameter of air chamber	420	250
Diameter of metaxylem	40	30
Width of protoxylem lacuna	65	42.5
Root		
Diameter of root	300	250
Endodermis radial wall thickness	7.5	5.0
Diameter of vascular bundle	170	125
Diameter of metaxylem	30	25

Structure of underground culm of *O.sativa* L. *var*. ADT-43 without associating cyanobacteria

The underground culm (rhizome) of control plant is circular in cross-sectional view with even and smooth surface. A wide circular air canal occupies the central core of the rhizome. Surrounding the central canal is a ring of smaller air chambers in the cortical region. The air chambers are separated by radial band of ground tissues. Outer to the air chambers adhere the

Plate 1

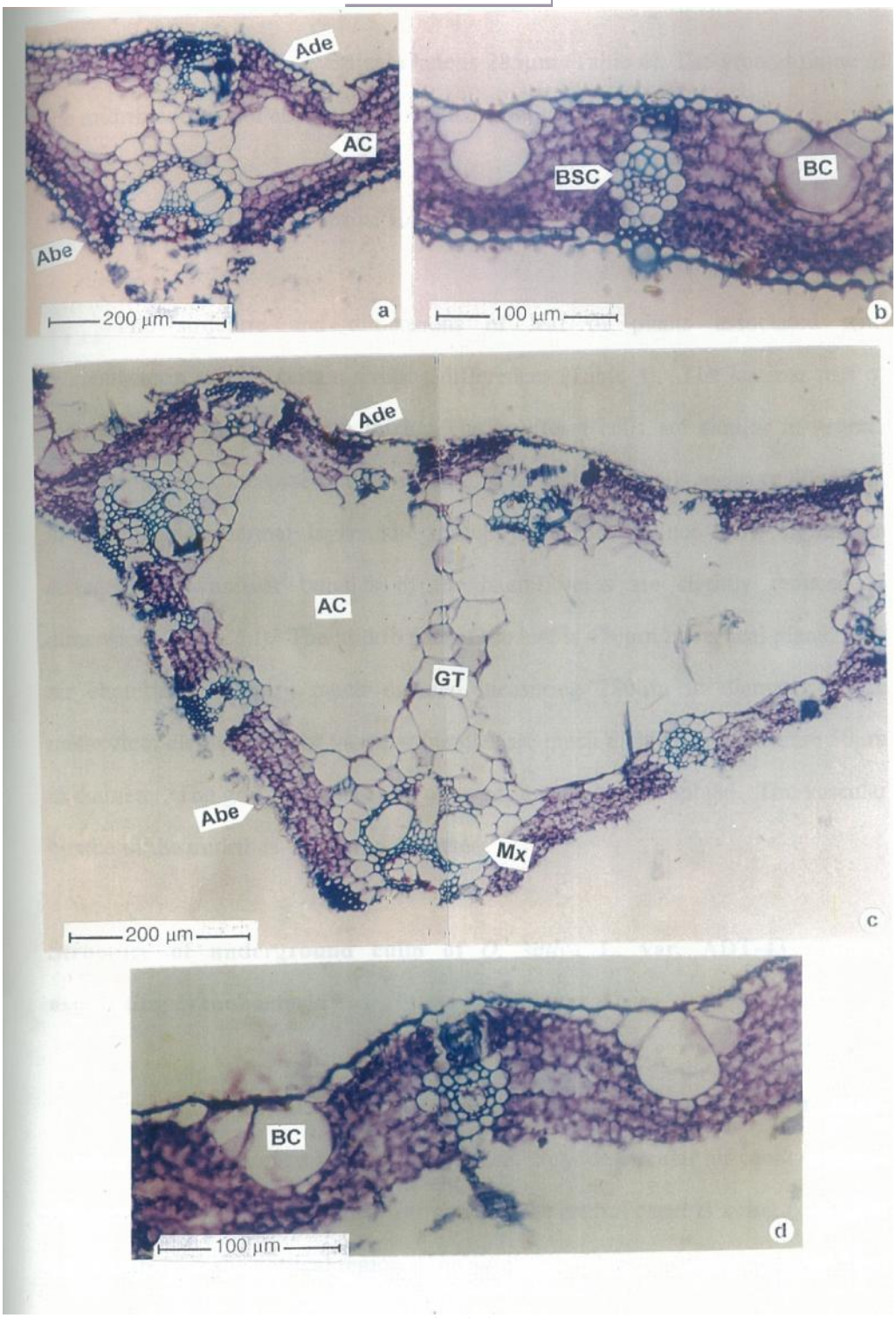

Plate: 1 Transverse section of leaf of *O. sativa* L. *var*. ADT-43

a. T.S. of leaf midrib of non-associated plant **b.** T.S. of lamina showing vascular bundle and bulliform cells **c.** T.S. of leaf midrib of *O. foreaui* associated plant **d.** T.S. of lamina of *O. foreaui* associated plant (Abe – abaxial epidermis, AC – aerenchymatous cortex, Ade – adaxial epidermis, BC – bulliform cells, BSC – bundle sheath cells, GT – ground tissue, Mx - metaxylem)

cortex and epidermis. The epidermal cells are small and cubical with thick radial and outer tangential walls. One/two layers of smaller, sclerenchyma elements follow the epidermis. The rest of the cortex consists of wide, thin-walled, three/four layers of compact parenchyma cells. Placed along the radial partitions are outer ring and inner ring of vascular bundles. The outer bundles are smaller than the inner ones. The bundles are collateral, closed with inner xylem elements and outer phloem. The vascular bundles are surrounded by thick-walled bundle sheath fibres (Plate 2a&b). The dimensions of different tissues of control culm are: diameter of vascular bundle

Plate 2

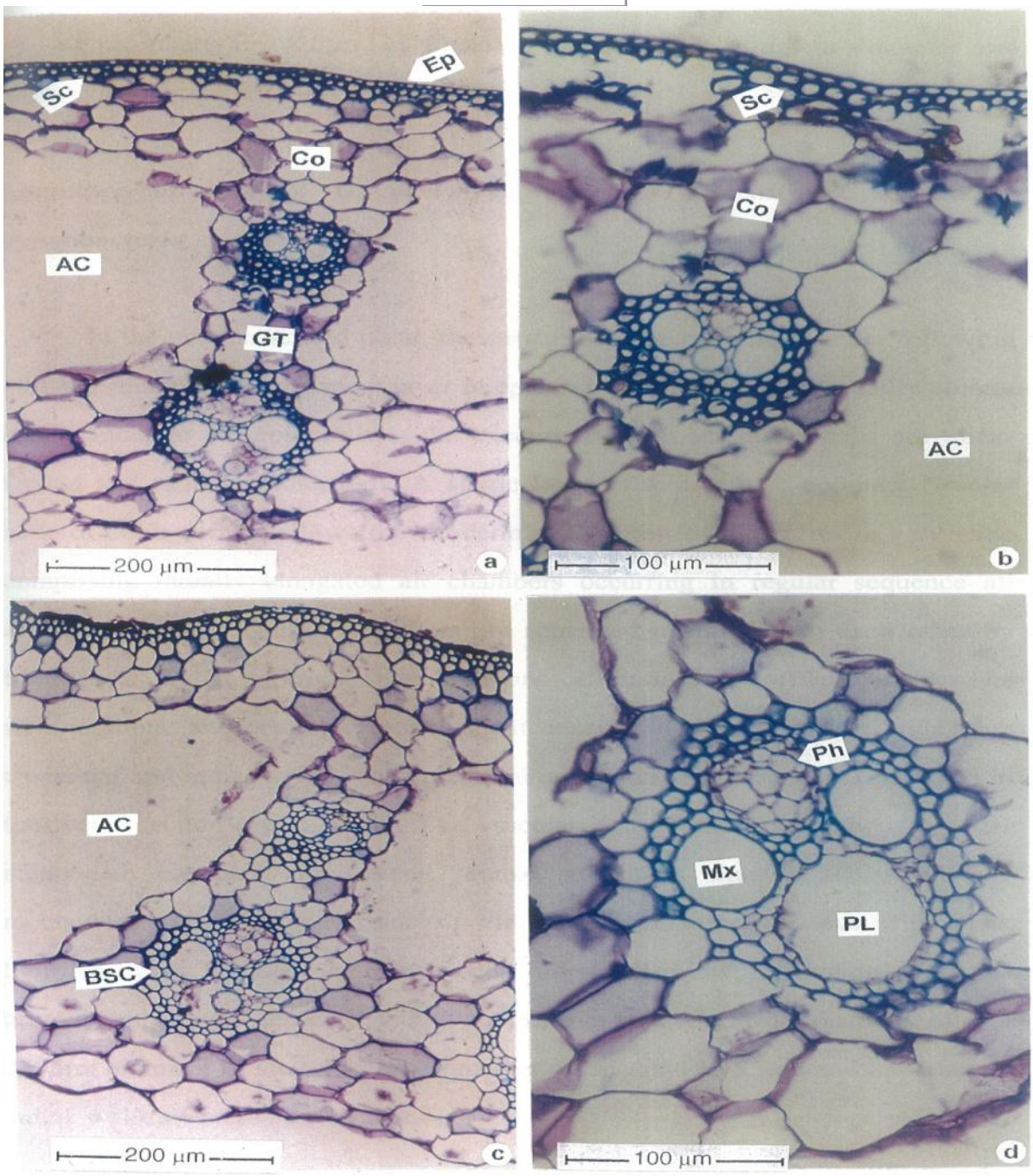

Plate: 2 Transverse section of underground culm of *O.sativa* L. *var*. ADT-43

a,c A sector of the culm of non-associated(a) and *O. foreaui* associated(c) plant b,d One vascular strand enlarged in non-associated(b) and *O. foreaui* associated(d) plant (AC – aerenchymatous cortex, BSC – bundle sheath cell, Co – cortex, Ep – epidermis, GT – ground tissue, Mx – metaxylem, Ph – phloem, PL – protoxylem lacuna, Sc - sclerenchyma)

115µm; vertical diameter of air chambers 250µm; diameter of metaxylem 30µm and width of protoxylem lacuna 42.5µm.

Structure of underground culm of *O.sativa* L. *var*. ADT-43 associated with *Oscillatoria foreaui*

The anatomical structure of culm of associated plant differs from the control plant in certain aspects. The most conspicuous difference is the reduction in the sclerenchymatous bundle sheath. In the associated plant, the bundle sheath fibres are thin-walled and the number of layers is also less (Plate 2c&d). Further, the metaxylem elements are very much dilated. Apart from these changes, the diameter of air chambers and diameter of vessels are increased. Thus, in the culm of associated plant, vascular bundles measure 230µm in diameter; the air chambers are 420µm in diameter; metaxylem is 40µm in diameter and protoxylem lacuna is 65µm wide (Table 1).

Plate 3

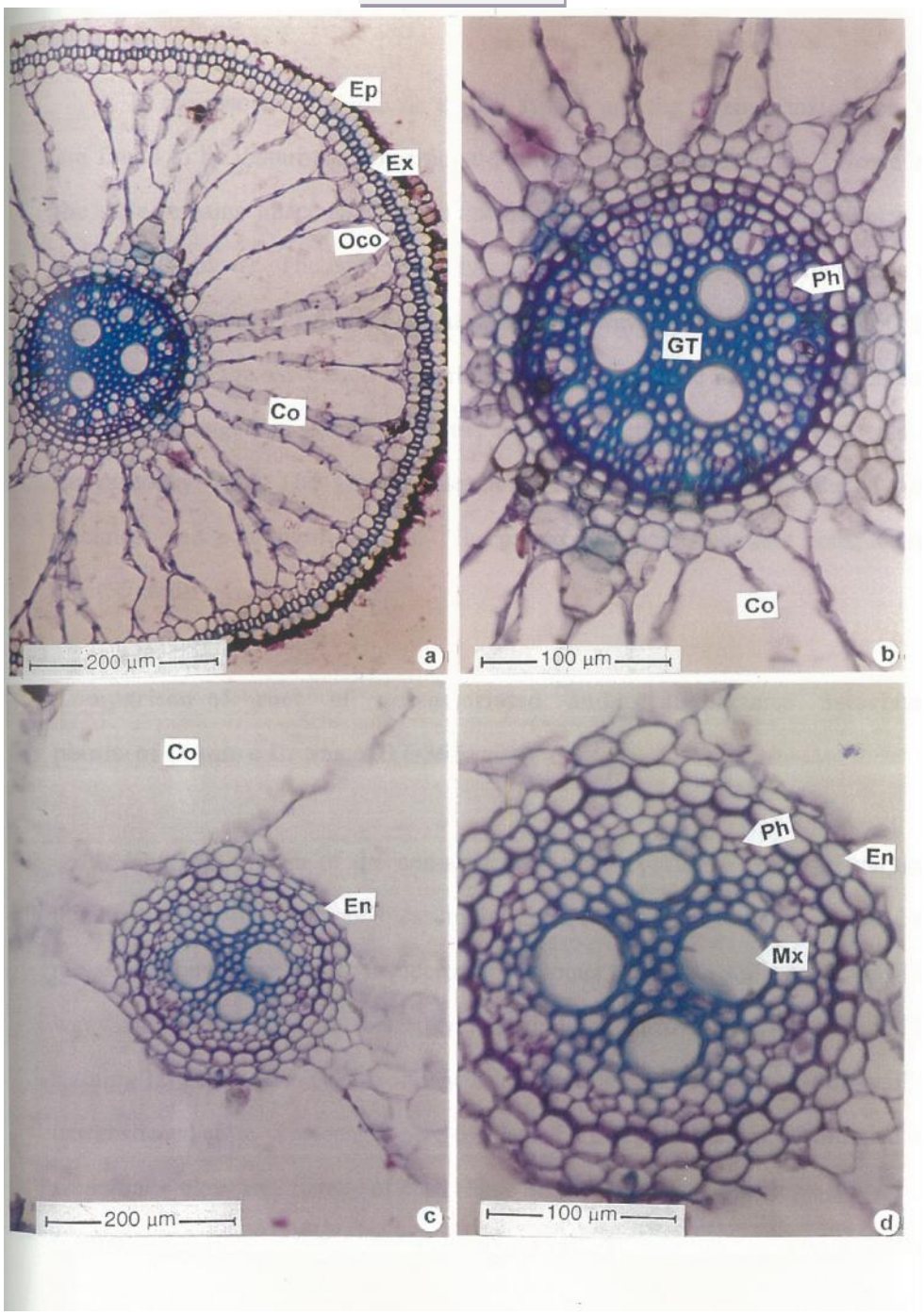

Plate: 3 Transverse section of root of *O. sativa* L. *var*. ADT-43

a. T.S. of non-associated root **b.** Stele of non-associated root enlarged **c & d** Stelar portion of root of *O. foreaui* associated plant under different magnifications (Co – cortex, En – endodermis, Ep – epidermis, Ex – exodermis, GT – ground tissue, Mx – metaxylem, Oco – outer cortex, Ph - phloem)

Structure of root of *O.sativa* L. *var*. ADT-43 without associating cyanobacteria

In the non-associated plant, the structure of the root exhibits the following features: There is an outermost layer of epidermis with somewhat radially oblong cells. Inner to the epidermis is the exodermal layer where the cells are thick-walled and radially elongated. A single layer of thin-walled, compact circular zone of outer cortex follows the exodermis. The rest of the cortex is very wide comprises radially elongated air chambers occurring in regular sequence all around the stele. The air chambers are separated laterally from each other by filamentous, radial partitions, which are one-cell in thickness (Plate 3a&b). The stele is single and central in position. It consists of an endodermis with thick tangential and radial walls. The individual endodermal cells appear 'U'

shaped in transverse section (Plate 3a&b). The vascular system consists of about 3 wider metaxylem elements and several protoxylem points situated all around the metaxylem. The ground tissue of the stele is sclerenchymatous with thick lignified walls. Phloem elements occur in small nests alternating with the protoxylem points. Measurements of control root are as follows: diameter of root 250µm; diameter of stele 125µm, diameter of metaxylem 25µm and endodermis radial wall thickness 5µm (Table 1).

Structure of root of *O.sativa* L. *var*. ADT-43 associated with *Oscillatoria foreaui*

In the associated plant, the dermal system and the aerenchymatous cortex are found to be disturbed, hence no structural details could decipher. However, the stele remains intact and exhibits some differences. The stele is 170 µm in diameter (Table 1). The endodermis has a characteristic 'U' shaped thickening of the inner tangential wall and radial walls. However, the radial walls of endodermis of associated root are thicker (7.5µm). The metaxylem elements remain unchanged in number, but the diameter of the metaxylem elements is increased by 5µm. The ground sclerenchymatous elements have reduced wall thickness and poor lignifications. The protoxylem points are less conspicuous (Plate 3c&d).

DISCUSSION

Among 17 strains of *Nostoc* tested for infection or association with *Gunnera manicata*, only 9 strains established symbiosis under laboratory conditions (Johansson and Bergman, 1994). It may be pointed out here that colonization or formation of cyanobacterial association is specific to individual isolates and hosts. This is also observed in studies on co-cultivation of strains of *Nostoc* and *Anabaena* with wheat, maize, bean, sugar beet and rice (Svircev *et al*., 1997). In the present study, which aims at the enhancement of total biomass of the paddy plant through subjecting the plant to biological association of cyanobacteria, cell constituents as well as cell structure have been taken as the yard sticks to assess the impacts of experimental association of the cyanobacteria with the plant. The significant result is with reference to the increased diameter of vessels in all organs of plants associated with cyanobacteria. Vessels are the principal water conducting conduits and these cells play significant role in the physiology of plant. Regarding the diameter of vessels, especially in perennial plants, it has been held that wider vessels are more efficient in water conduction. Cross-sections of roots exposed to Al concentrations were observed as more disordered than those of the control roots (Alvarez *et al*., 2012). Research in functional aspect of vessels has reached a consensus of opinion for the correlation between vessel diameter and efficiency in term of maximal water conductivity (Carlquist, 1975; Bass, 1976; Zimmermann, 1983). In the present study, the plants which become associated with the cyanobacterial colonies have uniformly wider vessels than those that are under control. For evaluating the conductivity of vessel, fourth power of a vessel diameter is to be calculated (Zimmermann, 1983). As per this generally accepted concept, a small increase in vessel diameter results in manifold increase in total water flow. This leads to a logic that the plants associated with cyanobacteria will have more efficient water transport system, which renders the plant active metabolic processes. The air chambers in paddy plant serve as the aerating system for gaseous exchange. Wider air chambers in cyanobacteria-associated plants are added advantage for the efficient physiological activities. Efficient associations between N_2 fixing cyanobacteria and non-legumes have been studied earlier by Allah et al. (2011). It may be inferred from the results of the anatomical data obtained from the present study that increase in vessel diameter as well as volume of the air-chambers may have a positive physiological correlation of the plants with the biological association of the cyanobacterial organism.

REFERENCES

Allah, F., El-Komy, H.M., Al-Harbi, N.A. and Sholkamy, E.N. 2011. *In vitro* creation of artificial nitrogen fixing cyanobacterium (*Nostoc muscorum*) association with wheat. *African J. of Microbiol. Res.* 5(3): 302-310

Alvarez, I., SAM, O., Reynaldo, I., Testillano, P., Risueno, M.D.C. and Arias, M. 2012. Morphological and cellular changes in rice roots (*Oryza sativa* L.) caused by Al stress. *Botanical Studies*, 53: 67-73.

Baas, P. 1976. Some functional and adaptive aspects of vessel member morphology. *Leiden Botanical Ser.* 3: 157-181.

Carlquist, S. 1975. Ecological strategies of xylem evolution. Univ. California Press, Berkeley. pp. 259.

Fogg, G.E., Stewart, W.D.P., Fay, P. and Walsby, A.E. 1973. The blue-green algae. Academic press, London, England.

Foster, A.S. 1934. The use of tannic acid and iron chloride for staining cell wall in meristematic tissue. *Stain Technol*. 9: 91-92.

Gantar, M. 2000. Co-cultivation of N_2-fixing cyanobacterium *Nostoc* sp. Strain 2S9B and wheat callus. *Symbiosis*. 29: 1-18.

Gantar, M., Kerby, N.W., Rowell, P. and Obreht, Z. 1991. Colonization of wheat (*Triticum vulgare* L.) by nitrogen fixing cyanobacteria: I. A survey of soil cyanobacterial isolates forming associations with roots. *New Phytol*. 118: 477-483.

Johansson, C. and Bergman, B. 1994. Reconstitution of the symbiosis of *Gunnera manicata* Linden: Cyanobacterial specificity. *New Phytol*. 126: 643-652.

Kannaiyan, S. 1985. Studies on the algal application for low land rice crop. *Tamil Nadu Agric. Univ. Bull.*, Coimbatore, TN pp. 16.

O'Brien, T.P., Feder, N. and Mc Cull, M.E. 1964. Polychromatic staining of plant cell walls by toluidine blue. *Protoplasma*. 59: 364-373.

Rai, A.N. and Bergman, B. 2002. Creation of new nitrogen-fixing cyanobacterial associations. Proceedings of the Royal Irish Academy 102B: 65-68.

Rippka, R., Deruelles, J., Waterbury, J.B., Herdman, M. and Stanier, R.Y.1979. Generic assignments, strain histories and properties of oure cultures of cyanobacteria. *J. Gen. Microbiol*. 111: 1-61.

Sass, J.E. 1957. Elements of botanical microtechnique. McGraw Hill Book Co. New York.

Singh, R.N. 1961. Role of blue-green algae in nitrogen economy of Indian Agriculture. ICAR, New Delhi. India pp. 175.

Svircev, Z., Tamas, I., Nenin, P. and Drobac, A. 1997. Co-cultivation of N_2-fixing cyanobacteria and some

agriculturally important plants in liquid and sand cultures. *Appl. Soil Ecol*. 6: 301-308.

Toledo, G., bashan, Y. and Soeldner, A. 1995. Cyanobacteria and black mangroves in North Western Mexico: colonization, diurnal and seasonal nitrogen fixation on aerial roots. *Can. J. Microbiol*. 41: 999-1011.

Venkataraman, G.S. 1981. Blue-green algae for rice production – A manual for its promotion. *FAO Soils Bulletin No*. 46, pp. 102.

Zimmermann, M.H. 1983. Xylem structure and the ascent of sap. Springer-Verlag Publ., Berlin pp. 143.

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