



ANATOMICAL RESPONSES OF RICE (*ORYZA SATIVA L.*) TO ALUMINIUM TOXICITY

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ABSTRACT

Anatomical response of rice (*Oryza sativa L.*) to aluminium toxicity grown in sand culture and half strength Hoagland solution revealed that aluminium stress caused a decrease in diameter of the root and the shoot. Aluminium toxicity reduced the number of metaxylem vessels in the root of rice. Number of sclerenchyma cells was more in aluminium-treated rice root. Under Al stress, smaller sized vascular bundles were found in the leaf of rice. Size and frequency of bulliform cells were increased in the leaf of Al-treated plants than that in control plants. Al treatment caused closure of stomata in rice leaves.

Key words: Aluminium toxicity, Anatomy, Rice, *Oryza sativa* and Sand culture.

INTRODUCTION

Aluminium is the most abundant metal and the third most common element in the earth's crust. In many acid soils throughout the world, aluminium is the most growth-limiting factor (Foy 1988), possibly affecting upto 70% of the world's arable land that is potentially usable for food and biomass production (Haug and Caldwell 1985).

Plants respond to various stresses through morphological, anatomical and physiological adjustments that help them to cope with the stress. The size of protoxylem and metaxylem vessels were smaller and showed deformed appearance in maize plant after aluminium treatment (Batista *et al.* 2012). Due to Al exposure, reduced length was found in the meristematic and elongation zones of barley root (Kochian 1995). Aluminium treatment resulted in closure of stomata (Rengel 1992). Presence of Al was found to reduce chlorophyll content in barley (Abdalla *et al.* 2008).

Rice is the staple food crop in Asia, and its productivity is drastically affected in acid upland

soils as well as acid sulfate soils due to Al toxicity (Matsumoto 2000).

Rice (*Oryza sativa L.* var. BRRI Dhan-53) was used as experimental plant material because there are only few reports on the changes in anatomical structure of rice caused by aluminium toxicity.

MATERIALS AND METHODS

Seeds of rice (*O. sativa* var. BRRI Dhan-53) were collected from Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur. The experiments were conducted in net house of Department of Botany, University of Dhaka, under the normal environmental conditions.

Plants were grown in sand culture (Hewitt 1966) and subjected to half strength Hoagland solution (pH 4.2) which served as control. Similarly, 150 and 300 μM AlCl_3 solution made in half strength Hoagland solution (pH 4.2) were applied to each pot containing 7-day-old seedlings which were used as treatments. Later on, half strength Hoagland solutions (pH 4.2) was applied to

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control plants and 150 and 300 μM AlCl_3 solutions (pH 4.2) were applied to respective Al-treated plants every day up to 28 days. The root, stem and leaf of rice plants were collected at 28-day of aluminium exposure. Stem and root segments were taken, respectively, 1 cm above and 1 cm below the transition region where the stem and root meet. Free hand sectioning was done and the sections were stained with saffranin. Transverse sections of the root, stem and leaf were studied with a compound microscope. Photographs of sections were taken using a camera (Axiocam ERc 5s) at different magnification (5X, 10X and 40X). Leaf stoma and trichome of 28-day-old control and Al-treated plants grown in sand culture were also studied.

RESULTS AND DISCUSSION

Effects of aluminium toxicity on anatomy of the root: In the root of rice, significant structural changes occurred due to aluminium stress. Under the stressed condition, root length was decreased as compared to that of control root (Plate 1). Silva *et al.* (2000) showed that within just 30 min, 100 μM Al entered in cell symplast, accumulating in the nucleus of meristematic cells of soybean roots, causing a decrease in root growth. In Al-treated root, the cortical cells presented a higher amount of intercellular space and its cells were elongated longitudinally (Plates 2 and 4). According to Batista *et al.* (2012), due to 75 and 300 $\mu\text{mol L}^{-1}$ Al treatment in maize root, the cortical parenchyma cells consisted of prominent intercellular spaces, which was a form of adaptation. Sclerenchymatous layer was uniseriate both in control and 150 μM Al treated plant but biserrate in 300 μM Al-treated plant (Plate 4). The number of metaxylem vessels was reduced than

that of the control plants. The number of metaxylem vessels were four whereas in 150 and 300 μM Al-stressed root, the number was reduced to three and two, respectively. On the other hand, in 300 μM Al-treated root, the diameter of metaxylem vessels was increased (Plates 2 and 3). Similarly, due to 300 $\mu\text{M L}^{-1}$ Al, Batista *et al.* (2012) found that, in the vascular bundle, the metaxylem and protoxylem had no secondary walls and their diameter was much smaller compared to that of control plants. This study demonstrated that higher concentration of Al caused structural impairment. At 300 $\mu\text{mol L}^{-1}$ Al, the metaxylem and protoxylem did not reach maturation so they lacked a definite shape, had a reduced diameter and a poorly developed perimedullar region.

Effects of aluminium toxicity on anatomy of the stem (internode): In the stem (internode) of Al-treated rice plant, few anatomical differences were observed in relation to the control plants. Adaxial to epidermis thick layer of sclerenchymatous cells were found in Al-treated plant (Plate 5). Sclerenchymatous layer was many in number in 300 μM Al-treated plant as compared to that of control plant. In the rice internode, two types of vascular bundles were found and they were arranged in two rings. The abaxial bundles were attached to the adaxial sclerenchyma cells of hypodermis. Size of the vascular bundles was decreased in Al-treated plant. In both small and large bundles, two metaxylem vessels, one protoxylem vessel were found. The protoxylem lacuna was found in large bundle, vascular bundles were covered by bundle sheath. The metaxylem vessels of Al-treated plant were smaller in size than that of control plant. Phloem area decreased in Al-treated plant (Plate 5).

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Effects of aluminium toxicity on anatomy of the leaf blade: In the control leaf (midrib region), parenchyma cells were well developed but in the leaf of Al-treated rice, poorly developed parenchyma cells were observed (Plate 6). Number of vascular bundles decreased in midrib region of Al-stressed plant. Vascular bundles of the leaf blade were completely surrounded by parenchymatous bundle sheath. Diameter of metaxylem vessel was also decreased in the leaf of Al-treated plant (Plate 7). Mesophyll tissue of the leaf was fewer in number in Al-treated plant. Amount of chlorophyll was also reduced in the leaf of Al-stressed plant as compared to that of control (Plate 8). Similarly, Mihailovic *et al.* (2008) observed in maize, that, chlorophyll content was severely affected by 200 μM Al and concluded that aluminium might affect on the uptake and transport of several essential mineral nutrients required for chloroplastidic pigment biosynthesis. Ruan *et al.* (2011) reported that Al toxicity caused cell dehydration, which resulted in reduced leaf thickness in *Acacia melanoxylon*. According to Qian *et al.* (2014), aluminium (0.1 and 0.3 mM) damaged the mesophyll cells of oilseed rape because aluminium-induced stress led to dissolved thylakoid membranes and disrupt all the organelles inside the cell.

Bulliform cells are large, thin-walled, colorless, bubble-shaped epidermal *cells* that occur in groups on the adaxial surface of the leaf of rice. In the leaf blade, bulliform cells were observed in the leaf of control and Al-treated rice plant. Size and frequency of bulliform cells were increased in the leaf of Al-treated plant. Midrib region lacked bulliform cells (Plate 8).

Effects of aluminium toxicity on stomata of leaf: Stoma is a tiny pore in a leaf surrounded by a

pair of guard cells. Graminaceous type of stomata were observed in the leaf of rice plant. In the leaf of Al-treated plant, the number of the stomata was increased whereas stomatal opening was reduced. Due to aluminium treatment, the guard and subsidiary cells also became reduced in size than those of control plant (Plate 9). Developing smaller but more densely distributed stomata is seen as an adaptation in leaves growing under conditions of different stresses, which allows a leaf to reduce transpiration by regulating stomatal mechanisms more rapidly (Larcher 1995). In tea, Al toxicity reduced stomatal opening and thus, reduced stomatal conductance culminated in decreased internal CO₂ and photosynthesis (Mukhopadhyay *et al.* 2012). Al (100 μM) treatment was found to induce stomatal closure in tobacco, which is usually considered as a sign of transpiration inhibition (Sivaguru *et al.* 1999).

CONCLUSION

Aluminium stress decreased the number and diameter of xylem vessels of the root, stem and leaf of rice plant (Plates 2, 3, 5 and 7). Aluminium-induced decrease in number and diameter of xylem vessels would decrease the translocation of ions from the root to the shoot and thus adversely affect the distribution of ions in different parts of the plant. This would, in turn, slow down the metabolic processes in different organs of plant because ions act as cofactors of enzymes involved in numerous metabolic reactions. Aluminium toxicity caused the closure of stomata in rice leaves (Plate 9). Al stress-induced closure of stomata might decrease the rate of photosynthesis due to the decrease in CO₂ diffusion through the stomata.

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Plate 1. Effects of aluminium toxicity on the root and shoot length of rice plants grown in sand culture.

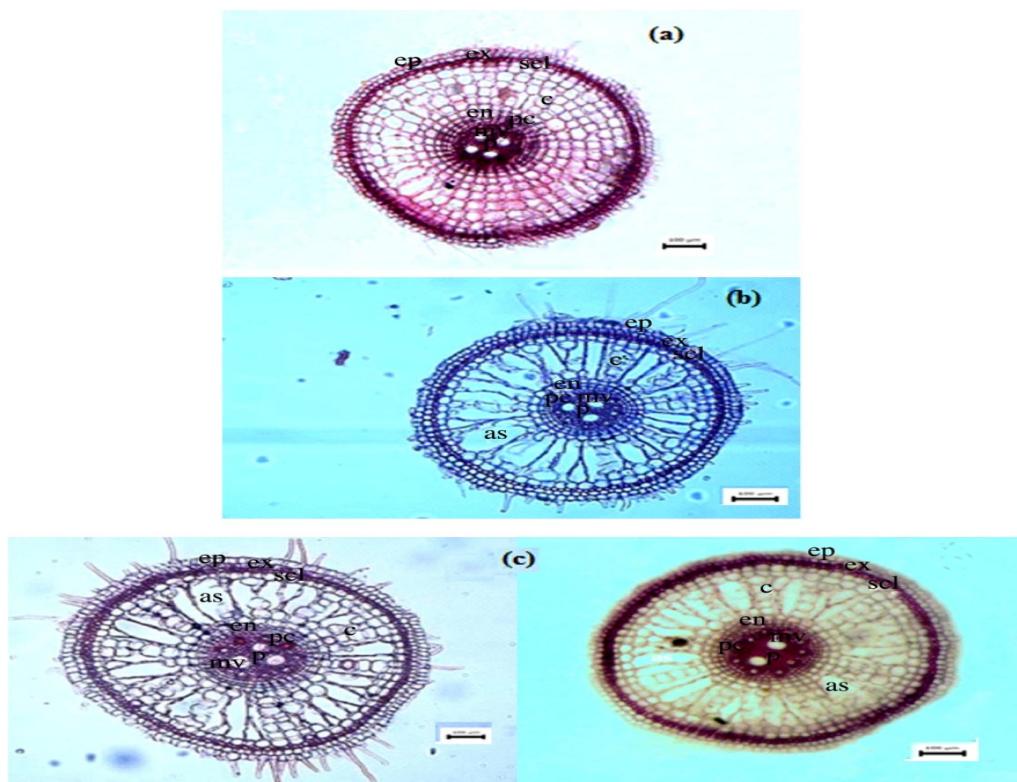


Plate 2. Transverse section of the root of rice (a) control, (b) 150 μM Al and (c) 300 μM Al-treated plant showing epidermis (ep), exodermis (ex), sclerenchyma (scl), cortex (c), endodermis (en), pericycle (pc), metaxylem vessel (mv), pith (p) and air space (as). Bar = 100 μm

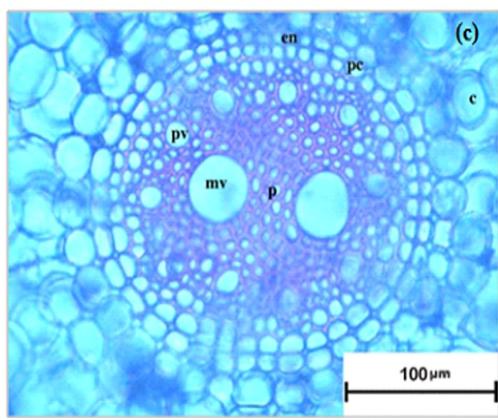
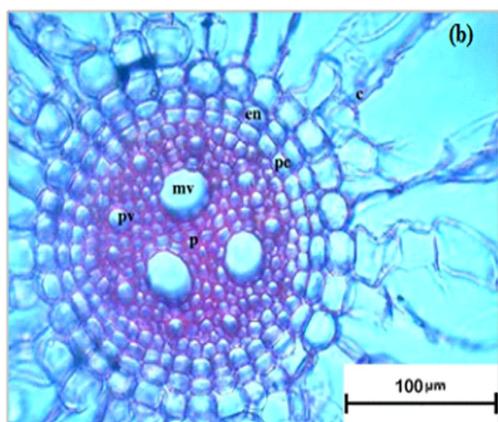
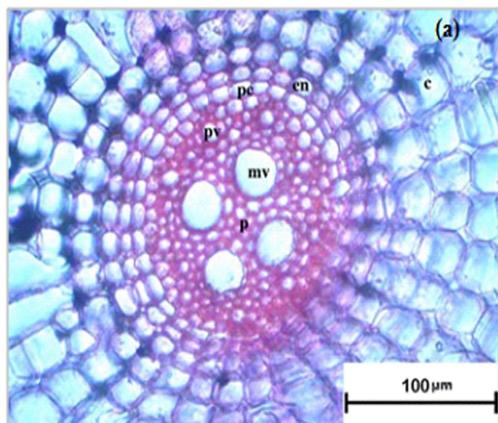


Plate 3. Same as plate 2 but of higher magnification showing cortex (c), endodermis (en), pericycle (pc), metaxylem vessel (mv), protoxylem (pv) and pith (p). Bar = 100 μ m.

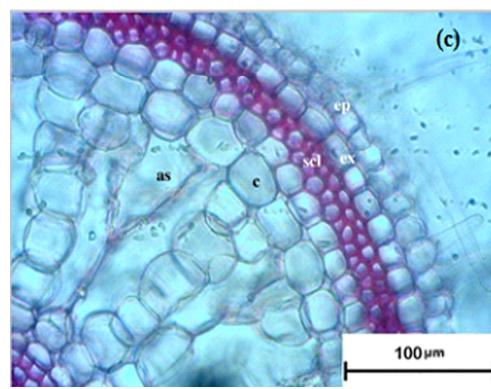
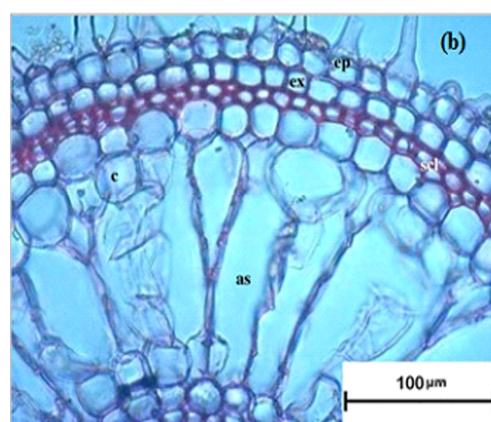
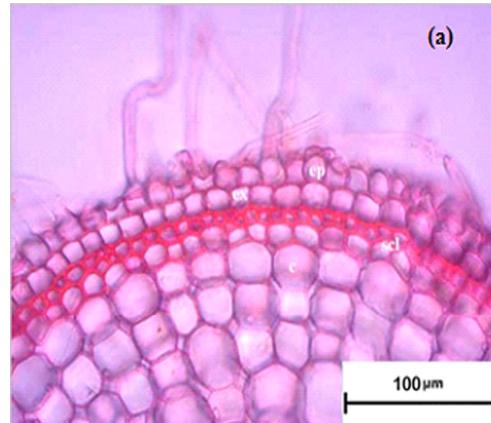


Plate 4. Same as plate 2 but of higher magnification showing epidermis (ep), exodermis (ex), sclerenchyma (scl), cortex (c) and air space (as). Bar = 100 μ m.

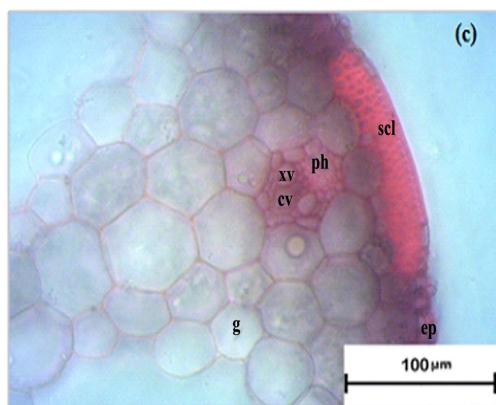
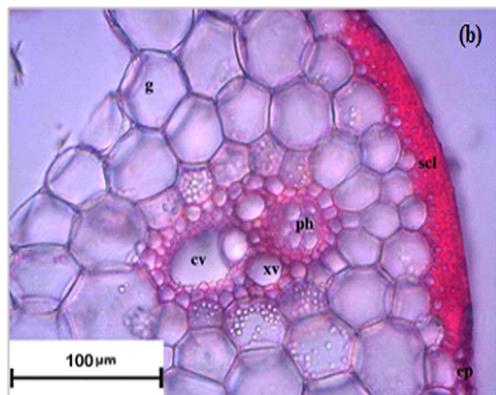
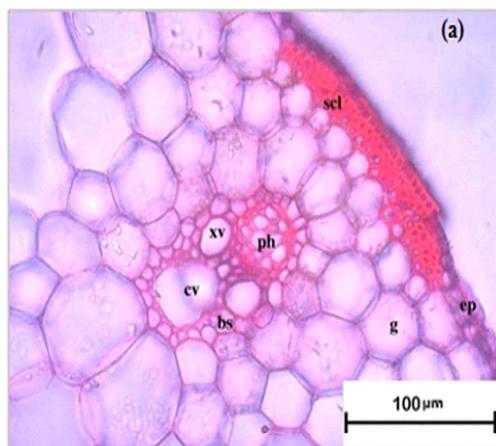


Plate 5. Transverse section of the stem (internode) of rice (a) control, (b) 150 μM Al and (c) 300 μM Al-treated plant showing epidermis (ep), sclerenchyma (scl), ground tissue (g), phloem (ph), xylem vessel (xv) and protoxylem cavity (cv). Bar = 100 μm .

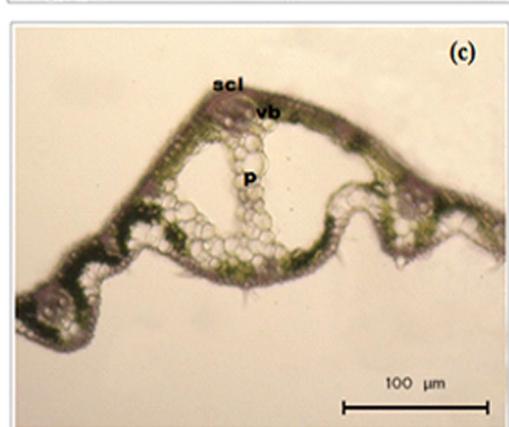
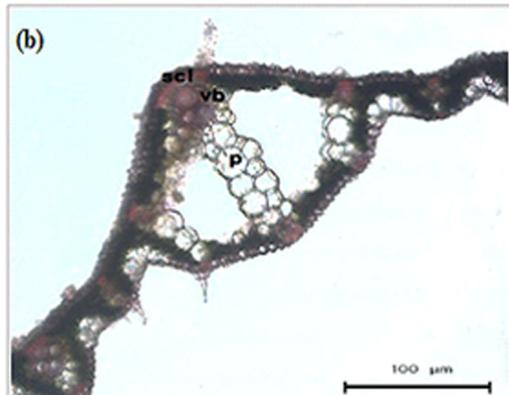
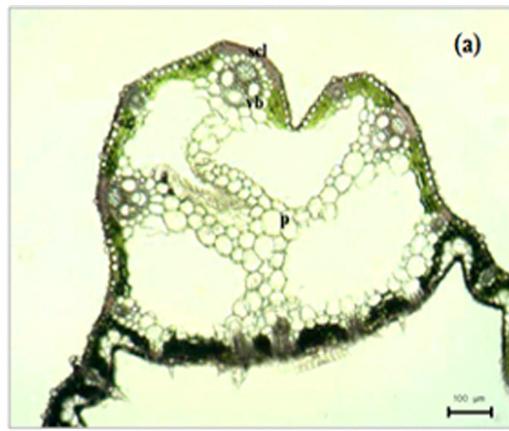


Plate 6. Transverse section of the leaf (midrib rea) of rice (a) control, (b) 150 μM Al and (c) 300 μM Al-treated plant showing sclerenchyma (scl), vascular bundle (vb) and parenchyma cell (p). Bar = 100 μm .

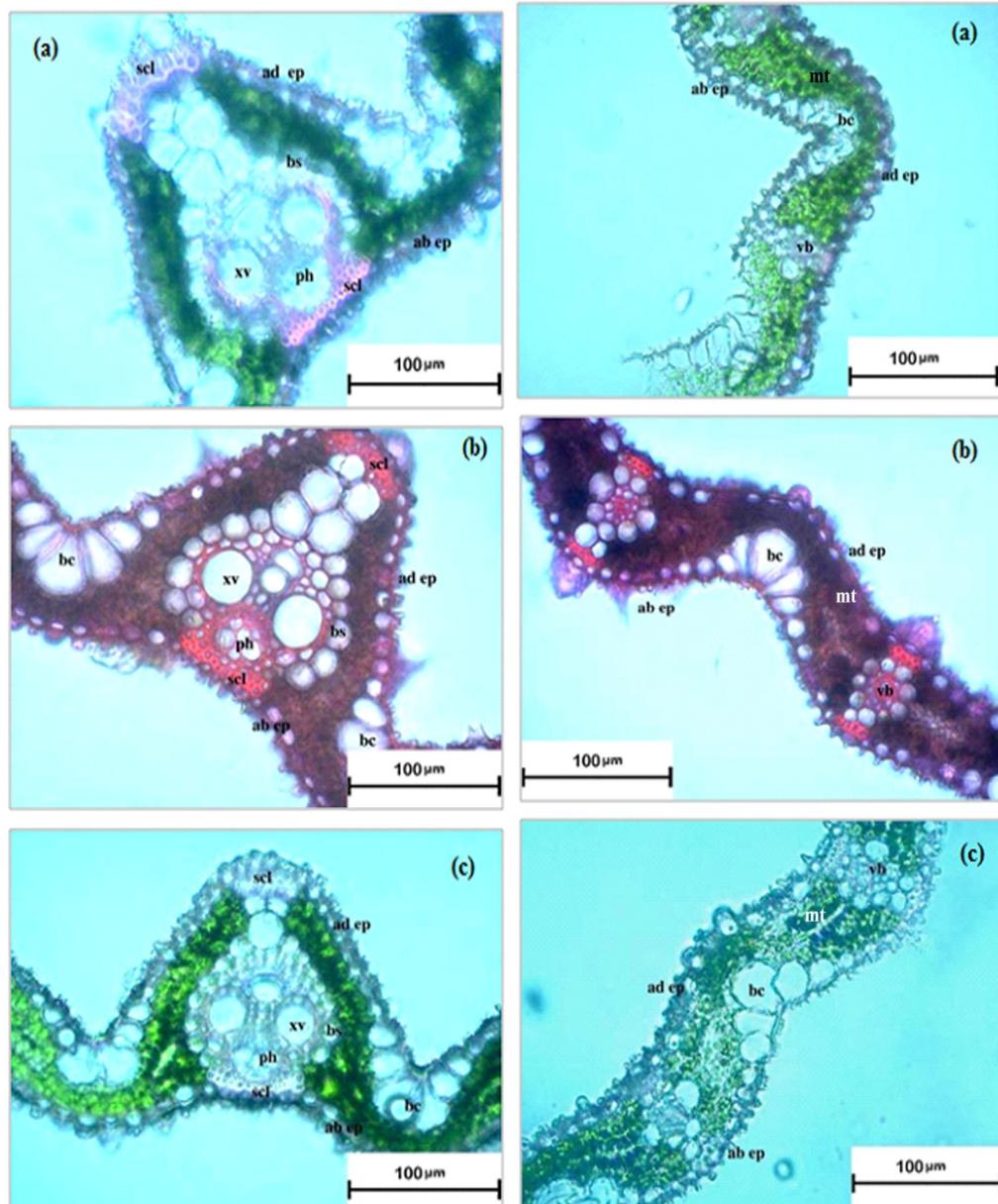


Plate 7. Transverse section of the leaf blade of rice (a) control, (b) 150 µM Al and (c) 300 µM Al-treated plant showing adaxial surface epidermis (ad ep), abaxial surface epidermis (ab ep), sclerenchyma (scl), xylem vessel (xv), phloem (ph), bundle sheath (bs) and bulliform cell (bc). Bar = 100 µm.

Plate 8. Transverse section of the leaf blade of rice (a) control, (b) 150 µM Al and (c) 300 µM Al-treated plant showing adaxial face epidermis (ad ep), abaxial face epidermis (ab ep), vascular bundle (vb), mesophyll tissue (mt) and bulliform cell (bc). Bar = 100 µm.

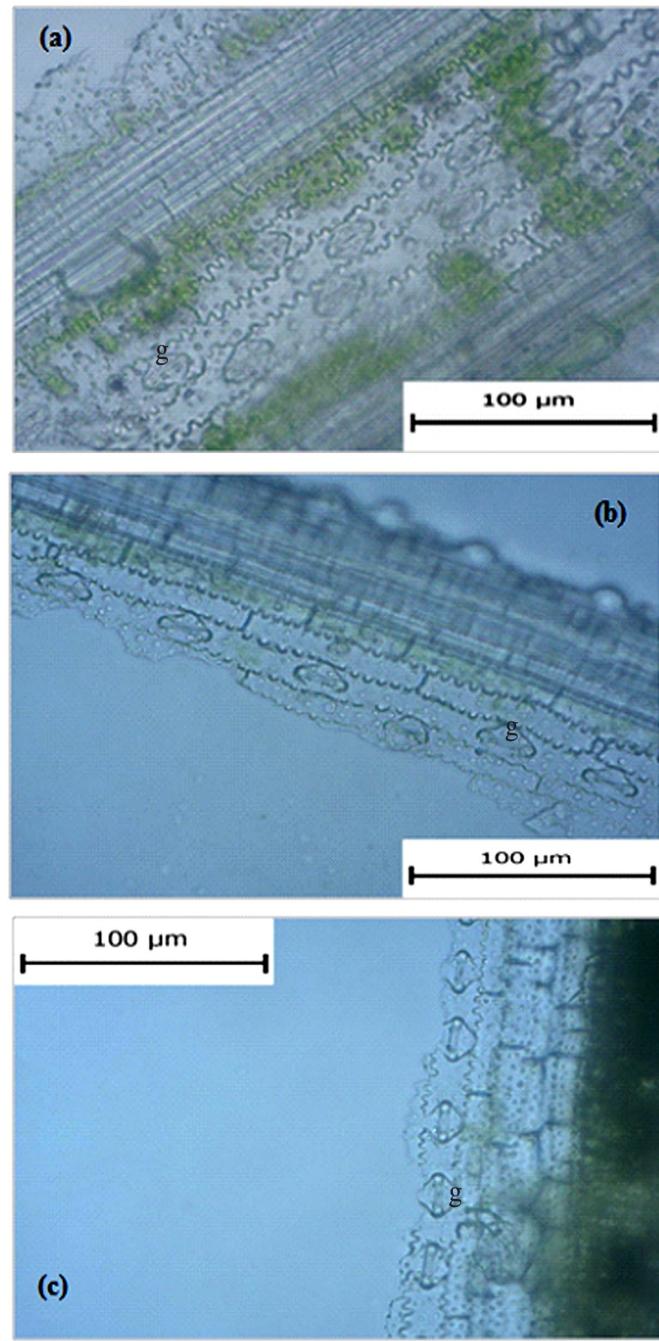


Plate 9. Peel of the leaf of rice (a) control, (b) 150 μM and (c) 300 μM Al-treated plant showing stomata and guard cell (g). Bar = 100 μm .