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ANATOMICAL CHANGES IN CHICKPEA (*CICER ARIETINUM* L.) UNDER ALUMINIUM STRESS CONDITION

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Abstract

An experiment was conducted to investigate the effect of aluminium (Al) toxicity on the anatomical changes in the root, stem and leaf of chickpea (*Cicer arietinum* L.) plants grown in sand culture. Toxicity of Al reduced the length of primary root and the number of lateral roots of chickpea than that of the control. Aluminium decreased the size and number of vessels in the root of chickpea. Larger area of sclerenchyma cells was noticed in the stem of Al-stressed plant. Number of palisade parenchyma was reduced in the leaf of chickpea. Aluminium treatment caused closure of stomata. Increased number of trichomes in chickpea leaves was also reported due to aluminium.

Introduction

Aluminium is the third most abundant element (after oxygen and silicon) of all elements in earth crust. The total Al concentration in the soil and the forms of Al species depend on the soil pH and the chemical environment of the soil solution(1). Under acidic conditions (pH <5), mineral Al solubilizes into trivalent Al^{3+}, which is highly toxic to organisms(2). To cope with the hostile environment, plants respond to various stresses through morphological, physiological and anatomical changes. Due to Al exposure, reduced length was found in the root cortex of maize(3). Gomes *et al.*(4) reported that, toxicity of Al, increased the leaf epidermis thickness of the adaxial and abaxial surface of *Brachiaria decumbens* (signal grass). The size of leaf mesophyll cells of pitch pine seedlings was reduced after Al treatment(5). In Roman nettle, stomatal sizes were significantly decreased due to Al stress compared to that of control(6).

Chickpea is one of the most popular legumes known for its rich protein contents that contribute significantly to human diets. Therefore, chickpea plays a major role in improving human health and nutrition.Reports on the effect of Al stress in chickpea on anatomical changes are rare. Hence, this research was carried out to assess the influence of Al stress on the anatomical structure of the root, stem and leaf of chickpea grown in sand culture.

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Materials and Methods

Seeds of chickpea (*Cicer arietinum* L. var. BARI Chhola-7) were procured from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur. The experiments were conducted in net house of Department of Botany, University of Dhaka, under normal environmental conditions.

Plants were grown in sand culture(7) and subjected to half strength Hoagland solution(8) (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. Control and each treatment had three replications. Later on, half strength Hoagland solution (pH 4.2) was applied to control plants and 150 and 300 µM $AlCl_3$ solution (pH 4.2) were applied to respective Al-treated plants every day up to 28 days. The root, stem and leaf of chickpea plants were collected at 28-day of Al exposure. 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 stomata 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: Due to aluminium treatment, the length of primary root and the number of lateral roots of chickpea were reduced than that of the control root (Plate 1). In the root of control chickpea plant, isodiametric single layered epidermis was observed but in the root of Al-treated plant, the broken epidermal layer was found (Plate 2a, b and c). Budíková *et al.*(9) reported that the epidermis and peripheral cortex layers were more affected than the central cylinder cells in Al treated root. In the root of Al-treated chickpea plant, cortex cells occupied smaller area than that of control root (Plate 2a, b and c). The cortex composed of 16-17 layers of cells in the root of control plant whereas its thickness was 12-13 layers of cells in the root of 300 µM Al-treated plant. Similar changes in cortical cells have also been reported in rice root caused by Al stress(10). The most significant structural changes were occurred in the vascular system. The number of metaxylem vessels were reduced in the root of Al-treated plant. Smaller sized and irregular structured metaxylem vessels were also found in the root of 150 and 300 µM Al-treated chickpea plant (Plate 3a, b and c). As compared to that of control root, larger group of sclerenchyma cells were superimposed upon the phloem tissue (Plate 4a, b and c). Phloem was also smaller in size in the root of Al-treated plant. No remarkable change was observed in pith in the root of Al-treated chickpea. Similarly, Batista *et al.*(11) found in corn plant, that in vascular bundle, the metaxylem and protoxylem had no secondary walls and their diameter was much smaller compared to that of control plants.

Plate 1. Effects of aluminium toxicity on the primary root and number of lateral roots of chickpea plants grown in sand culture.

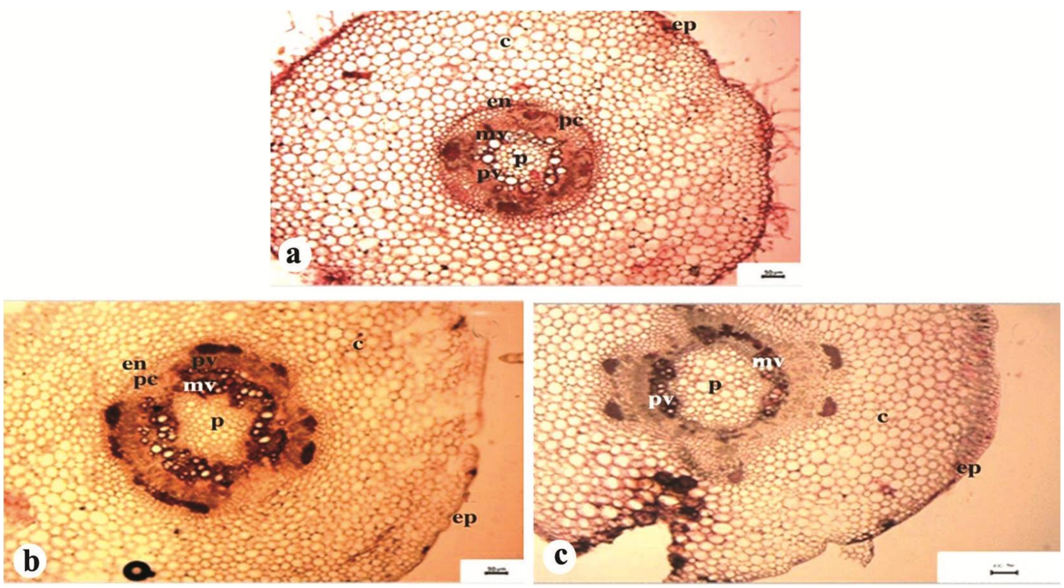
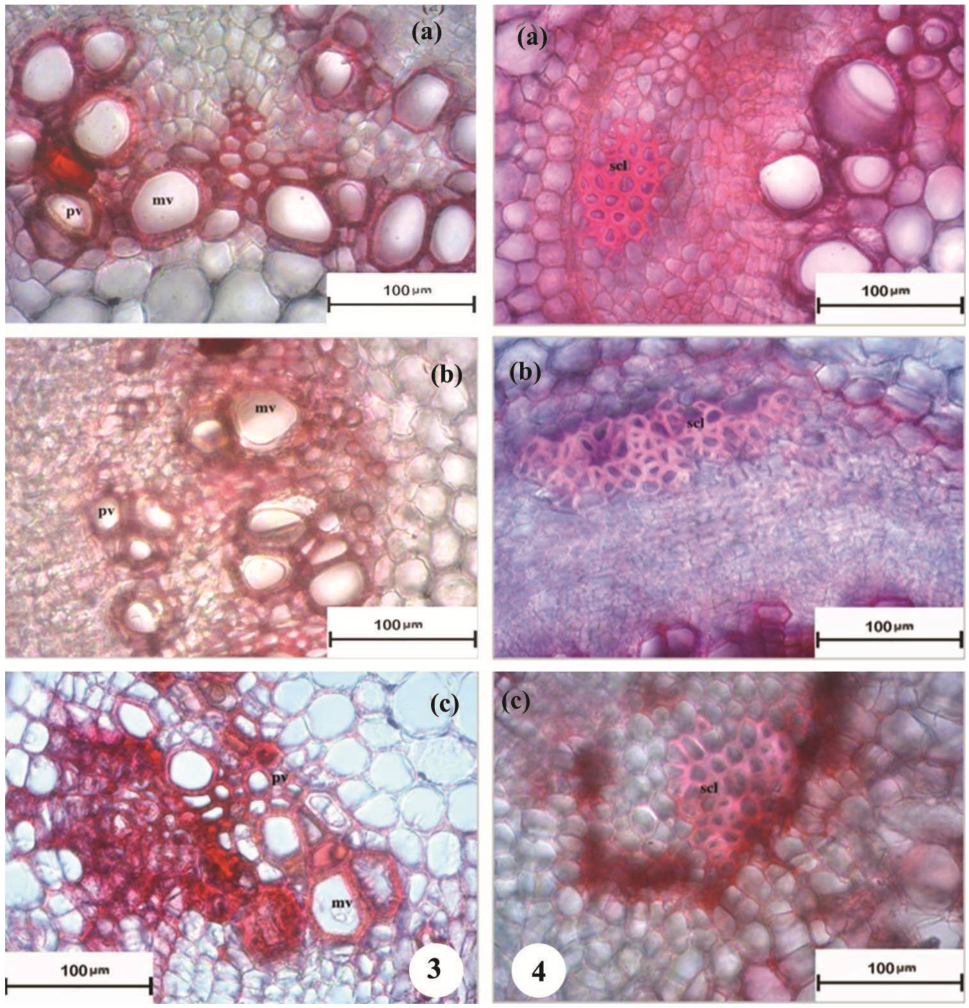
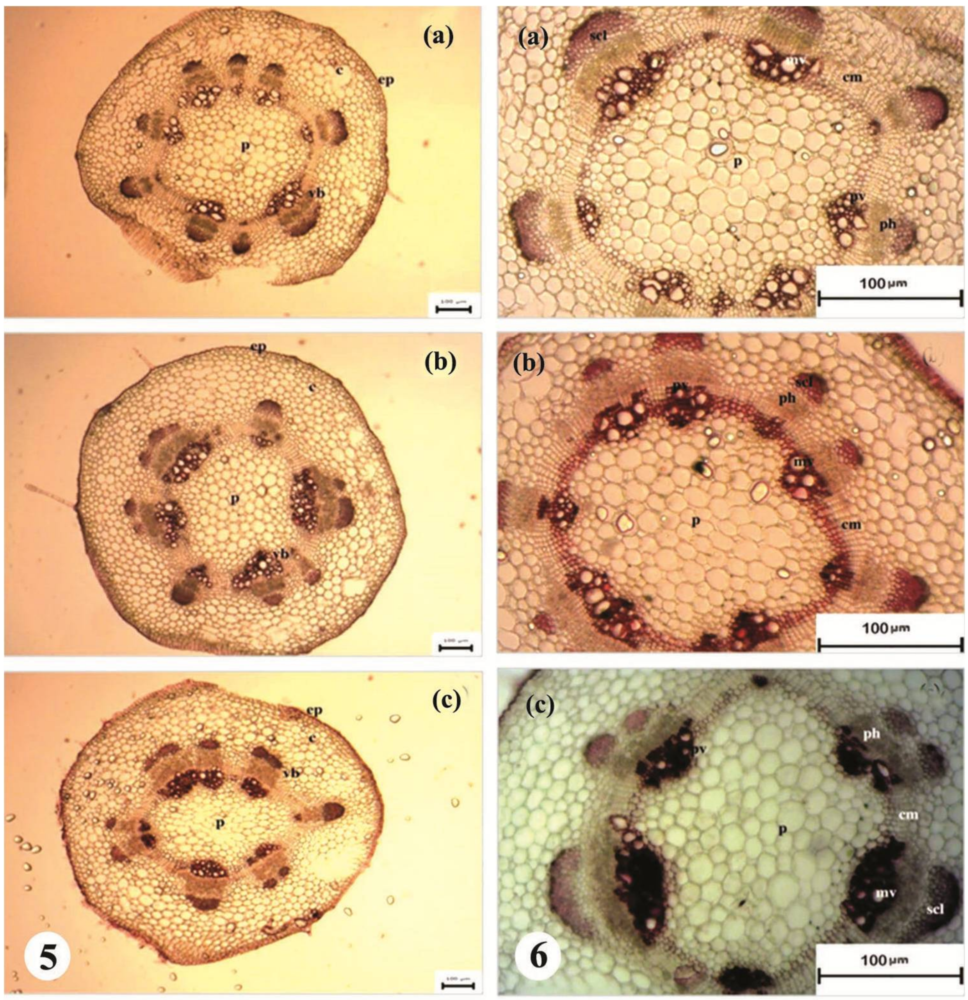


Plate 2. Transverse section of the root of chickpea (×5) (a) control, (b) 150 µM Al and (c) 300 µM Al-treated plant showing epidermis (ep), cortex (c), endodermis (en), pericycle (pc), metaxylem vessel (mv), protoxylem vessel (pv) and pith (p). Bar = 100 µm.

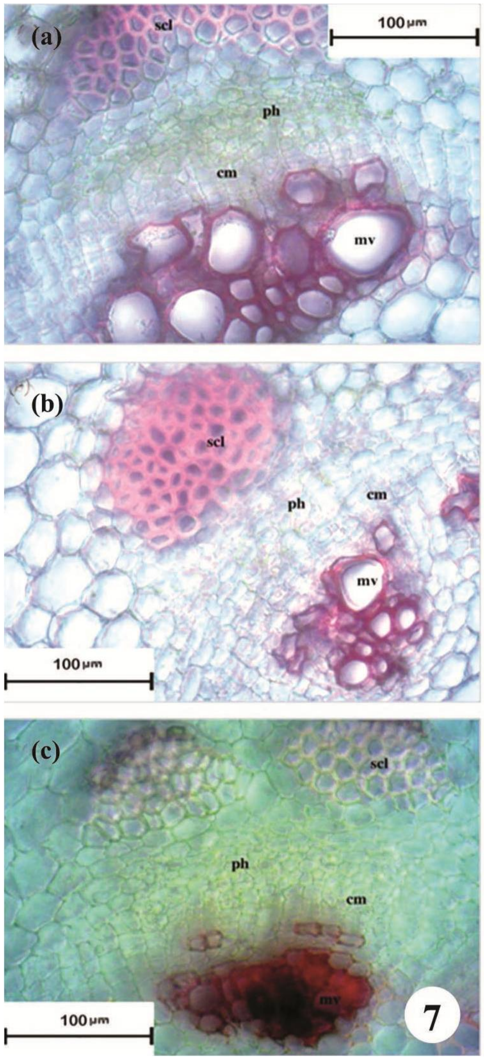


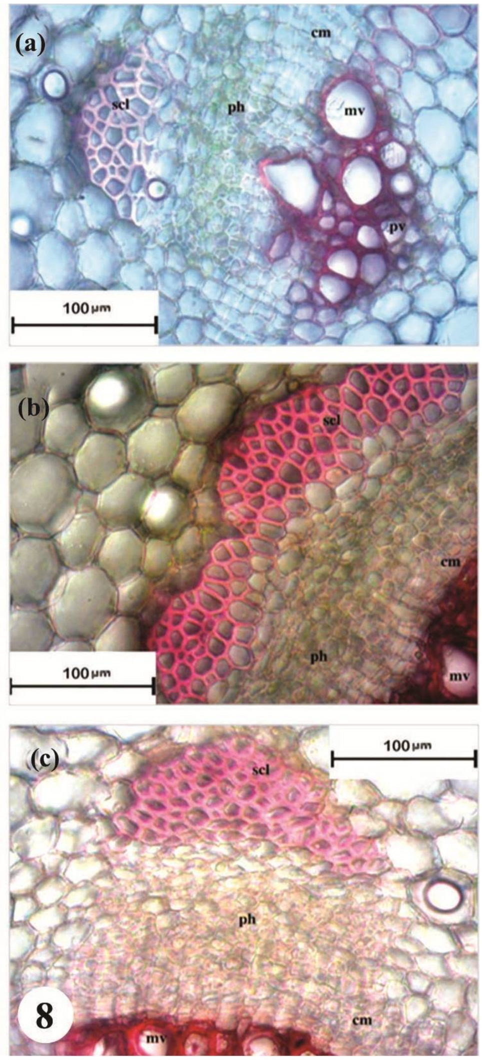
Plates 3-4: 3. Transverse section of the root of chickpea (×40) showing metaxylem vessel (mv) and protoxylem vessel (pv). Bar = 100 µm. 4. Same as plate 2 but of higher magnification (×40) showing sclerenchyma (scl). Bar = 100 µm.

Effects of aluminium toxicity on anatomy of the stem: In the stem (internode) of Al-treated chickpea plant, few anatomical differences were observed in relation to the control plants. The cortical cells of Al stressed stem occupied smaller area than that of the control stem in chickpea (Plate 5a, b and c). Application of Al reduced number of vascular bundles which were radially arranged. In some vascular bundles of Al-treated plant, there was poor development of xylem vessels (Plate 6a, b and c). Metaxylem vessels were reduced in the plant treated with aluminium than that of control plant. Reduced number and smaller sized metaxylem vessels were observed in the stem of Al-treated chickpea plant (Plate 7a, b and c). In Al-treated plant, cambium ring was thin compared to control. As compared to control, larger area of sclerenchyma cells was noticed in the stem of Al-stressed plant (Plate 8a, b and c). Smaller pith area was observed in Al-treated chickpea plant as compared to that of control plants (Plates 5a, b and c, and 6a, b and c). The region of the pith has also been found to be reduced and poorly developed in corn root due to Al toxicity(11).



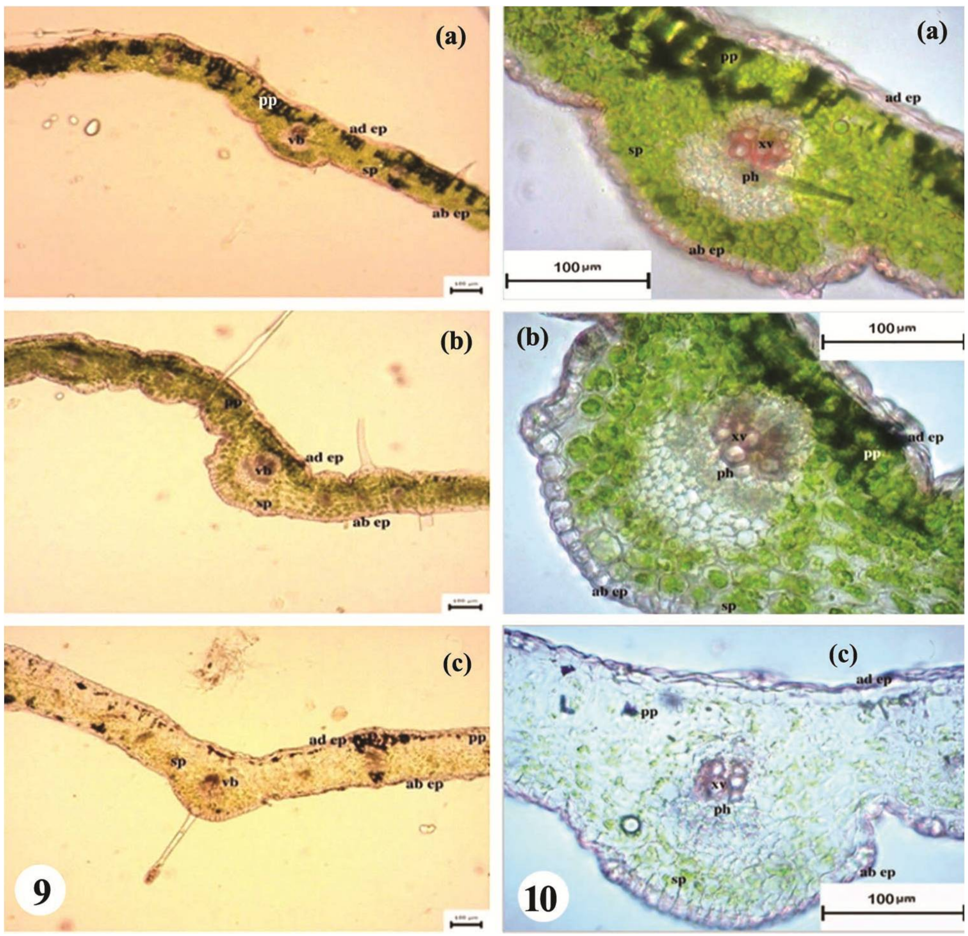
Plates 5-6: 5. Transverse section of the stem of chickpea (×5) (a) control, (b) 150 µM Al and (c) 300 µM Al-treated plant showing epidermis (ep), cortex (c), vascular bundle (vb) and pith (p). Bar = 100 µm. 6. Same as plate 5 but of higher magnification (×10) showing sclerenchyma (scl), metaxylem vessel (mv), protoxylem vessel (pv), cambium (cm), phloem (ph) and pith (p). Bar = 100 µm.





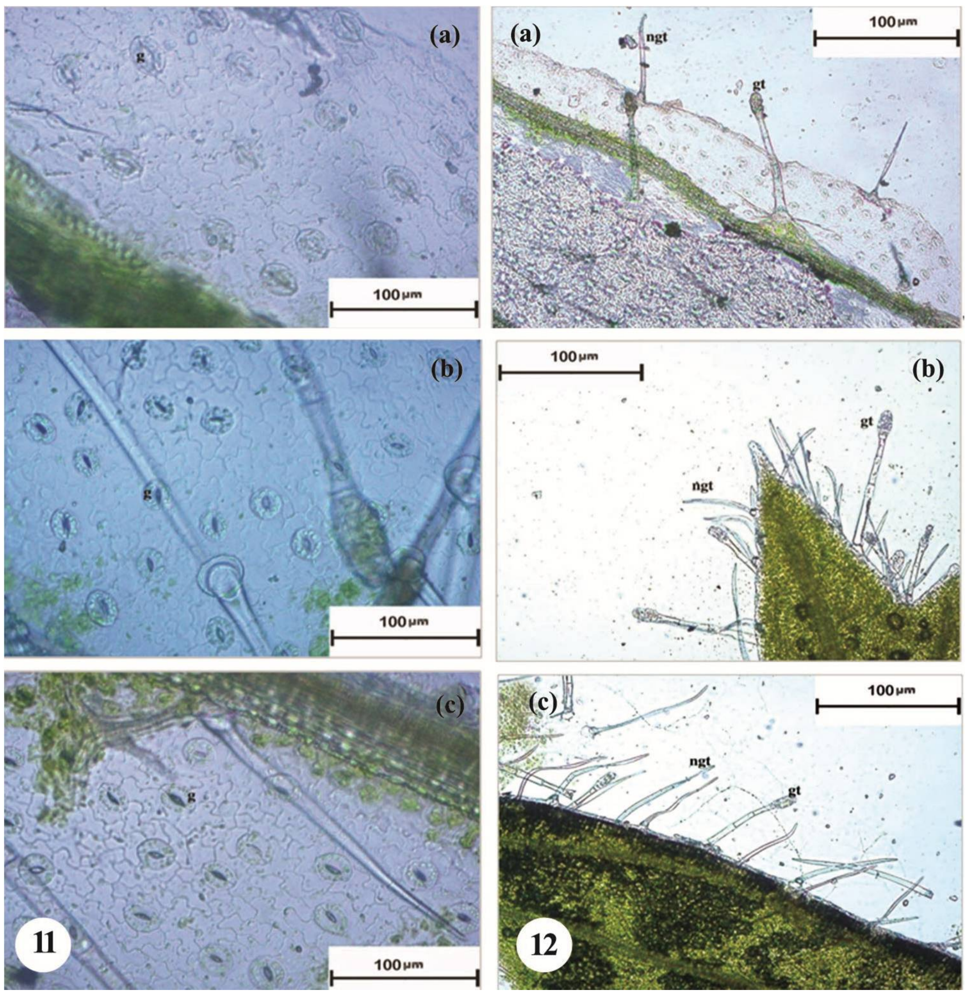
Plates 7-8: 7. Transverse section of the stem of chickpea (×40) showing sclerenchyma (scl), metaxylem vessel (mv), cambium (cm) and phloem (ph). Bar = 100 µm. 8. Same as plate 5 but of higher magnification (×40) showing sclerenchyma (scl), phloem (ph), cambium (cm), metaxylem vessel (mv) and protoxylem vessel (pv). Bar = 100 µm

Effects of aluminium toxicity on anatomy of the leaf: As compared to control plant, the epidermal cells were irregular and disorganized in size and shape in Al-stressed plants. In the leaf of Al-treated plants, the epidermal cells were comparatively smaller than those of the leaf of control plant (Plate 10a, b and c). On the other hand, Özyiğit *et al.*[12] observed no significant differences between epidermis (adaxial and abaxial) of control and Al-treated cotton plants. In the leaf of chickpea, amount of palisade and spongy parenchyma decreased following Al treatments (Plate 9a, b and c). In the leaf of Al-treated chickpea plants, both palisade and spongy parenchyma were reduced (Plate 9a, b and c, and 10a, b and c). Similarly, Özyiğit *et al.*(12) found that after Al exposure a structural degradation of palisade and spongy parenchyma was caused in cotton. The phloem area in Al-treated leaf of chickpea became smaller in relation to that of the leaf of control plant. In 300 µM Al-treatment, the number of xylem vessel in the leaf was reduced as compared to that of control plant (Plate 10a, b and c).



Plates 9-10: 9. Transverse section of the leaf of chickpea (×5) (a) control, (b) 150 µM Al and (c) 300 µM Al-treated plant showing adaxial surface epidermis (ad ep), abaxial surface epidermis (ab ep), palisade parenchyma (pp), spongy parenchyma (sp) and vascular bundle (vb). Bar = 100 µm. 10. Same as plate 9 but of higher magnification (×10) showing adaxial face epidermis (ad ep), abaxial surface epidermis (ab ep), xylem vessel (xv), phloem (ph), palisade parenchyma (pp) and spongy parenchyma (sp). Bar = 100 µm.

Effects of aluminium toxicity on stomata and trichomes of leaf: In the leaf of 28-day-old control plant, almost all the stomata were open whereas Al treatment caused closure of stomata (Plate 11a, b and c). In Al-sensitive plants, presence of Al was found to reduce stomatal conductance in tea after 8 weeks of treatment[13]. Aluminium treatment was found to induce stomatal closure[14]. In Al-treated leaf, number of trichome was higher as compared to that of the leaf of control plant. Both glandular and non-glandular trichomes were more common on the leaves of chickpea plant (Plate 12a, b and c).



Plates 11-12: 11. Peel of the leaf of chickpea (×10) (a) control, (b) 150 µM and (c) 300 µM Al-treated plant showing open and closed stomata and guard cell (g). Bar = 100 µm. 12. Peel of the leaf of *Cicer aeriatinum* L. (×10) (a) control, (b) 150 µM and (c) 300 µM Al-treated showing glandular (gt) and nonglandular trichomes (ngt). Bar = 100 µm.

Conclusion

Aluminium-induced decreased number and diameter of xylem vessels (Plates 2, 3, 5, 6, 7, 10) would decrease the translocation of ions from the root to the different parts of the plant. So, changes in the root system may affect nutrient uptake, which can lead to nutritional deficiencies in shoot and leaves. Closure of stomata might lower the transpiration rate and also decrease the rate of photosynthesis due to decrease in CO_2 diffusion through the stomata. To minimize the negative effects of Al toxicity, chickpea plants responded by changing the anatomical structure of the root, stem and leaf and by closing maximum number of stomata. Increased number of trichomes in the leaf (Plates 12) functioned as defense mechanism to protect the chickpea plants against Al stress.

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