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ENVIRONMENTAL FACTORS AFFECT CALCIUM OXALATE CRYSTALS FORMATION IN *TRADESCANTIA PALLIDA* (COMMELINACEAE)

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Abstract

Tradescantia pallida has major types of calcium oxalate crystals i.e., raphide, prismatic, and druse that are widely distributed within collenchmya, cortical and vascular parenchyma in the stem. However, mechanisms involved in crystal formation in response to stress conditions are not properly understood. In order to evaluate formation of these crystals in response to heavy metals i.e., mercury, sections of control (untreated) and mercury treated plants were prepared, stained with toluidine blue and photographed (infinity software). Mercuric chloride at high doses increased all types of crystals as compared with low doses; suggesting that mercury stress increases metabolic activities of *Tradescantia* that produce crystals may be, in order to defend themselves. So in *T. pallida*, crystal formation is influenced by mercury stress that increased raphide and prismatic crystals in the treated plants. However, biochemical aspects involved in oxalic acid formation and release of Ca by Hg need to be explored more.

Introduction

Tradescantia pallida, a monocot herbaceous plant is one of the members of family Commelinaceae that grows usually in vicinity of large trees. Leaves are the prominent part of plants and appear purple/blue color due to anthocyanin pigments, flavonoid compounds that are soluble in water and give plants, flowers and fruits their brilliant colors.

In *Tradescantia*, crystals of different shapes are seen in parenchyma and collenchmya of vegetative organs. They are minerals in form of calcium oxalate (CaOx) crystals and form the most widely distributed mineralized system particularly in seed plants (Nakata, 2003). Crystals occur in various shapes in plants and classified into five major types based on their morphology (i) raphide (bundle-shaped or needle like), (ii) styloid (elongated), (iii) druse (spherical aggregate), and (iv) prismatic (rhombohedral shape). Shape and growth of crystals is determined by intravacuolar matrices of macromolecules. CaOx crystals are accumulated in specific cells, idioblasts and crystal formation in plants is related with calcium regulation and protection against herbivory (Franceschi & Nakata, 2005; Vincent & Nakata, 2005; Kostman *et al*., 2001). One of the models involved in crystal formation states that due to initial random distribution of dissolved calcium and oxalate ions in the crystal chamber, clusters of Ca and oxalate break up and reform easily. Growth of crystals is accelerated with the increase in concentration of dissolved ions. During the formation of one crystal nucleus, concentration of dissolved ions becomes low that causes other surrounding nuclei to dissolve, thus consequently resulting in formation of one crystal per chamber.

Calcium oxalate in *Tradescantia* occurs in two forms, monohydrate (CaC_2O_4. $2H_2O$) and dihydrate (CaC_2O_4. $2H_2O$). Monohydrates are characterized by three crystallographic axes that are unequal in length having one oblique intersection and while other two intersections are 90°. However, the three axes of tetragonal system are at right angles to each other with the two equal lateral axes and the third, vertical axis may be longer or shorter. Whewellites normally exist as raphides, druse and weddellite exist as prismatic shaped crystals.

Biochemical pathways involved in crystal formation include hydrolysis of oxaloacetate, glycolate/glyoxylate oxidation or oxidation cleavage of L-ascorbic acid (Keates *et al*., 2000; Debolt *et al*., 2007). Crystal shape and formation is influenced by many factors like proteins, polysaccharides, lipids and membrane structure (Webb, 1999).

Mercury is one of the heavy metals with high density and exists as metallic, organic and inorganic; however, the most common form of organic mercury is methyl mercury. Metal pollution from industrial or agricultural activities or motor vehicles cause serious impact on surrounding areas (Neculita *et al*., 2005; Luan *et al*., 2008; Cenkci *et al*., 2010; Khattak & Jabeen, 2012, Shafeeq *et al*., 2012 and Yasar *et al*., 2012). Fertilizers, use of metal-contaminated sludge, hazardous solid waste, all contributes to mercury pollution of soil and crops. Cations of organic mercury form salt with organic and inorganic acids and react with biologically important ligands (sulphur group) (Patra & Sharma, 2000). Mercury uptake in plants upset their growth and cell division and causes numerous structural irregularities that interfere cell wall and cytoplasmic membrane synthesis and function (Mor *et al*., 2002). Substitution of the central atom of chlorophyll, magnesium, by Hg results in breakdown of photosynthesis (Patra & Sharma, 2000).

Mercuric cations have a high affinity for sulphydryl (-SH) group and almost all proteins have sulphydryl groups; so mercuric ions can disturb the function of proteins. The possible binding sites for mercury ions may be two sites of a protein molecule without deforming the chain, binding the neighboring chains together, or binding of mercury ions even results in protein precipitation. Two different uptake routes followed by Hg ions may be passive uptake, due to concentration gradient across the membrane and other may be inducible substrate-specific and ATP-dependent uptake (Williams *et al*., 2000).

Materials and Methods

T. pallida plants of equal lengths were collected from campus of Forman Christian University, Lahore. Plants were placed in beakers containing water (control), 25 ppm, 50 ppm and 100ppm $HgCl_2$ for one week. They were placed at a place where they could get sufficient sunlight. After one week, sections of shoot from all the treatments were cut with razor blade passed though ethanol grades and then stained with toluidine blue. Since crystals are lost during sectioning, so experiments were repeated thrice to ensure maximum reliable results.

In transverse section, crystals were observed for their shape, number and distribution in the collenchmya, cortical and vascular parenchyma of stem. At least five replicates from each treatment were taken into consideration. Prismatic and bundle shaped crystals were randomly counted than druse due to their consistency of occurrence. Microphotographs were taken through infinity software and images processed in Adobe Photoshop 7.0.

Result and Discussion

Although CaOx crystal formation and shape is genetic characteristic but many factors like light, water, temperature, soil, and heavy metals stress also control calcium oxalate formation in plants. Crystal formation is a constant process including cellular specialization in *T. pallida* and forms the important basis of chemotaxonomy. These crystals are formed in specialized cells known as crystal idioblasts vacuole as a result of increasing concentration of oxalic acid, a metabolic by-product that is toxic in high concentration. Idioblasts are as important as single-celled organs and operate as single physiological entities (Kostman *et al*., 2001).

T. pallida exhibits great diversity of crystals shapes and crystals of three major shapes i.e., prismatic, raphide, and druse were prominent in stem (Figs. 1, 2, 3 and 4). In *Tradescantia*, prismatic and raphide crystals were predominant and average number of these crystals was 19-22 and 24-28 in cortical parenchyma of stem, while the 100 ppm $HgCl_2$ treated plants showed the average number of prismatic and raphide crystals 38-42 and 42-46 respectively (Figs. 5, 6 and 7). Likewise, collenchmya and vascular parenchyma also showed significant number of both types of crystals. Druse was rare and did not show constant distribution so raphide and prismatic crystals were considered to be more reliable feature. Crystals are usually seen throughout plant body both in vegetative and reproductive parts; however, in *T. pallida* they are abundant in the cortical parenchyma than in collenchmya and vascular parenchymatous cells (Figs. 1, 2, 3 and 4).

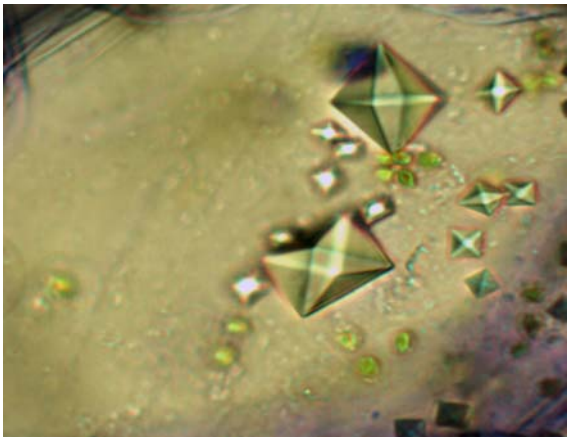

Fig. 1. Prismatic crystals in the vascular parenchyma of untreated stem of *Tradescantia pallida* (10x).

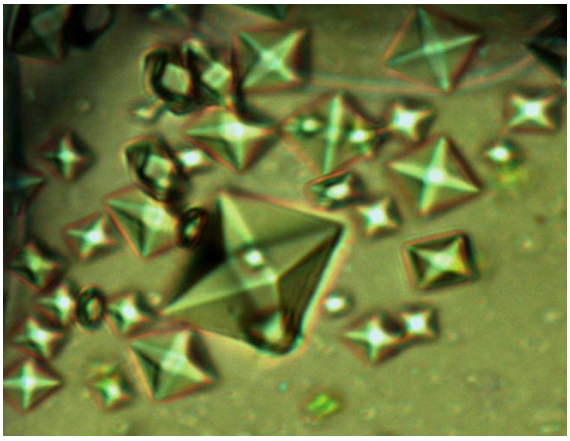

Fig. 2. Crystals in stem treated with 100ppm $HgCl_2$ (10x).

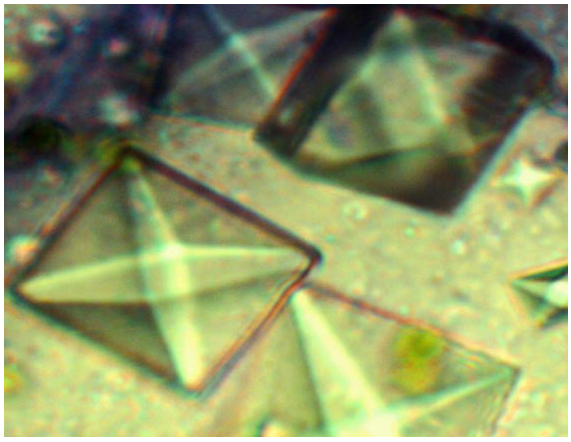

Fig. 3. Prismatic crystals in 100ppm$HgCl_2$ observed at 60x.

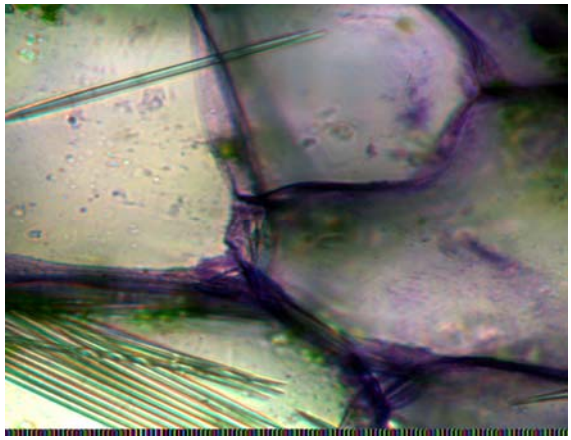

Fig. 4. Raphide crystal in 100ppm $HgCl_2$ treated stem.

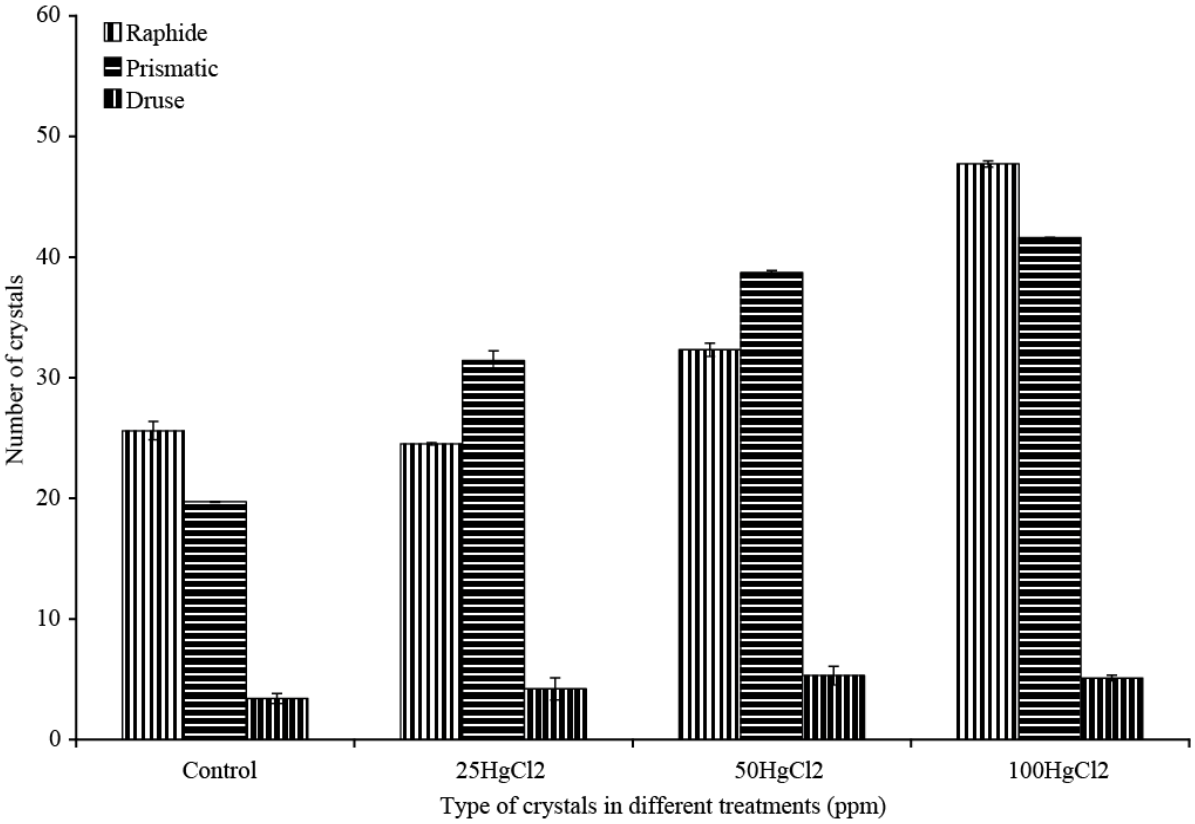

Fig. 5. A comparison of calcium oxalate crystals in the cortical parenchyma of *T. pallida* stem in comparison with $HgCl_2$ treatments (10x).

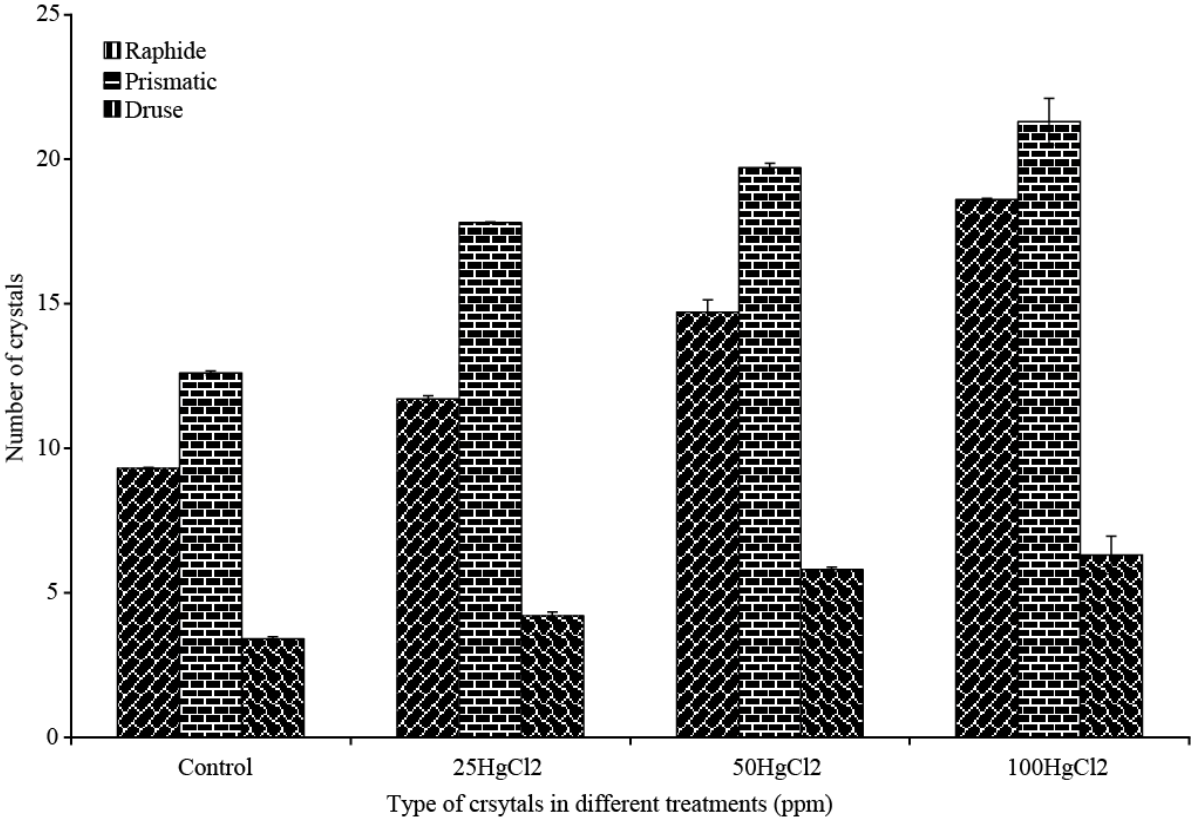

Fig. 6. A comparison of calcium oxalate crystals in the collenchmya of *T. pallida* stem in comparison with $HgCl_2$ treatments.

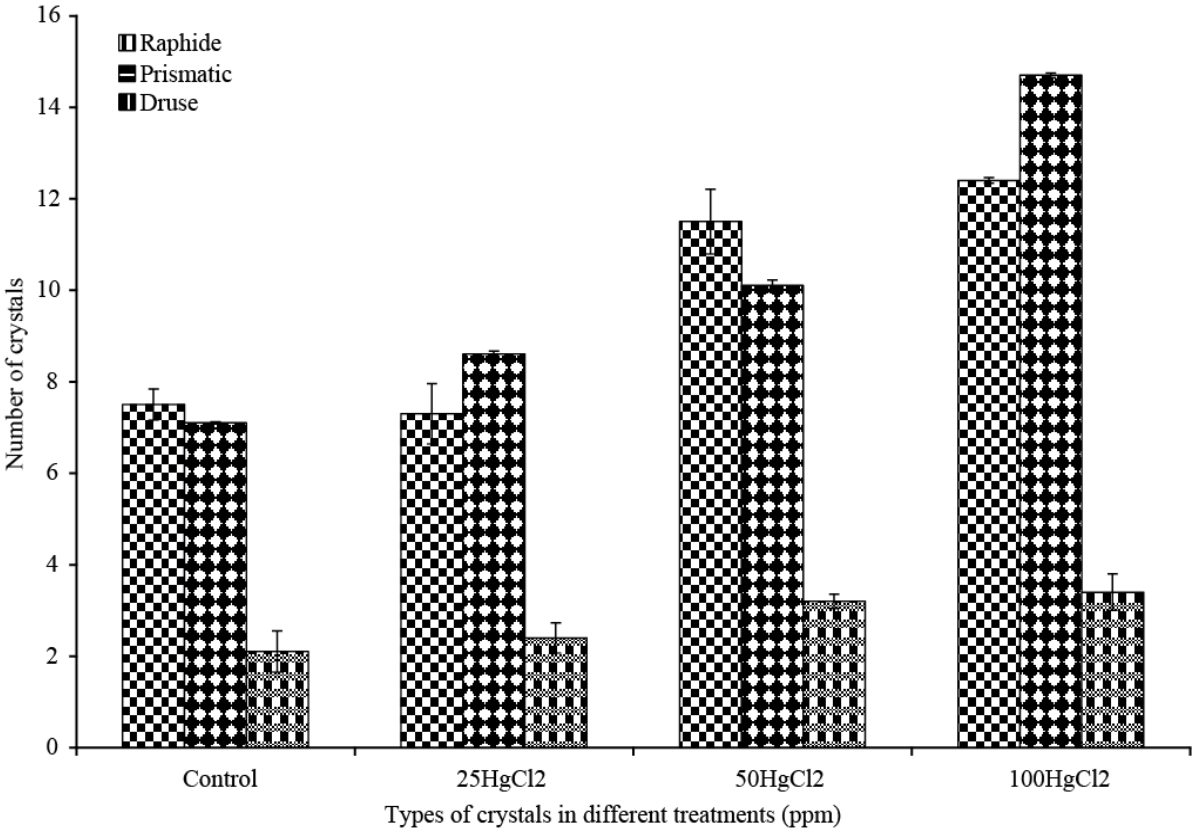

Fig. 7. A comparison of calcium oxalate crystals in the vascular parenchyma of *T. pallida* stem in comparison with $HgCl_2$ treatments.

Increase in number of prismatic (weddellite) and raphide crystals (whewellite) with dose of 50 and 100 ppm Hg in all replicates **(**Figs. 6 and 7) indicate that Hg increases oxalic acid formation in *Tradescantia* plants. Many pathways are proposed for oxalic acid formation in plants, however, one suggesting ascorbate as precursor of oxalic acid is widely accepted (Loewus, 1999). One of these involves oxidation of glycolate, which takes place in two steps one, with glyoxylic acid as an intermediate and in second glycolic acid oxidase as the enzyme. Glyoxylic aid is produced by enzymatic cleavage of isocitric acid (Fig. 8). Availability of oxalic acid increases the possibility of formation of CaOx crystals (Fig. 9) Anti-feeding role of oxaloacetic acid was demonstrated by abrasion of mouthparts of larva following grazing on oxalate containing *Medicago* through SEM (Korth *et al*., 2006). Another reason is that plants growing under Hg stress have high metabolic rate that consequently leads to increase in oxalic acid formation and also their formation may be due to effort of plants to maintain their ionic equilibrium (Fig. 10). One of the possibilities is that their accumulation is an indication that these crystals are doing effective role in sequestration of metal ions (Franceschi & Nakata, 2005). High concentrations of mercury in plants negatively affects their important physiological functions and causes detrimental effects on enzymes functions, vitamins and hormones synthesis (Mor *et al*., 2002; Neculita *et al*., 2005). Two different uptake routes followed by Hg ions may be passive uptake, due to concentration gradient across the membrane and other may be inducible substrate-specific and energy-dependent uptake (Williams *et al*., 2000). Hg upsets ionic balance of plant thereby increasing oxalate synthesis. Another possible reason for the increase in number of crystals may be the accumulation of Hg in epidermal, cortical and vascular regions (Figs. 4, 5 and 6). There is lot of evidence that suggests mercury accumulation in cortical and vascular region where it adversely affects the physiological activities of cells, upsetting the whole plant body (Mor *et al*., 2002; Patra *et al*., 2004; Neculita *et al*., 2005). Root tips or root hairs are the possible sites through which mercury ions enter the plant when plant roots are exposed to Hg**.** Symplastic transport of mercury ions occur from cortex to stele, particularly when whole wall of endodermal cell is suberized. Distance between formation of suberin lamellae and the root tip is another important factor that influences the influences the translocation of metals (Lux *et al*., 2004). Uptake of mercury ions delays cell division and also deprives plants of some nutrients required for their growth and metabolism consequently causing overall reduction in plants. Hg ions at higher concentration follow symplastic pathway and diffuse into cells where they directly interfere with plant metabolic activities and upset organelles (nucleolus) causing deleterious effects as Hg is known to cause chromosomal, nucleus and nucleolus irregularities.

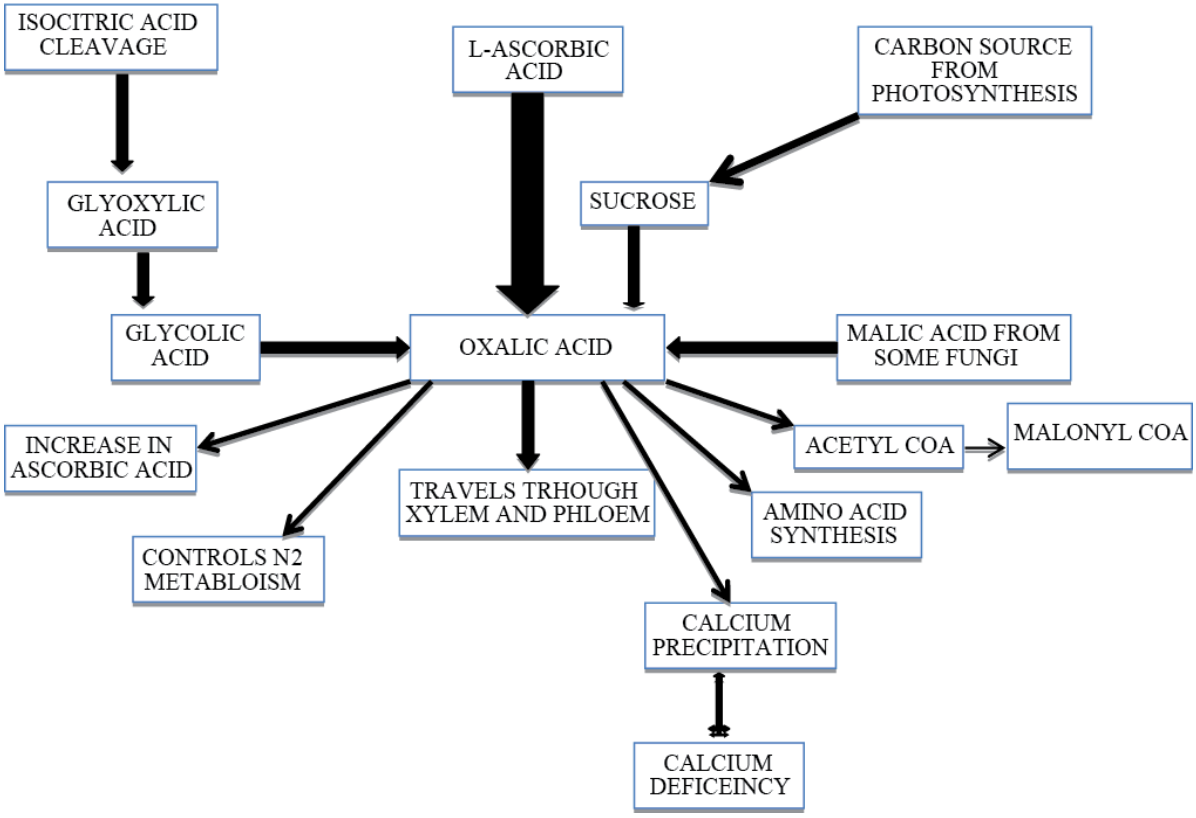

Fig. 8. An Overview of Oxalate synthesis in plants and its functions.

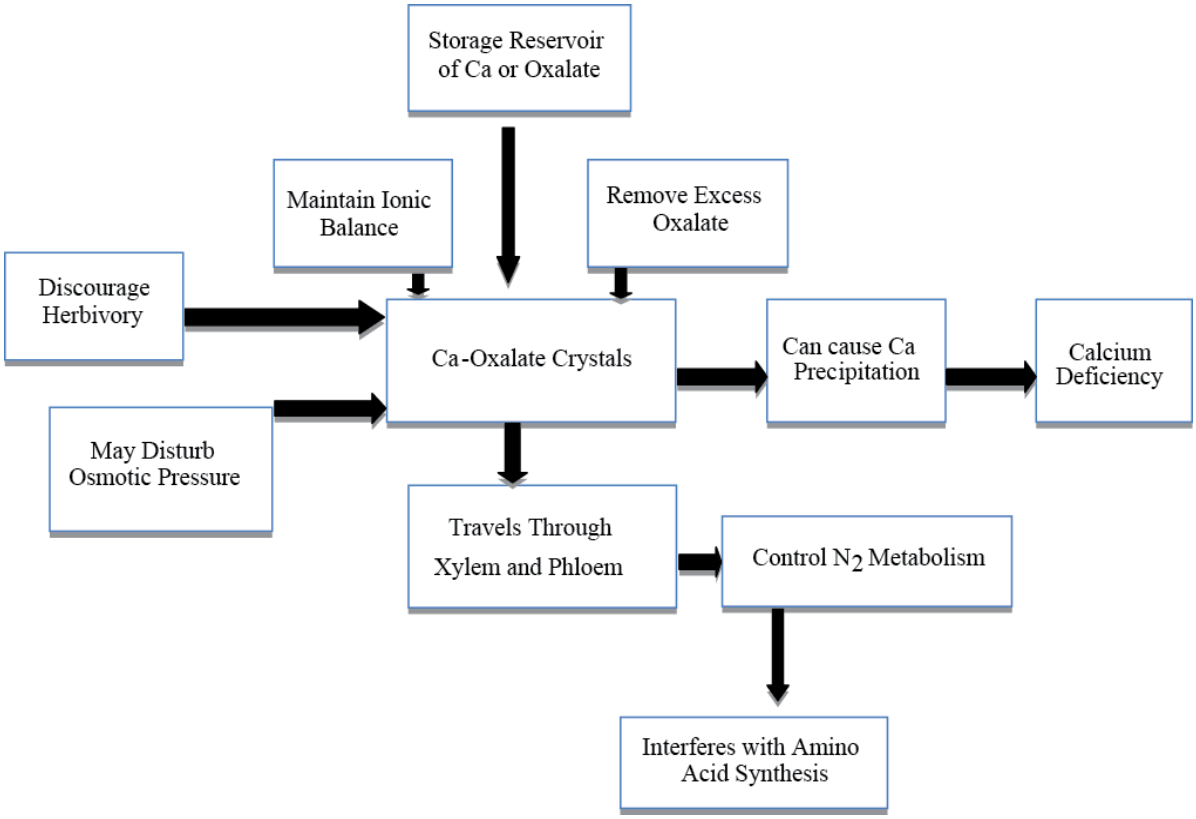

Fig. 9. Scheme showing Ca-oxalate crystals formation.

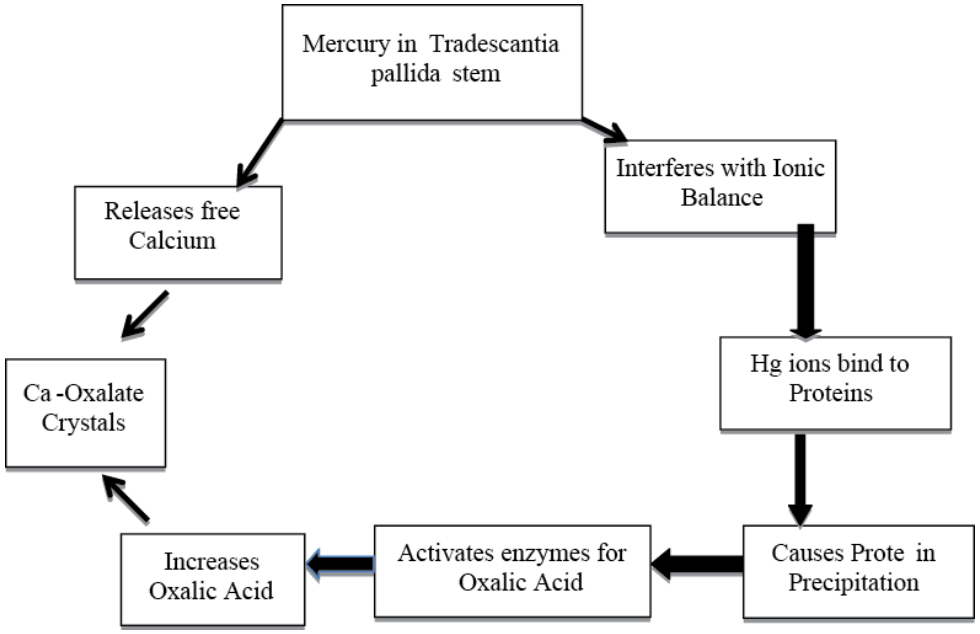

Fig. 10. Proposed scheme of mercury action on oxalate and Ca in *T. pallida*.

Conclusion

In current work, increase in number of raphide and prismatic crystals with higher doses of Hg (100ppm) might be due to its reactivity with oxalic acids and Ca, accumulation, interference with metabolic and increase in physiological activities of *T. pallida*.

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