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EFFECT OF CADAVERINE ON CULTIVARS OF TOMATO [*LYCOPERSICON ESCULENTUM* (L.) EM. THELL] UNDER MULTIPLE STRESS

Charu Rajpal and Pushpa C. Tomar*

Department of Biotechnology, Faculty of Engineering and Technology, Manav Rachna International Institute of Research and Studies, Faridabad-121001, Haryana, India

*Corresponding author: pushpa.fet@mriu.edu.in

Abstract

Abiotic stresses are a major threat for agricultural productivity. Several strategies have been developed to improve such stresses like crop salt tolerance and heavy metal stresses, and in regard to this the effects of polyamines (PA) have been well established. Cadaverine (Cad), a structurally different diamine as that of putrescine and having an independent biosynthetic pathway with respect to other polyamines is regarded as a growth regulator and follows the same mode of action. In day today life, consumption of tomato remains as a main source because of its advantageous constituents like β-carotene, lycopene and other indispensable vitamins. It has been demonstrated for its anticipatory nature in numerous types of cancer and cardiovascular diseases. In this regard, this study was conducted using three different varieties of tomato (*Lycopersicon esculentum Mill.*) vis. *Pusa Rohini, Pusa Ruby* and *Pusa Sadabahar* under multiple abiotic stresses like salinity and heavy metals. Salinity possesses a negative impact on plant growth as it causes plant intoxication and osmotic stress which interferes with the crop development. While heavy metals inhibit nitrate reductase activity and decrease organic nitrogen and protein contents. Their higher concentrations can severely distress the growth of plant and biomass yield. Cad improves crop salt tolerance; improvise heavy metal stress, acts as a plant growth regulator that further activates antioxidant defense system. This study is undertaken to provide some insight into Cad's regulatory mechanism on the growth of the tomato plant. Germination rate, speed of germination, pigment estimation (chlorophyll a, chlorophyll b and carotenoid) of roots and shoots were the parameters assayed and an analysis of Cad's potential to mitigate the adverse responses induced under multiple stresses.

***Keywords*:** Cadaverine, Growth parameters, Heavy metals, *Lycopersicon esculentum*, Salinity

Introduction

Environmental stresses are a major threat for agricultural productivity. But certain biotic and abiotic stresses like salinity and heavy metals, especially Cd or Pb are the main cause of soil degradation and inhibit germination, speed of germination, dry mass accumulation, photosynthetic pigments and change in biomass partitioning due to perturbed physiochemical processes resulting decline in growth and related metabolites. Abiotic and biotic environmental stresses adversely affect crop yield (Yuan *et al.,* 2016). One of the leading abiotic stresses is the salinity in the soil that owes to both natural reasons and an unsuitable agronomic operation. Salinity emphasizes three times effects on reducing water potential causing toxicity and osmotic imbalance. While heavy metals accumulation has become a global concern due to crop irrigation by contaminated water by inhibiting nitrate reductase activity and decreasing organic nitrogen and protein contents in the leaf tissue (Hussain *et al.,* 2015). Reportedly, among all vegetables, tomato (*Lycopersicon esculentum*), is slightly sensitive to salinity and it can help to combat several life threatening conditions like cancer, certain cardiovascular diseases, osteoporosis etc. This may be because of the presence of "Lycopene" and certain bioflavonoids that can eradicate the free radicals formed during metabolism. Tomato being the most significant crop is required to sustain stress condition is still a topic that remains fascination and yet to be explored in plant sciences. To improve crop salt tolerance and heavy metal stresses, several strategies have been developed and in regard to this the effects of polyamines (PA) have been well established. Tolerance to salt is a complex characteristic which is responsible for osmotic adjustments, exclusion of toxic ions, compartmentalization and morphological modifications (Munns & Tester, 2008). A few recent studies have emphasized the effect of shoot tissue responses towards the excess salt concentration, by minimizing the accumulation of toxic ions in the leaf resulting in hampered growth and productivity of plant (Julkowska *et al.,* 2014). Excess accumulation of salt ions present in the soil exert osmotic pressure that reduces the uptake of water by the roots of the plants leading to excessive accumulation of reactive oxygen species (ROS) (Jiang *et al.,* 2016), and causes root membrane disruption (Gupta & Huang, 2014). And in case of heavy metals, it is reported that heavy metal concentration was higher in upper parts of plants like fruits as compared to the lower parts like leaves, roots etc. (Ammar *et al.,* 2008). Cadmium taken up by plants ranges between 0.1-2.4 mg/kg (Alloways, 1995). Higher concentrations can strictly distress the growth of plant and biomass yield (Hussain *et al.,* 2015). Gratão *et al.* in 2008 when treated tomato plants with cadmium stated amplified peroxidation of lipids, catalase activity, glutathione reductase activity and reduced activity of glutathion peroxidase enzyme (Ammar *et al.,* 2007). Polyamines (PA) are small aliphatic polycations, ubiquitously present in all organisms. Functionally, these molecules exhibit their role in wide range of biological processes. Since last few years, molecular genetic studies have contributed immensely in understanding the transformed activity of enzymes in the synthesis of PA and their probable biological functions in plants. Previous studies have focused mostly on three biogenic amines: putrescine (Put), spermidine (Spd) and spermine (Spm), and their derivatives. The application of PA applied effects the patterns of senescence and morphogenesis providing a restrictive role of polyamines in these processes (Galston &

Sawhney, 1990). Cadaverine (Cad), a structurally different diamine which has an independent biosynthetic pathway for its synthesis also comes under the family of PA. It is known as a growth regulator with similar mode of action. Cad also tends to accumulate in higher plants under several biotic and abiotic stresses. Over-expression of genes indulged in polyamine biosynthesis results in building up of PA and therefore, more stress tolerance. Thus, the Cad exogenous application under stress likely to exert some positive response in stressed plants, which is not elucidated well. The present study will be undertaken to provide some insight into Cad's regulatory mechanism on the growth of the tomato plant and its potential to mitigate the adverse responses induced under multiple stresses. Cad also ameliorates the growth of the leaves and root tissues under multiple stress conditions and these stress induced proteins in the presence of Cad may help plant to grow under stress conditions (Tomar *et al*., 2017).

Materials and Methods

Surface sterilization of seeds of tomato (*Lycopersicon esculentum* var. *Pusa Ruby, Pusa Rohini and Pusa Sadabahar*) with 0.1% sodium hypochlorite solution was done for five minutes and then rinsed with double distilled water thrice and then sown in Whatman's filter paper lined petri-plates. The seedlings will be grown under controlled conditions (light 75 Wm^2, 25±2℃, 65% RH) and watered with full strength Hoagland nutrient solution containing 50mM NaCl, 1 mM Cd or Pb and blended with 1 mM Cad as per the experimental plan. The pH 6.4 of the nutrient solution containing salts and Cad was kept constant for all the treatments (T_0: Control, T_1: NaCl, T_2: Cd, T_3: Pb, T_4: Cd+NaCl, T_5: Pb+NaCl, T_6: Pb+Cd) with and without Cad. The seed germination was counted after 4^{th}, 8^{th}, 12^{th} and 16^{th} day of sowing. Random sampling of morphologically and physiologically similar seedlings was done on 16^{th} day for various measurements. Biomass was estimated after drying the seedlings for 72 hours at 65℃. Chlorophyll contents (Strain & Svec 1966), carotenoid (Ikan 1969) estimation was done. The data are mean of three replicates along with ±SD. The significance of the treatments and data analysis Student's 't' test was used.

Results

Germination and growth under multiple stress with and without Cad

The Germination was examined for all the treatments on 4^{th}, 8^{th}, 12^{th} and 16^{th} days (Figure 1-3). It was observed that either salinity or Pb or Cd alone or in combination reduced the seed germination as examined after 16 days (Figure 4). Though no germination was seen in *Pusa Sadabahar* on 4^{th} day (Figure 1) while the germination pattern of seeds under each type of stress after 16 days remained almost same. But salinity and metal stress in combination decreased the germination further. The Cad application showed protective effect on seed germ ability under all stress except Cd plus salinity (T4). The pattern of root /shoot elongation in the seedling was similar under any of the stress, salinity or metal alone or in combination with or without Cadaverine treatment. However, salinity alone or in combination with Pb or Cd severely reduced root/shoot elongation (Figure 5, 6) while in the presence of Cad the root and shoot length was comparatively longer as that of former.

Photosynthetic pigments under multiple stress

Total chlorophyll content in leaf tissues increased with Cad treatment over control (Figure 7, 8). The Cadaverine treatment of the seedlings elevated the carotenoid content also in the seedlings under all stress conditions except NaCl stressed seedlings and remained consistent under multiple stresses also (Figure 9).

Discussion

Deteriorating effects of some biotic and abiotic stresses can be ameliorated by exogenous application of polyamines. The functions of PA's against stresses have been explained in many plants. Though their accumulation can be considered as a marker under abiotic stresses but it depends upon the age of the plant and its developmental conditions. In case of humans, Cad that is synthesized endogenously is considered important in several biological functions while in case of plants, the stressed conditions individually or in combination hinder the physiological process depending upon the stress type. The Cad controlled the inhibition of germination and growth caused by stresses (Figure 4). Non receptiveness of Cad in the presence of Pb and salinity may lead to strong inhibition of hydrolytic enzymes engaged in germination or excessive accumulation of Pb ions in the embryonic tissues (Pang *et al.,* 2007). Cad supplementation to the stressed plants exhibited positive effects on the length of shoots and roots depending upon severity of the stress (Figure 5, 6). The roots of seedlings tend to upsurge the surface area to manage with the scarcity of water under salinity.

The overall growth inhibition by stresses linked with pigment estimation like chlorophyll and carotenoids deteriorate in leaves (Koryo, 2006 & Ahmad *et al*., 2010) were tested significantly with Cad. (Figure 7-9). The putrescine preserves the enzyme activity when blended with salinity (Singh, 2002) and by other polyamines in maize (Shankar *et al*., 2001). The higher translocation of Cd than Pb in shoot was also dropped by Cad (Tomar *et al*., 2013). However the distinct accumulation pattern of Cd and Pb was related to that of rice (Rubio *et al*., 1994). The salinity improved metal accumulation leads to change in the bio-membranes (Choudhari & Choudhari, 1993) which otherwise might be sheltered by Cad. Verma and Mishra in 2005 have proposed that putrescine stabilizes the membrane of the plants stressed with salinity. It seems that the Cad intensely upholds the stressed plant growth through preserving the metabolites.

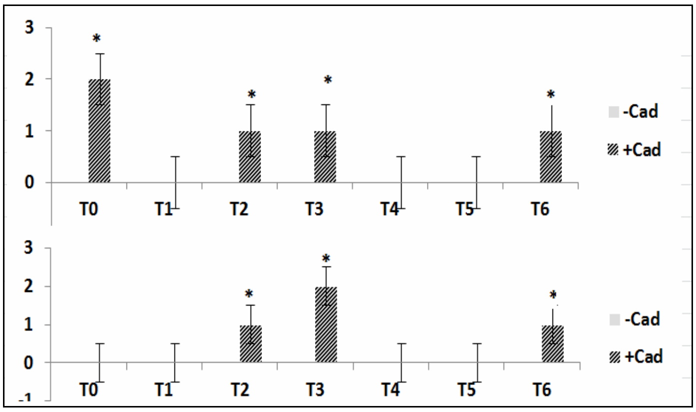

Fig. 1 : Tomato seed germination rate under salinity, heavy metals and twin stress with or without Cad after 4 days where T0=Control, T1= NaCl (50mM), T2=Cd (1Mm), T3= Pb (1mM), T4= Cd+NaCl, T5= Pb+NaCl, T6= Pb+Cd. Values are mean ± SD from 3 replicates. *P* value: *<0.05%, <**0.01, <***0.001.

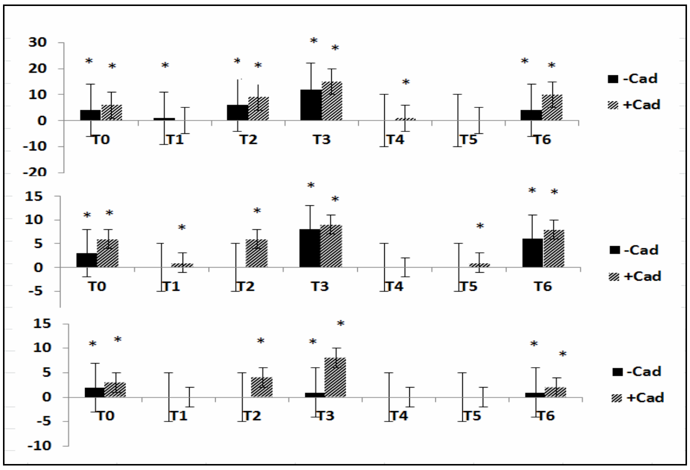

Fig. 2 : Tomato seed germination rate under salinity, metal and twin stress with or without Cad after 8 days (other details are same as in Figure 1).

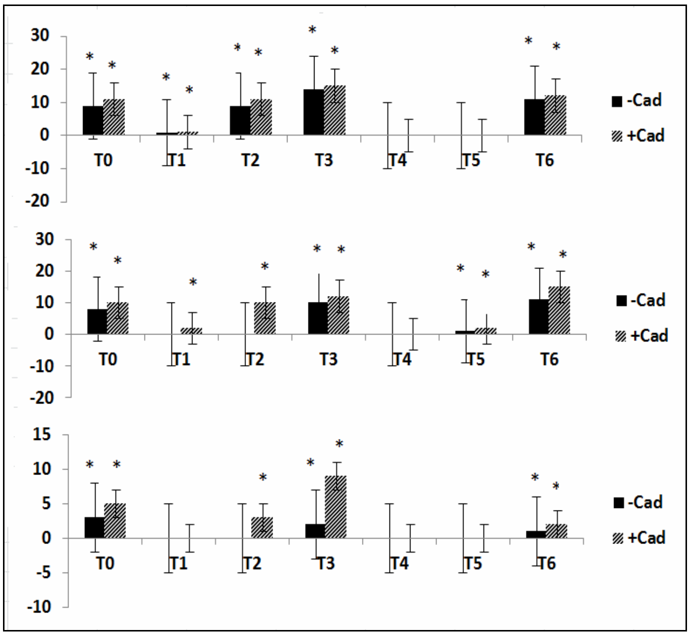

Fig. 3 : Tomato seed germination rate under salinity, metal and twin stress with or without Cad after 12 days (other details are same as in Figure 1).

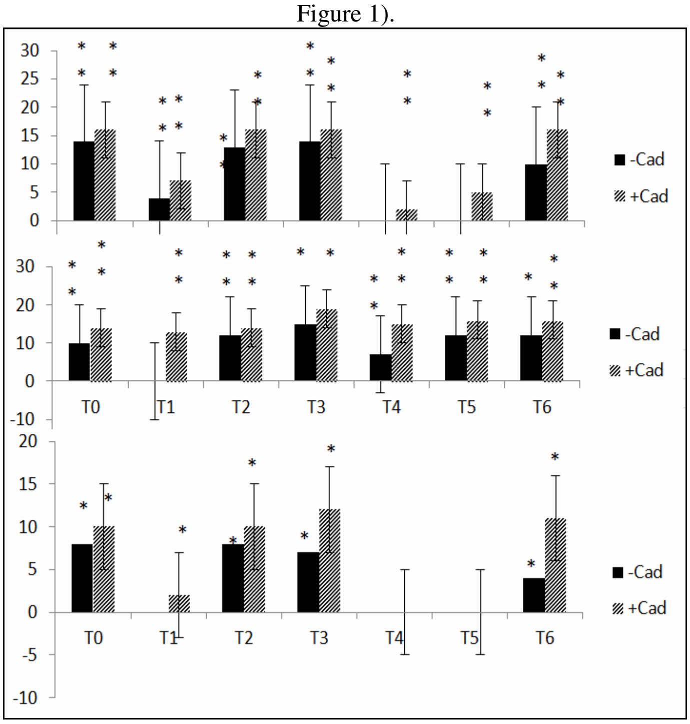

Fig. 4: Tomato seed germination rate under salinity, metal and twin stress with or without Cad after 16 days (other details are same as in Figure 1).

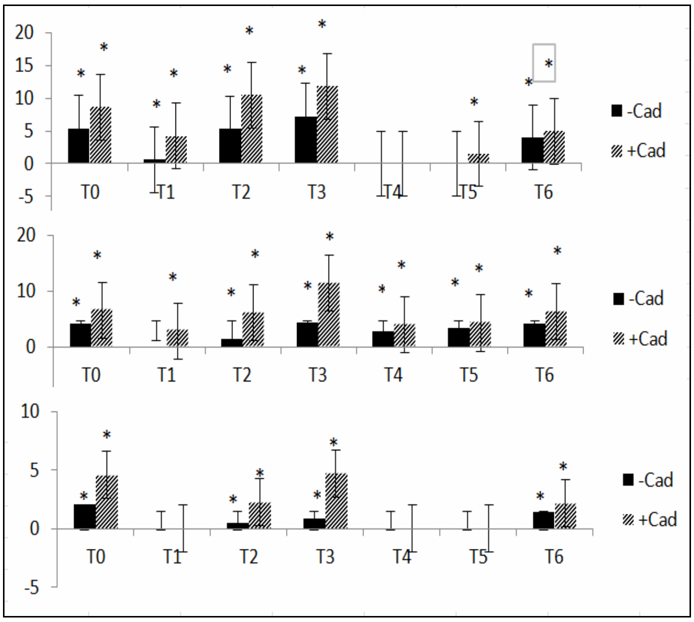

Fig. 5: Root length under salinity, metal and twin stress with or without Cad after 16 days (other details are same as in Figure 1).

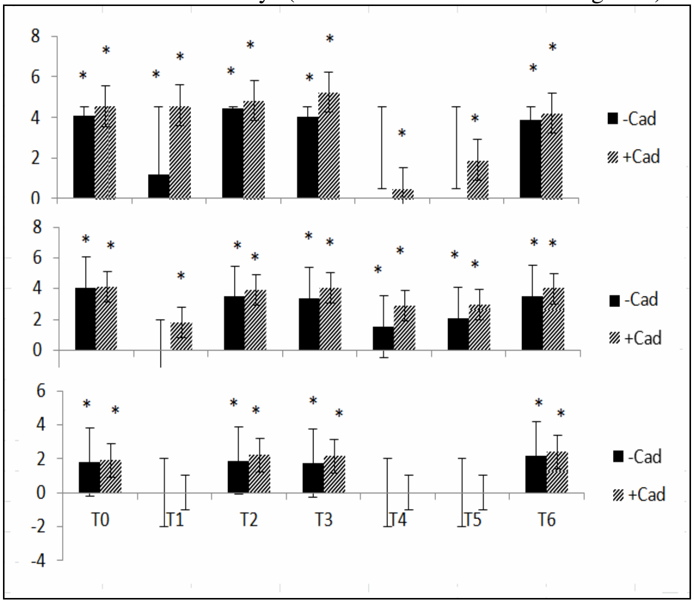

Fig. 6: Shoot length under salinity, metal and twin stress with or without Cad after 16 days (other details are same as in Figure 1).

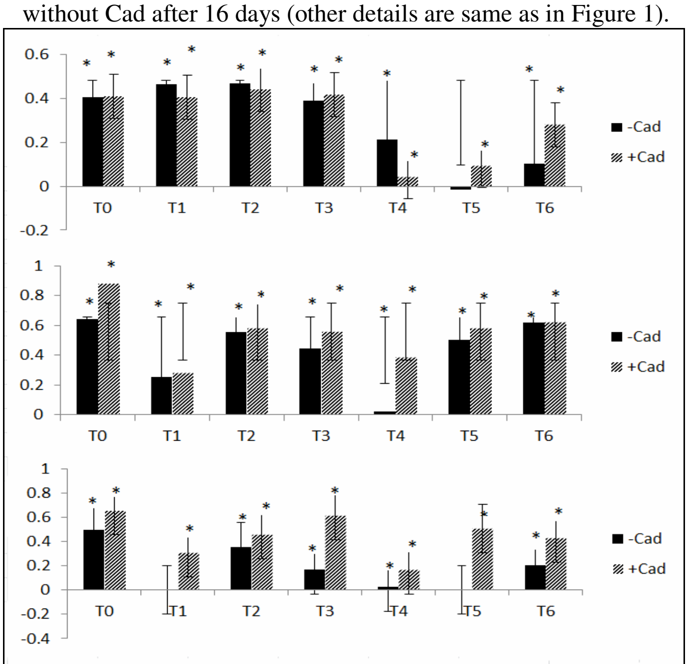

Fig. 7 : Chlorophyll (A) content in leaf tissues of 16th day old seedlings under stress with or without Cad (other details are same as in Figure 1).

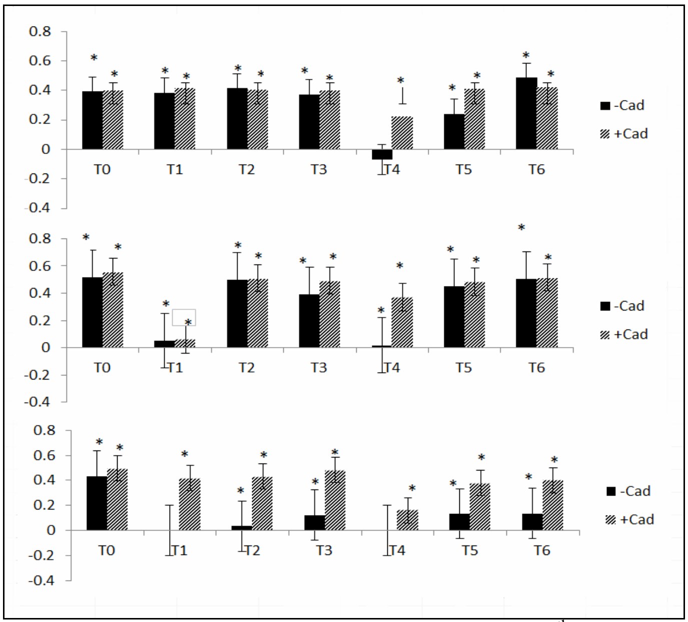

Fig. 8: Chlorophyll (B) content in leaf tissues of 16th day old seedlings under stress with or without Cad (other details are same as in Figure 1).

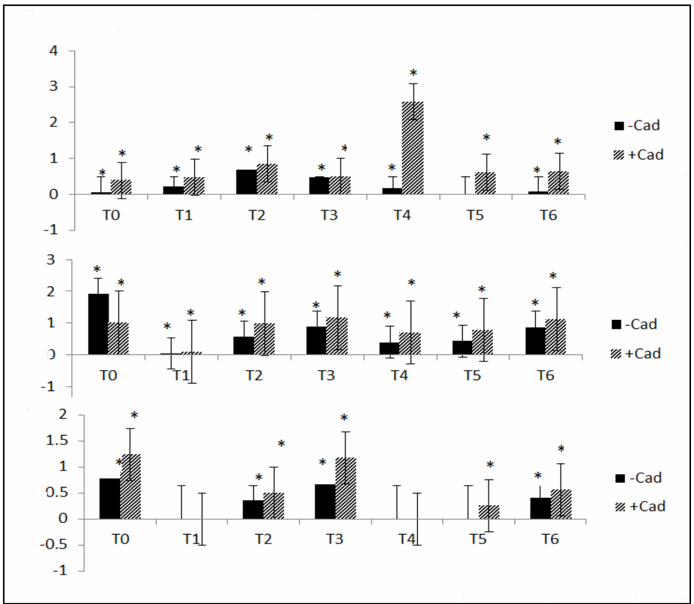

Fig. 9: Carotenoid content in leaf tissues of 16th day old seedlings under stress with or without Cad (other details are same as in Figure 1).

References

Ahmad, M.S.A.; Ashraf, M. and Ali, Q. (2010). Soil salinity as a selection pressure is key determinant for the evolution of salt tolerance in blue panic grass (*Panicum antidotale Retz.*). Flora, 205:37.

Alloways, B.J. (1995). Heavy metals in soils, 2nd ed., Springer Science & Bussiness Media.

Ammar, W.B.; Mediouni, C.; Tray, B. and Ghorbel, M. (2008). Glutathione and phytochelatin contents in tomato plants exposed to cadmium., Biol Plantarum., 52, 314-320.

Ammar, W.B.; Nouairi, I.; Zarrouk, M. and Jemal, F. (2007). Cadmium stress induces changes in the lipid biosynthesis in tomato (*Lycopersicon esculentum* Mill.) leaves. Plant Growth Regul., 53, 75-85.

Choudhari, K. and Choudhari, M.A. (1993). Effect of short term NaCl salinity stress on free radical mediated membrane damage in two jute species. Indian J. Exp. Biol., 31: 327.

Galston, A.W. and Sawhney, R.K. (1990). Polyamines in plant physiology. Plant Physiol., 94: 406-410.

Gratão, P.; Monteiro, C.; Antunes, A. and Peres, L. (2008). Acquired tolerance of tomato (*Lycopersicon esculentum* cv. Micro-Tom) plants to cadmium - induced stress. Ann. Appl. Biol., 153: 321-333.

Gupta, B. and Huang, B. (2014). Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. Int J Genomics., 6: 727-740.

Hussain, M.M.; Saeed, A.; Khan, A.A.; Javid, S. and Fatima, B. (2015). Differential responses of one Hundred tomato genotypes grown under cadmium stress. Genet Mol Res., 14(4): 13162-13171.

Ikan, R. (1969). (Natural Products): (A laboratory guide), Academic Press, New York, 101.

Jiang, K.; Moe-Lange, J.; Hennet, L. and Feldman, L. (2016). Salt stress affects the redox status of Arabidopsis root meristems. Front Plant Sci., 7:8.

Julkowska, M.M.; Hoefsloot, H.C.J.; Selena, M.; Richard, F.; GertJan, D.B. and Haring, M.A. (2014). Capturing Arabidopsis root architecture dynamics with ROOT-FIT reveals diversity in responses to salinity. Plant Physiol., 166: 1387-1402.

Koryo, H.W. (2006). Effects of salinity on growth, photosynthesis, water relations and solute composition of the potential cash crop halophyte planlago coronopus (L.). Environ. Exp. Bot., 56: 136.

Munns, R. and Tester, M. (2008). Mechanisms of salinity tolerance. Annu Rev Plant Biol., 59: 651-681.

Pang, X.; Zhang, Z.; Wen, X.; Ban, Y. and Moriguchi, T. (2007). Polyamines, all-purpose players in response to environment stresses in plants. Plant stress, 1: 173.

Rubio, M.I.; Escrig, I.; Martinez-Cortina, C.; Lopez-Benet, F.J. and Sanz, A. (1994). Cadmium and Nickle accumulation in rice plants effects on mineral nutrition and possible interactions of abscisic acid and gibberellic acids. Plant Growth Regu., 14: 151.

Shankar, N.; Khan, S.R. and Srivastava, H.S. (2001). The response of NRA and nitrate assimilation in maize roots to growth regulators at acidic pH. Biol. Plant, 44: 599.

Singh, D.B.; Verma, S. and Mishra, S.N. (2002). Putrescine effect on nitrate reductase activity, inorganic nitrogen, protein and growth in heavy metal and salinity stressed mustard seedlings. Biol. Plant, 45: 605.

Strain, H.H. and Svec, W.A. (1996). Extraction, separation, estimation and isolation of the Chlorophyll, in *Chlorophyll*, Ed. Vermon L.P. and Seely G.R. Academic Press, New York, 101.

Tomar, P.C.; Lakra, N. and Mishra, S.N. (2013). Effect of Cadaverine on *Brassica juncea* (L.) under multiple stress. Indian J. Exp. Biol., 51: 758-763.

Tomar, P.C.; Mishra, S.N. and Rajpal, C. (2017). Effect of Cadaverine on protein profiling of *Brassica juncea* (rh-30) seedlings under multiple stress. *IJAIST*, 6(3): 49-62.

Verma, S. and Mishra, S.N. (2005). Putrescine alleviation of growth in salt stressed *Brassica juncea* by including antioxidative defense system. J. Plant Physiol., 162: 669.

Yuan, Y.; Zhong, M.; Shu, S.; Du, N.; Sun, J. and Guo, S. (2016). Proteomic and physiological analyses reveal putrescine response in roots of cucumber stressed by NaCl. Front Plant Sci., 7: 1035.