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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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学位論文題目：
Title of Dissertation

Effect of L-glutamic acid on enhancing abiotic stress tolerance through modulation of antioxidant defense system in lentil (*Lens culinaris* Medik.)
(レンズマメにおける抗酸化防御系の調整を介した非生物ストレス耐性強化に及ぼすL-グルタミン酸の効果)

学位論文要約：
Dissertation Summary

Background

Over the last century, the rate of increasing population of the world became enhanced and expected to be 9.3 billion by 2050. Food demand is also being anticipated to grow substantially by 2050 for the overgrown population and will be 77% greater than 2007 (Linehan et al., 2012, Fig.1). Therefore, farmers of worldwide need to increase crop production in future for fulfill the requirement of food of those increasing population.

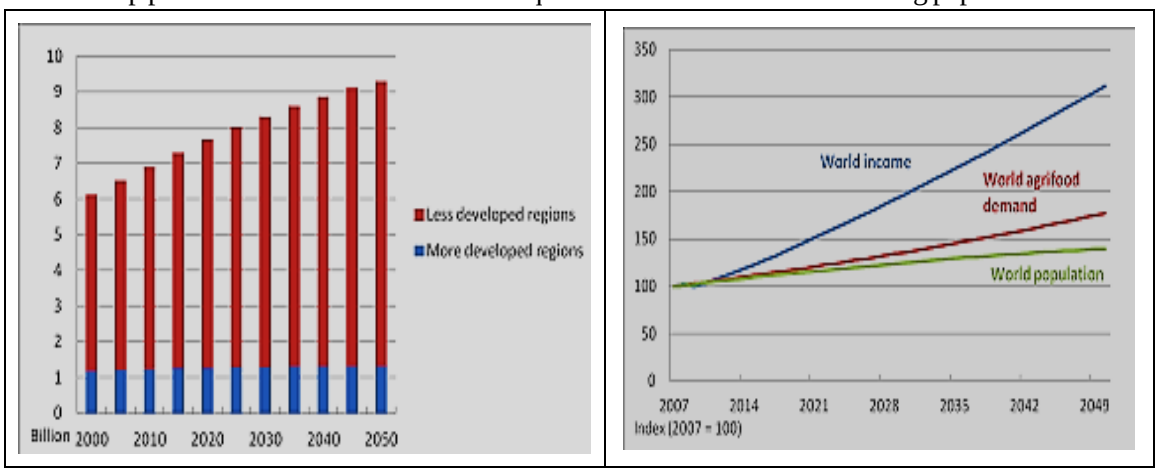

Fig. 1 The projected world population and world food demand in the year of 2050

However, a variety of environmental factors, emerged either by naturally or by human activities creates a hostile environment for crop growing on that condition. Those factors are salinity, drought, heavy metal and metalloid toxicity, high temperature, low temperature, and high and low light intensity, among others and also known as abiotic stressors; accounting for great yield loss of crop (Wang et al., 2003; Wania et al., 2016).

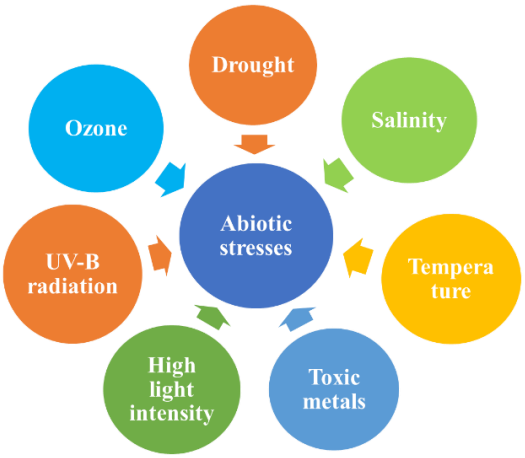

Fig. 2 Types of abiotic stresses

Among abiotic stresses, salinity stress greatly affects agricultural crops, around 20% irrigated land of world affected through salinity and generated huge crop yield losses which ultimately restrict global food security (Munns and Tester, 2008). To understand the mechanisms of plant tolerance, which is responsible to salinity stress, it is better to know the ways of affecting plant through salt stress. Salinity stress affect the plant in two ways, a) osmotic stress and b) ionic stress (Munns and Tester, 2008) (Fig. 3). Under salt affected land, crops first experiences with osmotic stress due to higher salt concentration around the root area caused lower osmotic potential. With time passes, plant falls on ionic stress with the increasing accumulation of toxic ions, mainly Na^+ which perturb many physiological and biochemical properties of plants (Munns and Tester, 2008; Ismail and Horie, 2017). Excess sodium in the root area also disrupts ion homeostasis specially increases potassium leakage. Severe potassium leakage from plant leads to a disturbance of vital enzyme activity, photosynthesis and accelerate cell death process (Shabala and Pottison, 2014). Therefore, both of osmotic and ionic stress also generates reactive oxygen species (ROS) which subsequently causes oxidative damage and cell death of plant. Furthermore, cadmium considered as a highly toxic pollutant which pollutes soil through increasing urbanization, sewage sludge disposing into the environment and using of excessive amount of phosphate fertilizers into soil (He et al., 2016; Khan et al., 2017). As cadmium has no biological function in plants, so plants growing in Cd-polluted soil severely affected its growth and development even at lower concentration (5-10µg g^{-1} dry weight) (Qadir et al., 2014; Khan et al., 2017). In addition to, Cd can easily be transported to other edible portions of plants, which poses a great risk to human health (Ismael et al., 2019; Mishra et al., 2019). The noticeable damage caused by toxic Cd in plants includes growth reduction, photosynthesis and respiration restriction, ROS generation, oxidative damage and leaf chlorosis (Yotsova et al., 2018; Xin et al., 2019). Copper (Cu), an essential micronutrient, plays an effective role in plant developmental processes including photosynthesis, respiration, redox reaction, and detoxification (Rai et al., 2021). However, extensive use of pesticides, industrial waste, smelting, and mining increases the level of Cu in soil (Ballabio et al., 2018). Furthermore, Cu concentrations in long-contaminated soils can be varied, ranging from 500 to 3000 mg kg^{-1}, depending on the severity of the polluted areas (Adrees et al., 2015). Plants grown in Cu-contaminated soil, greatly reduces the physiological and biochemical attributes (Huang et al., 2020). Excess Cu is also considered as lethal to animal and human when it comes to them through consuming toxic Cu affected plant and plant product (Vieira-Filho and Monteiro, 2022). The common response of toxic Cu is leaf chlorosis and necrosis, stunted growth, ROS generation, causing oxidative stress and cellular death.

ROS primarily form with hydroxyl radical ($^{\cdot}OH$), superoxide ($O_2^{\cdot-}$), and hydrogen peroxides (H_2O_2) (Foyer and Noctor, 2005). Under normal environment, ROS produced sustainedly in cells of plant. However, overproduction of ROS can be caused through different environmental factors such as salinity, Cd and Cu stress condition

(Roychoudhury and Basu, 2012). Furthermore, oxidative stress remains the main reason for loss of ROS scavenging system. As a result, inactivation and denaturation of protein, disruption of membrane integrity and cell death occur in plant (Gill and Tuteja, 2010).

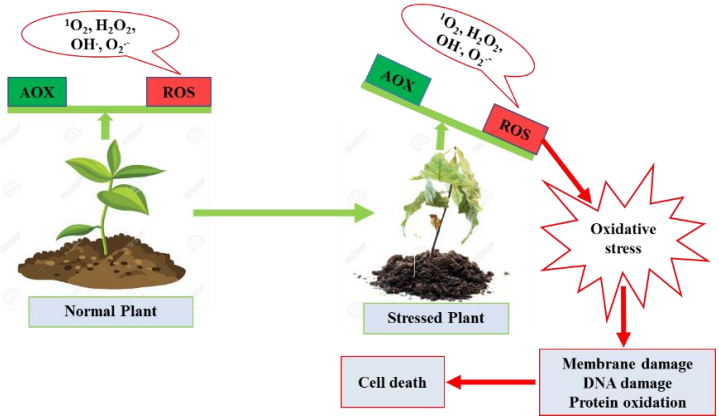

Fig. 3 ROS-mediated damage in stressed plants. AOX, antioxidant defense system; ROS, reactive oxygen species; $^1O_2$, singlet oxygen; $O_2^{\cdot-}$, super oxide radical, $^{\cdot}OH$, hydroxyl radical and H_2O_2, hydrogen peroxide.

Plants have an innate capacity to balance ROS homeostasis by maintaining the antioxidant defense system composed of non-enzymatic antioxidants such as ascorbate (AsA) and reduced glutathione (GSH) and enzymatic antioxidants including ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), and glutathione *S*-transferase (GST) (Shanying et al., 2017). Although plants trigger the activity of antioxidant defense system and increased the accumulation of osmolytes to keep the ROS in equilibrium rate during normal environmental condition (Taiz et al., 2015). However, an immense rate of ROS generating situation, especially under salinity, Cd and Cu stress condition, the efficiency of antioxidant defense system become inadequate for plant to detoxify them (Vieira-Filho and Monteiro, 2022). However, by any means, stimulation of this pathway may further increase the effectiveness of this system. For example, comparative studies reveal that tolerant variety possess better functioning of antioxidant defense system (Tiwary and Sarangi, 2017). In addition, transgenic plants with any of the enzymatic component of this system increases the tolerance of plants under stress condition (Nguyen et al., 2018).

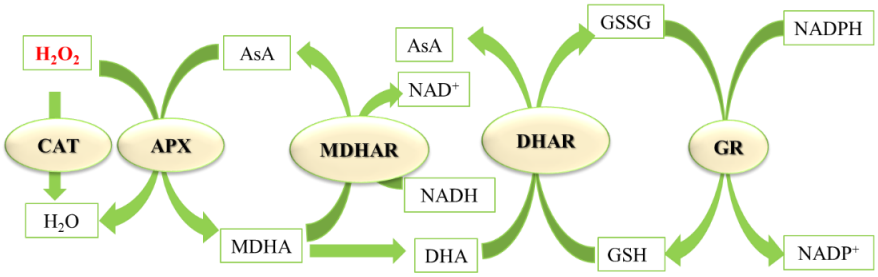

Fig. 4 Antioxidant defense system in plants. Catalase; CAT, ascorbate peroxidase; APX, monodehydroascorbate reductase; MDHAR, dehydroascorbate reductase, DHAR and glutathione reductase, GR; ascorbate, AsA; glutathione, GSH and oxidized glutathione, GSSG.

Lentil is a valuable crop for humanity with its high protein content. Besides its nutritive value, it has an agricultural value of nitrogen fixation and thus reduced the fertilizer use which further helps in soil enrichment (Romano et al., 2021). Lentil is a cool season crop which is best in soil with pH 6.0-8.0 and cannot tolerate waterlogging, flooding or high soil salinity and heavy metal toxicity. However, there is limited research have done regarding the enhancement of abiotic stress tolerance in lentil compared to other main crop including rice,

maize, wheat etc.

Different techniques have been employed to enhance multiple stress tolerance, such as conventional breeding (time-consuming technique) and plant genetic modification (unacceptable in many countries). Another way to treat the situation of enhancing stress tolerance in an eco-friendly way is using different types of chemicals which helps to mediate the physiological and biochemical changes to plants without any genetic modification (Savvides et al., 2016; Nguyen et al., 2018). Numerous studies have already done while using various chemicals including amino acids (proline), hormones (salicylic acid), reactive oxygen-nitrogen-sulfur species (RONSS) and water. Still, chemicals are being screened for more effectiveness. Recently, glutamate (Glu) a non-essential amino acid, functioned as a signaling role in many physiological development and abiotic stress adaptation of plant (Forde et al., 2014; Kong et al., 2015; Cheng et al., 2018; Li Z. et al., 2019). Furthermore, exogenous L-glutamic acid (L-Glu) application mitigate the adverse effect of salinity stress, drought stress and toxic cadmium in plant (Sh Sadak et al., 2015; La et al., 2020; Fardus et al., 2021a,b). Consistently, as an amino acid, L-Glu seems to be eco-friendly in nature due to having easy digestion ability by animal and plant (Kan et al., 2017). Considering the potential of L-Glu, we hypothesized that L-Glu could enhance other abiotic stress tolerance.

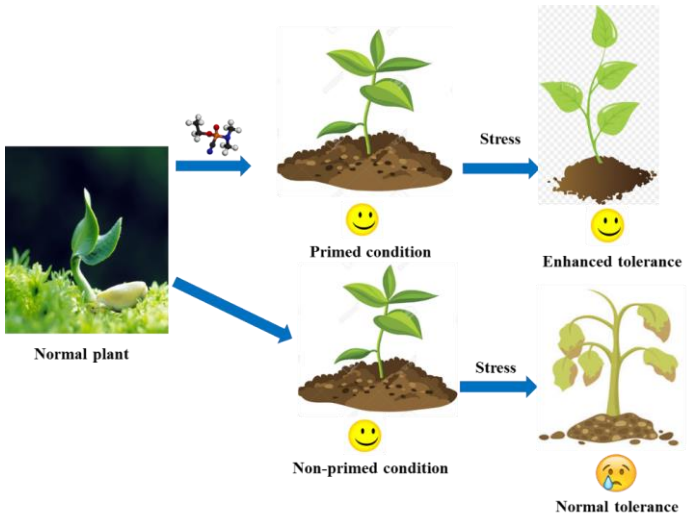

Fig. 5 Chemical priming enhances stress tolerance in plants

Going through the published research articles in the field of abiotic stress tolerance, I realized that upregulation of antioxidant defense system could enhance multiple stress tolerance in plants. To enhance stress tolerance, I was interested to focus on chemical biology approach as it could be convenient, low cost and ecofriendly. Therefore, the objectives of my study

1) To investigate the stress tolerance mechanism in economically important crop, lentil under salinity, Cd and Cu stresses.

2) To identify a potential chemical that can mitigate stressed-induced damages in lentil seedlings.

To accomplish these objectives, several experiments were conducted, and the findings are described in five different titles as follows–

1. Modulation of the Antioxidant Defense System by Exogenous L-Glutamic Acid Application Enhances Salt Tolerance in Lentil (*Lens culinaris* Medik.)

2. Potential role of L-glutamic acid in mitigating cadmium toxicity in lentil (*Lens culinaris* Medik.) through modulating the antioxidant defence system and nutrient homeostasis

3. L-glutamic acid modulates antioxidant defense systems and nutrient homeostasis in lentil (*Lens culinaris* Medik.) under copper toxicity

Experiment 1: Modulation of the Antioxidant Defense System by Exogenous L-Glutamic Acid Application Enhances Salt Tolerance in Lentil (*Lens culinaris* Medik.)

Meterials and methods

Plant materials and test conditions

Uniform seeds of lentil (*Lens culinaris* Medik cv. BARI Masur-7) were surface sterilized for 5 min using 70% ethanol, then dipped in distilled water for 24 h and placed on moistened six layers of paper towels in the Petri dishes and kept in dark conditions for 72 h. Keeping 40 germinated seedlings per Petri plate, Petri plates were then transferred into the growth chamber under a photon flux density of 350 µmol m^{-2} s^{-1} with continuous illumination and 25 ± 1 ˚C temperature. After 24 h, two sets of Petri plate were provided with 10 mM L-Glu along with Hyponex nutrient solution (Hossain et al., 2020). Another two sets of Petri plate were provided with only Hyponex (Tokyo, Japan) nutrient solution. Then 6-day-old seedlings were treated with NaCl (110 mM) with or without L-Glu pretreatment. The dose of L-Glu and salt stress was selected based on our preliminary trial and former report (Fig. 7) (Kan et al., 2017). After 2 days of stress treatment, seedlings were allowed to recover by supplying nutrient solution only. Thus, the treatments were control (without NaCl), 10 mM L-Glu, 110 mM NaCl (S), and 110 mM NaCl with 10 mM L-Glu (S+L-Glu), and each treatment had three replications. Finally, physiological, and biochemical parameters were measured from the seedlings.

Determination of physiological and biochemical parameters

Eleven-day-old seedlings were used to determine plant height, shoot fresh weight (SFW) and dry weight (SDW), root fresh weight (RFW) and dry weight (RDW), water content (WC), proline content (Pro), photosynthetic pigments content (Chl a, Chl b, Chls (a+b) and carotenoid; Car), oxidative stress markers contents (MDA, H_2O_2, and electrolyte leakage; EL), non-enzymatic antioxidant contents (AsA, reduced glutathione; GSH, oxidized glutathione; GSSG and GSH/GSSG), enzymatic antioxidant activities (CAT, APX, MDHAR, DHAR, GR, GPX, and GST), and minerals contents (Na^+, K^+, Ca^{2+} and Mg^{2+}) by following established standard methods.

Statistical analysis

XLSTAT v.2020 software (Addinsoft, Paris, France) using Fisher's least significant difference (LSD) at 5% probability ($P \leq 0.05$) used to evaluate the comparable mean difference of three replications and the analysis of variance (ANOVA).

Results

The effect of salt stress and the role of L-Glu in mitigating salt-induced damage in lentil seedlings were investigated by initially exposing the seedlings to 110 mM salt stress with or without different doses of L-Glu. The stressed plants became stunted and wilted and some leaves turned yellowish in color, but the plants pretreated with L-Glu were healthier and greener as they recovered from the imposed salt stress (Fig. 6a,b). Salt stress inhibited the growth and reduced the photosynthetic pigment (Chl a, Chl b, Chls (a+b) and Car) level, WC, and survival of lentil seedlings during recovery from the stress. Salt stress also induced oxidative damage, as indicated by higher H_2O_2 and MDA contents and EL (%), by interrupting the antioxidant defense system and promoting the accumulation of toxic levels of Na^+ (Fig. 7). However, L-Glu pretreatment mitigated the salt-induced damage in lentil seedlings by reducing the accumulation of Na^+, maintaining ion homeostasis, and increasing the activities of antioxidant enzymes (CAT and APX) (Fig. 7). As a result, salt-induced oxidative damage was reduced, seedling growth and photosynthetic pigment contents were enhanced, and the survival rate of the lentil seedlings was improved in response to salt stress, indicating an ameliorative role for L-Glu in lentil seedling growth under salt stress.

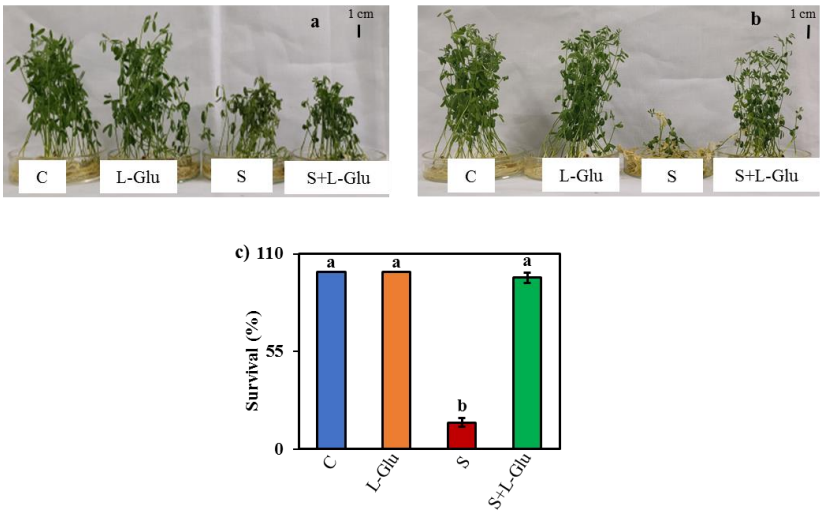

Fig. 6 Effect of L-Glutamic acid (L-Glu) on (a) the phenotypic appearance of lentil seedlings after 2 days of recovery, (b) the phenotypic appearance of lentil seedlings after 7 days of recovery and (c) the survival percentage of lentil seedlings after 7 days of recovery. The treatments were control (C), 10 mM L-Glu, 110 mM NaCl (S) and 110 mM NaCl + 10 mM L-Glutamic acid (S+L-Glu). The above mean (±SE) was calculated from three replications. Values of different letters indicate statistically significant differences at $P \leq 0.05$ (Fisher's LSD test).

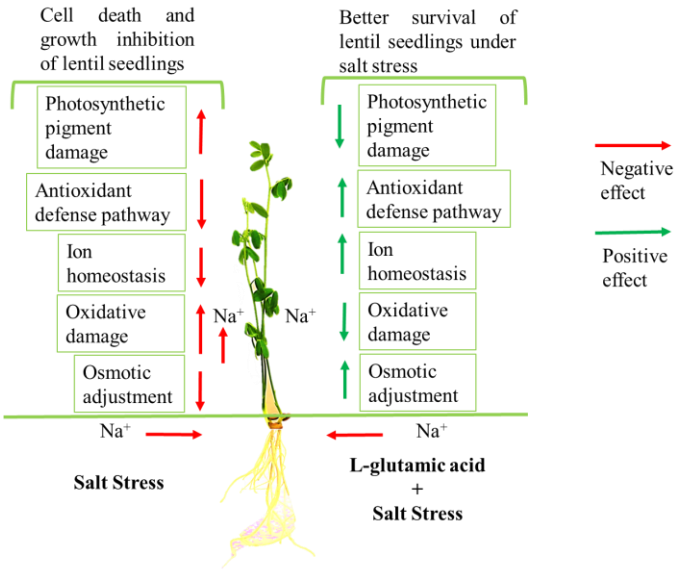

Fig. 7 Possible mechanisms of L-Glutamic acid in improving salt tolerance in lentil seedlings based on the present investigation.

Experiment 2: Potential role of L-glutamic acid in mitigating cadmium toxicity in lentil (*Lens culinaris* Medik.) through modulating the antioxidant defence system and nutrient homeostasis

Materials and methods

Plant materials and test conditions

Healthy lentil (*Lens culinaris* Medik cv. 'BARI Masur-7') seeds were surface sterilized by soaking them in 70% ethanol for 5 min. The disinfected seeds were then washed and soaked in distilled water for 24 h. The next day, the soaked seeds were washed again with distilled water and kept in a dark condition for 72 h for gemination in

Petri dishes containing six layers of wetted paper towels. Forty germinated seedlings were kept in each Petri dish and placed in a cultivation chamber under continuous illumination at 350 µmol m^{-2} s^{-1} photon flux density and 25 ± 1 °C. Hyponex (Tokyo, Japan) nutrient solution with the concentration of 0.2 mL L^{-1} was supplied to the seedlings with or without 10 mM L-Glu on the following day and left for another 48 h. The dose of L-Glu was selected based on the previous reports of Fardus et al. (2021) and Kan et al. (2017). The seedlings from four sets of Petri dishes with or without L-Glu were then exposed to 1- and 2-mM cadmium chloride ($CdCl_2$). The doses of Cd were chosen based on a preliminary trial testing a series of Cd concentrations (0.3-3 mM). Stress treatments were continued for five days and changed on alternate days. The 9-day-old seedlings were then used to determine the physiological and biochemical attributes. Three replications were used for each treatment.

Determination of physiological and biochemical parameters

Plants (11 days) were used to determine SFW and SDW, RFW and RDW, WC, Pro content, photosynthetic pigments content (Chl a, Chl b, Chls (a+b) and Car), oxidative stress markers contents (MDA, H_2O_2, and EL), non-enzymatic antioxidant contents (AsA, GSH, GSSG and GSH/GSSG), enzymatic antioxidant activities (CAT, APX, MDHAR, DHAR, GR, and GST), Cd and minerals contents (K^+, Ca^{2+} and Mg^{2+}) by following established standard methods.

Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA) of three replications and XLSTAT v.2020 software. Fisher's least significant difference (LSD) with a probability of 5% was used to assay the mean difference of those replications.

Results

Exposing the lentil seedlings to the liquid solution of Cd (1 and 2 mM $CdCl_2$) clearly affected the phenotypic appearance of the seedlings, including growth reduction and leaf chlorosis. Conversely, compared with the Cd stressed plants, exogenous pre-treatment with 10 mM L-Glu reversed the phytotoxic effects of Cd by reviving the leaves and improving the phenotypic appearance of the lentil seedlings (Fig. 8). A high dose of Cd negatively affected the SDW, RDW, and photosynthetic pigments (Chl a, Chl b, Chls (a+b) and Car). Furthermore, Cd stress induced severe oxidative damage, a reduction in CAT activity and AsA content, and accumulation of Cd in both the roots and shoots (Fig. 9). Adding L-Glu protected the photosynthetic pigments of the lentil seedlings and thus improved the growth of the seedlings. In addition, L-Glu pre-treatment enhanced the AsA content; increased the activity of enzymes such as CAT, APX, MDHAR, and GPX (Fig. 9). L-Glu was also reduced Cd uptake and translocation, which in turn alleviated the oxidative damage in the Cd-stressed seedlings indicated the potential role of this chemical. Results suggest that pre-treatment with L-Glu reduces Cd toxicity in lentil seedlings by inhibiting Cd accumulation and by reducing oxidative damage.

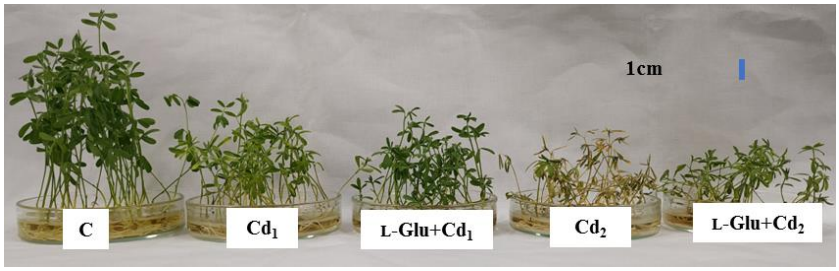

Fig. 8 Effect of L-glutamic acid on the phenotypic appearance of the lentil seedlings under Cd stress. The treatments were control (C), 1 mM $CdCl_2$ (Cd_1), 10 mM L-glutamic acid + 1 mM $CdCl_2$ (L-Glu+Cd_1), 2 mM $CdCl_2$ (Cd_2), and 10 mM L-glutamic acid + 2 mM $CdCl_2$ (L-Glu+Cd_2).

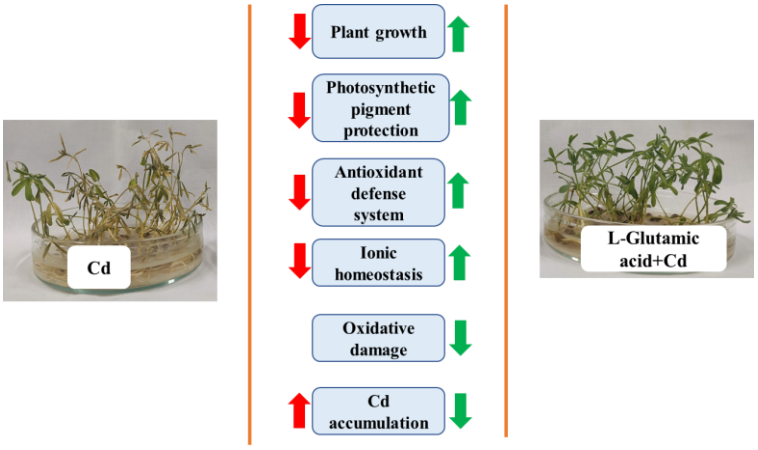

Fig. 9 Possible mechanisms of L-Glutamic acid in improving Cd toxicity tolerance in lentil seedlings based on the present investigation.

Experiment 3: L-glutamic acid modulates antioxidant defense systems and nutrient homeostasis in lentil (Lens culinaris Medik.) under copper toxicity

Materials and methods

Growth condition and stress treatments

Vigorous seeds of lentil (*Lens culinaris* Medik cv. 'BARI Masur-7') were soaked in distilled water for 24 h after surface sterilization with 70% ethanol and repeated washing. The soaked seeds were then sown in petri dishes with six layered wetted paper towel and kept in the dark at 28 ± 2 ˚C for 3 days to germinate. After germinations, uniform seedlings (40 seedlings per petri dish) were transferred into growth chamber at 25 ± 1 ˚C temperature. On the following day, 0.2 mL L^{-1} diluted hyponex (Tokyo, Japan) solution with or without 10 mM L-Glu were provided to the seedlings. Previous reports of Fardus et al. (2021) and Kan et al. (2017) were followed to select the dose of L-Glu. At day 7 after sowing, L-Glu pretreated and non-pretreated seedlings were then subjected to 2- and 3-mM copper sulphate pentahydrate ($CuSO_4.5H_2O$) in the diluted hyponex solution. The concentration of $CuSO_4$ were chosen based on the preliminary trial with a range of 0.5-4 mM $CuSO_4$ concentration. The hyponex and treatment was renewed in every two days. After 7-days of first treatment, different physiological and biochemical parameters were determined. Therefore, our experimental detailed treatments as follows: (a) Control (C), (b) 2 mM $CuSO_4$ (Cu_2), (d) 10 mM L-glutamic acid + 2 mM $CuSO_4$ (L-Glu+Cu_2), (e) 3 mM $CuSO_4$ (Cu_3), (f) 10 mM L-glutamic acid + 3 mM $CuSO_4$ (L-Glu+Cu_3) and each treatments had three replications.

Determination of physiological and biochemical parameters

Eleven-day-old seedlings were used to determine SFW and SDW, RFW and RDW, WC, Pro content, photosynthetic pigments content (Chl a, Chl b, Chls (a+b), and Car), oxidative stress markers contents (MDA, H_2O_2, and EL), non-enzymatic antioxidant contents (AsA, GSH, GSSG and GSH/GSSG), enzymatic antioxidant activities (CAT, APX, MDHAR, DHAR, GR, GPX, and GST), Cu and minerals contents (K^+, Ca^{2+} and Mg^{2+}) by following established standard methods.

Statistical analysis

Statistical analysis was executed through applying XLSTAT software (Addinsoft, Paris, France) where data were subjected to analysis of variance (ANOVA) and different letters indicate significant differences at 5% probability ($p \leq 0.05$) according to Fisher's least significant difference (LSD).

Results

To determine the feasible effects of L-Glu in mitigating the toxic effect of Cu, seedlings of lentil were grown with exposing 2 and 3 mM concentration of $CuSO_4.5H_2O$ in the presence or absence of L-Glu. Visible symptoms of toxic Cu such as retarded growth, leaf chlorosis, reduced branching and thickening, gradually noticed by the treatment of toxic Cu in a Cu concentration-dependent manner, as comparable to control (Fig. 10). Exposure to toxic Cu depleted photosynthetic pigments (Chl a, Chl b, Chls (a+b), and Car), imbalanced water content and other essential nutrients, increased oxidative stress and reduced enzymatic and non-enzymatic antioxidants (Fig. 11). However, pretreatment of L-Glu improved phenotypic appearance of lentil seedlings, which was distinctly appeared by higher biomass production, water balance maintaining and increasing photosynthetic pigments when exposed to toxic Cu. L-Glu also protected the seedlings from Cu-induced oxidative stress by reducing the oxidative stress marker, specifically by the efficient action of enzymatic and non-enzymatic antioxidants specially AsA, CAT, MDHAR and GPX and maintaining redox balance (Fig. 11). Furthermore, L-Glu kept maintaining the homeostasis of Cu and other nutrient to the roots, shoots, and leaves of lentil. Collectively, our results provide an evidence of the mechanism of L-Glu-mediated protective role in lentil against Cu toxicity, thus proposed as a potential chemical for controlling Cu toxicity not only in lentil but also other plants.

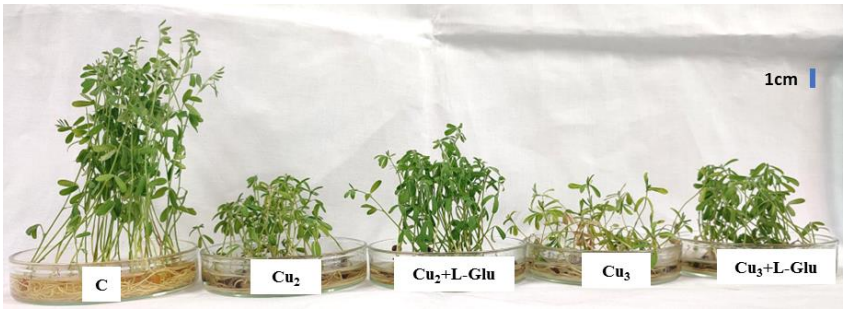

Fig. 10 Effect of L-glutamic acid on the phenotypic appearance of the lentil seedlings under Cu stress. The treatments were control (C), 2 mM $CuSO_4$ (Cu_2), 10 mM L-glutamic acid + 2 mM $CuSO_4$ (L-Glu+ Cu_2), 3 mM $CuSO_4$ (Cu_3), and 10 mM L-glutamic acid + 3 mM $CuSO_4$ (L-Glu+Cu_3).

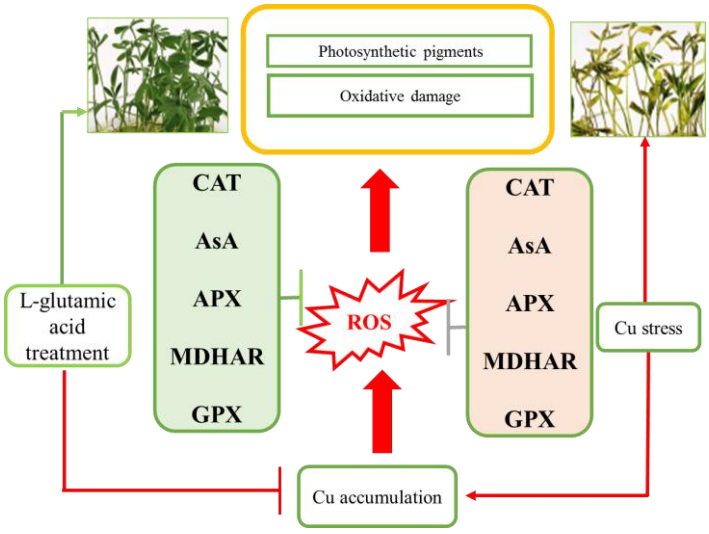

Fig. 11 Possible mechanisms of L-Glutamic acid in improving Cu toxicity tolerance in lentil seedlings based on the present investigation.

Summary

Based on our result, I can summarize that, exposure to NaCl and toxic concentrated Cd and Cu in an extended period resulted in severe reduction of physiological and biochemical content of lentil seedlings. In contrast, pretreatment of L-Glu improved seedlings phenotype while reducing the toxicity of NaCl, Cd and Cu by i) reducing accumulation and translocation of Na, Cd and Cu to the root to shoot to leaves, ii) improving nutrient homeostasis and photosynthetic pigmentation, iii) maintaining water balance and proline uptake, iv) decreasing oxidative damage through the detoxification of ROS by maintain the active participation of antioxidants, especially AsA, CAT, and MDHAR. Taken together, a coordinating action of physiological and biochemical systems requires to induce tolerance against heavy metal toxicity conditions. Therefore, our present study make advancement to understand that mechanism of diminishing the adverse effects of NaCl, Cd and Cu integrating to lentil seedlings. However, long term effect along with molecular mechanisms of L-Glu in increasing tolerance against NaCl, Cd and Cu should be considered to determine in our further studies.

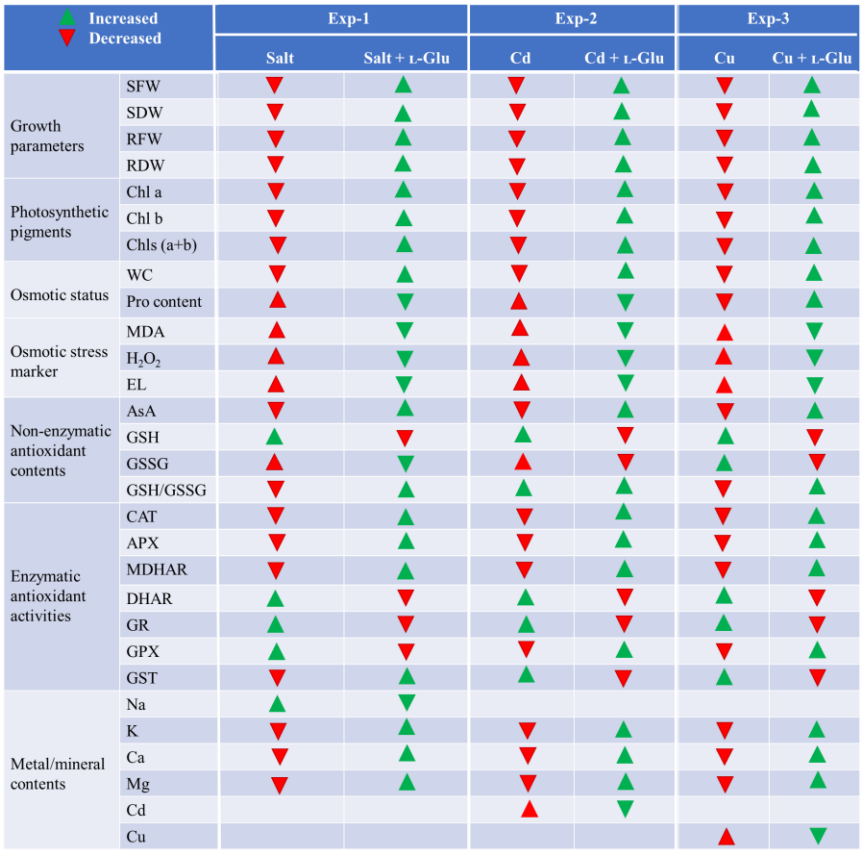

Fig. 12 Overall summaries on possible mechanisms of L-Glutamic acid for improving plant tolerance under abiotic stress conditions based on the present investigation.

References

Addinsoft. XLSTAT v. 2020.1.1: Data analysis and statistics software for Microsoft Excel. Addinsoft, Paris 2020.

Lineham, V., Thorpe, S., Andrews, N., Kim, Y. and Beaini, F. Food Demand to 2050: Opportunities for Australian Agriculture, Algebraic Description of Agrifood Model. ABARES 2012.

Wang, W., Vinocur, B. and Altman, A. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta 2003, 218, 1-14.

Wania, S. H., Kumar, V., Shriram, V., and Sah, S. K. Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. Crop J. 2016, 4, 162–176.

Munns, R. and Tester, M. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 2008, 59, 651–681.

Ismail, A.M. and Horie, T. Genomics, physiology, and molecular breeding approaches for improving salt tolerance. Annu. Rev. Plant Biol. 2017, 68, 405–434.

He, F., Arce, A.L., Schmitz, G., Koornneef, M., Novikova, P., Beyer, A. and De Meaux, J. The footprint of polygenic adaptation on stress-responsive cis-regulatory divergence in the Arabidopsis genus. Mol. Biol. Evolut. 2016, 33, 2088-2101.

Khan, M.A., Khan, S., Khan, A. and Alam, M. Soil contamination with cadmium, consequences and remediation using organic amendments. Sci. Total Environ. 2017, 601, 1591-1605.

Ismael, M.A., Elyamine, A.M., Moussa, M.G., Cai, M., Zhao, X. and Hu, C. Cadmium in plants: Uptake, toxicity, and its interactions with selenium fertilizers. Metallomics 2019, 11, 255-277.

Mishra, P., Bhoomika, K. and Dubey, R.S. Differential responses of antioxidative defense system to prolonged salinity stress in salt-tolerant and salt-sensitive Indica rice (*Oryza sativa* L.) seedlings. Protoplasma 2013, 250, 3–19.

Nguyen, H.C., Lin, K.H., Ho, S.L., Chiang, C.M. and Yang, C.M. Enhancing the abiotic stress tolerance of plants: From chemical treatment to biotechnological approaches. Physiol. Plant. 2018, 164, 452–466.

Qadir, S., Jamshieed, S., Rasool, S., Ashraf, M., Akram, N.A. and Ahmad, P. Modulation of plant growth and metabolism in cadmium-enriched environments. Rev. Environ. Contam. Toxicol. 2014, 51-88.

Savvides, A., Ali, S., Tester, M. and Fotopoulos, V. Chemical priming of plants against multiple abiotic stresses: Mission possible? Trend. Plant Sci. 2016, 21, 329–340.

Shabala, S. and Pottosin, I. Regulation of potassium transport in plants under hostile conditions: implication for abiotic and biotic stress tolerance. Physiol. Plant. 2014, 151, 257–279.

Sh Sadak, M., Abdelhamid, M.T. and Schmidhalter, U. Effect of foliar application of amino acids on plant yield and some physiological parameters in bean plants irrigated with seawater. Acta Biol. Colomb. 2015, 20, 141–152.

Xin, J., Zhao, X.H., Tan, Q.L., Sun, X.C., Zhao, Y.Y. and Hu, C.X. Effects of cadmium exposure on the growth, photosynthesis, and antioxidant defense system in two radish (*Raphanus sativus* L.) cultivars. Photosynthetica 2019, 57, 967-973.

Rai, S., Singh, P.K., Mankotia, S., Swain, J. and Satbhai, S.B. Iron Homeostasis in Plants and its Crosstalk with Copper, Zinc, and Manganese. Plant Stress 2021, 100008.

Yotsova, E.K., Dobrikova, A.G., Stefanov, M.A., Kouzmanova, M. and Apostolova, E.L. Improvement of the rice photosynthetic apparatus defence under cadmium stress modulated by salicylic acid supply to roots. Theor. Experim. Plant Physiol. 2018, 30, 57-70.

Ballabio, C., Panagos, P., Lugato, E., Huang, J.H., Orgiazzi, A., Jones, A., Fernández-Ugalde, O., Borrelli, P. and Montanarella, L. Copper distribution in European topsoils: An assessment based on LUCAS soil survey. Sci. Total Environ. 2018, 636, 282-298.

Adrees, M., Ali, S., Rizwan, M., Zia-ur-Rehman, M., Ibrahim, M., Abbas, F., Farid, M., Qayyum, M.F. and Irshad, M.K. Mechanisms of silicon-mediated alleviation of heavy metal toxicity in plants: a review. Ecotoxicol. Environ. Saf. 2015, 119, 186–197.

Huang, W.L., Wu, F.L., Huang, H.Y., Huang, W.T., Deng, C.L., Yang, L.T., Huang, Z.R. and Chen, L.S. Excess copper-induced alterations of protein profiles and related physiological parameters in citrus leaves. Plants 2020, 9, 291.

Vieira-Filho, L.O. and Monteiro, F.A. Silicon improves photosynthetic activity and induces antioxidant enzyme activity in *Tanzania Guinea* grass under copper toxicity. Plant Stress 2022, 3, 100045.

Foyer, C. H. and Noctor, G. Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. Plant Cell 2005, 17, 1866–1875.

Gill, S.S. and Tuteja, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 2010, 48, 909–930.

Roychoudhury, A., and Basu, S. "Ascorbate-Glutathione and plant tolerance to various abiotic stresses," in Oxidative Stress in Plants: Causes, Consequences and Tolerance, eds N. A. Anjum, S. Umar, and A. Ahmad (New Delhi: IK International Publishers), 2012, 177–258.

Shanying, H.E., Xiaoe, Y.A.N.G., Zhenli, H.E. and Baligar, V.C. Morphological and physiological responses of plants to cadmium toxicity: A review. Pedosphere 2017, 27, 421-438.

Taiz, L., Zeiger, E., Møller, I.M. and Murphy, A. Plant physiology and development (No. Ed. 6). Sinauer Associates Incorporated, 2015.

Tiwari, S. and Sarangi, B.K. Comparative analysis of antioxidant response by *Pteris vittata* and *Vetiveria zizanioides* towards arsenic stress. Ecol. Eng. 2017, 100, 211-218.

Romano, A., Gallo, V., Ferranti, P. and Masi, P. Lentil flour: Nutritional and technological properties, in vitro digestibility and perspectives for use in the food industry. Curr. Opin. Food Sci. 2021, 40, 157-167.

Li, Z.G., Ye, X.Y. and Qiu, X.M. Glutamate signaling enhances the heat tolerance of maize seedlings by plant glutamate receptor-like channels-mediated calcium signaling. Protoplasma, 2019, 256, 1165-1169.

Kong, D., Ju, C., Parihar, A., Kim, S., Cho, D. and Kwak, J.M. Arabidopsis glutamate receptor homolog3.5 modulates cytosolic Ca^{2+} level to counteract effect of abscisic acid in seed germination. Plant Physiol. 2015, 167, 1630-1642.

Fardus, J., Hossain, M. and Fujita, M. Modulation of the antioxidant defense system by exogenous L-glutamic acid application enhances salt tolerance in lentil (*Lens culinaris* Medik.). Biomolecules 2021, 11, 587.

Fardus, J., Hossain, M.S. and Fujita, M. Potential role of L-glutamic acid in mitigating cadmium toxicity in lentil (*Lens culinaris* Medik.) through modulating the antioxidant defence system and nutrient homeostasis. Not. Bot. Horti Agrobot. Cluj-Napoca, 2021, 49, 12485-12485.

Cheng, Y., Zhang, X., Sun, T., Tian, Q. and Zhang, W.H. Glutamate receptor homolog3. 4 is involved in regulation of seed germination under salt stress in Arabidopsis. Plant Cell Physiol. 2018, 59, 978-988.

Kan, C.C., Chung, T.Y., Wu, H.Y., Juo, Y.A. and Hsieh, M.H. Exogenous glutamate rapidly induces the expression of genes involved in metabolism and defense responses in rice roots. BMC Genom. 2017, 18, 186.

La, V.H., Lee, B.R., Islam, M., Mamun, M., Park, S.H., Bae, D.W. and Kim, T.H. Characterization of Glutamate-Mediated Hormonal Regulatory Pathway of the Drought Responses in Relation to Proline Metabolism in *Brassica napus* L. Plants 2020, 9, 512.

Hossain, M.S., Abdelrahman, M., Tran, C.D., Nguyen, K.H., Chu, H.D., Watanabe, Y., Hasanuzzaman, M., Mohsin, S.M., Fujita, M. and Tran, L.S.P. Insights into acetate-mediated copper homeostasis and antioxidant defense in lentil under excessive copper stress. Environ. Pollut. 2020, 258, 113544.

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