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THE POTENTIAL OF THIOUREA IN AMELIORATION OF NICKEL TOXICITY IN BARLEY (*HORDEUM VULGARE* L.) CULTIVARS

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Abstract

Nickel (Ni) stress is vital environmental stress which has reduced plant productivity worldwide. Thiourea (TU) is a key regulator of plant development which improves physiological and biochemical mechanisms of plants under Ni toxicity. To explore the part of TU in mitigating Ni toxicity, we investigated various levels of TU (50, 100 mM) for 40 days on phenotypic, photosynthetic, antioxidant activity and organic and inorganic osmolytes of barley cultivars. The results depicted that Ni stress (50 mM) significantly reduced all studied growth parameters, photosynthetic content, antioxidative activity, organic osmolytes (soluble proteins and sugars), and inorganic osmolytes (K^+ and Ca^{2+}) in shoot and root of barley cultivars. However, the application of TU (100 mM) highly improved above mentioned growth attributes, photosynthetic content, antioxidative activity, and organic and inorganic osmolyte content and proved very effective in reversing the Ni toxicity effect. Thiourea application proved very effective in balancing endogenous metabolite levels under Ni stress conditions. The cultivar, Sultan-17 proved Ni-tolerant, while Jou-17 exhibited Ni sensitivity. On the whole, TU at 100 mM proved very effective in enhancing barley growth under Ni stress. The outcomes of current study may have good inferences for growing barley under Ni-toxic conditions.

Key words: Antioxidants; Barley; Growth; Nickel toxicity; Osmolytes; Oxidative stress; Thiourea

Introduction

Nickel is considered an important micronutrient in low concentration because it activates various enzymes employed in plant metabolism under stressful conditions. Nonetheless, in high concentrations, it develops toxins in plants by lowering various metabolic pathways and ultimately reducing plant growth and development. Nickel concentrations are about 10 - 100 mg/kg in normal soils, whereas they range 200-26,000 mg/kg in contaminated soil and about 1 ppm in plants (Sreekanth *et al*., 2013). Nickel at high levels causes growth inhibition and delays time required for 50% germination (Ahmad & Ashraf, 2012). Nickel toxicity reduces plant growth by decreasing fresh weight, leaf area and inhibition of cell division at the root meristem (Shaw *et al*., 2004). At the physiological level, it decrease the uptake of essential nutrients of plants (Ahmad *et al*., 2011; 2022a), CO_2 intake, chlorophyll content, and photosynthetic pathways (Yusuf *et al*., 2011; Sreekanth *et al*., 2013). High Ni content also causes the accumulation of hydroxyl ion (OH^-), hydrogen peroxide (H_2O_2), superoxide (O^{2-}), peroxyl, and alkoxyl. Nickel indirectly disturbs the activities of antioxidative enzymes, because this metal is not capable of undergoing redox reactions and therefore does not generate reactive oxygen species (ROS) directly (Chen *et al*., 2009). High Ni levels can lead to the production of oxidative stress by changing the metabolisms of enzymes, which directly causes protein damage and induce alteration in sugars and amino acids (Ahmad & Ashraf, 2012). This is essential to improve more precise ways to counteract the harmful effects of Ni.

A sufficient quantity of mineral nutrients is needed to plants for surviving under adverse environmental conditions. The nutritional status of plants affects their ability to mitigate the detrimental effects imposed by stressful situations (Waqas *et al*., 2019). Among naturally occurring plant growth regulators, TU, a nitrogen-containing growth-regulating substance, has been specifically used in stimulating crop productivity worldwide (Farooq *et al*., 2009; Perveen *et al*., 2016). Because of its significant water solubility and rapid absorption by biological tissues, it enhances stress tolerance. Applying TU through foliar spraying led to a reduction in the presence of detrimental ions, an elevation in active nutrient concentration, and a decrease in H_2O_2, MDA, and membrane permeability levels (Perveen *et al*., 2013). Functioning as a fertilizer rich in nitrogen and sulfur, it acts as a plant growth stimulant, promoting seed germination, growth, and mitigating stress in plants (Wahid *et al*., 2017). Foliar spray of TU could stimulate photosynthesis, and regulates metabolic activities of plants, nutrient absorption, and assimilation which increases tolerance against metal stress and improves growth in Ni-treated plants (Waqas *et al*., 2019). The use of TU is more effective in enhancing plant growth by increasing soluble sugars, proteins, and free amino acids (Amin *et al*., 2013), photosynthetic pigment contents (Wahid *et al*., 2017) and antioxidants, which increase length and greater fresh and dry weight of plants (Hassanein *et al*., 2015).

Barley (*Hordeum vulgare* L.) is a crucial annual crop which holds the fourth position in global rankings after wheat, corn, and rice. Barley grains contain around 70%

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carbohydrate, 2-3% free lipids, 1-2.5% minerals (Ca, Mg, P, Se, Mn, Zn), 10-20% protein, 11-34% fiber and 5-10% -glucan (w/w) (Farag *et al*., 2022). Due to its short growing season, barley is indeed an essential cereal grain. According to the 2016 global scenario, the annual production of barley is 147.4 million tones worldwide. In Pakistan, the total land area dedicated to barley cultivation encompasses approximately 60,000 hectares, resulting in a production of 58,000 metric tons and an average yield of 0.95 tons per hectare. This average yield is lower than the global average yielding of 3.0 t/ha (Ali, 2020). Heavy metals stand out as a paramount factor among various environmental stressors, significantly impacting the growth, nutrient uptake and ultimately barley yield (Haddad *et al*., 2021). To fulfill the rising demands of world, such practices that increase the crop's ability to withstand stress should be used.

From the above description it is clear that Ni stress disturbs plant growth and productivity. It is assumed that application of TU may reduce the toxic effects of Ni by modulating inorganic and organic osmolytes and changing antioxidant activity. In this study appropriate level of TU was determined which can lower Ni toxicity and improve overall plant growth.

Material and Methods

Experimental procedure and application of treatment: A study was carried out on barley (*Hordeum vulgare* L.) cultivars (Sultan-17 and Jou-17) acquired from Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan. This study aimed to examine the impact of TU on barley plants under conditions of nickel (Ni) stress. For the experimentation, seeds were planted in pots containing 8 kg of soil per pot, with a pH below 8.5 and an electrical conductivity exceeding 4.0 mmhos/cm. The potted plants were kept under sunlight with a ~20.2/~9±2°C day/night temperature, RH ~55/~65±2%, and photosynthetic photon flux density of 400 to 850 µmol $m^{-2}s^{-1}$. The concentration of Ni in this soil was 10-20 mg/ kg (ppm). After the germination of seeds, thinning was practiced and five uniform and healthy seedlings were left in each pot. To one week old plants, Ni stress (50 mM) was applied by using $NiCl_2$. $6H_2O$. Two different levels of TU (H_2NCSNH_2; F.W. 76.12) 50 and 100 mM were used as a foliar spray following one week of stress application. The first harvest was taken upon 15 days of foliar spray to determine growth parameters. There was a final harvest of 40 days old seedlings, and several vegetative, physiological also biochemical attributes were studied.

Growth parameters: The plants were carefully uprooted from the soil, and the shoot and root segments were separated to measure their lengths individually. A measuring tape was used to assess the lengths of the shoot and root. An electronic weight balance was employed to measure the fresh weight of the shoot and root. After a week, the samples were kept in an oven at 75°C to determine the dry weights of the shoot and root. The leaves were counted accurately per plant, and their average values were calculated.

Photosynthetic pigments: The photosynthetic attributes were assessed by following Arnon (1949 Fresh leaves (0.1 g) were extracted using 5 mL of 80% acetone. The sample was kept at room temperature overnight to facilitate thorough extraction. Then, the solution was centrifuged for 15 min at a speed of 14000 rpm. Optical density of the mixture was noted by using a Spectrophotometer UV-1100 Hitachi 220 (Japan). Absorbance readings were recorded at wavelengths of 663 nm, 645 nm, and 480 nm for chlorophyll a, chlorophyll b, and carotenoids, respectively.

$$\text{Chl. } a \text{ (mg mL}^{-1}) = [12.7 \text{ (OD 663)} - 2.69 \text{ (OD645)}] \times V/1000 \times W$$

$$\text{Chl. } b \text{ (mg mL}^{-1}) = [22.9 \text{ (OD 645)} - 4.68 \text{ (OD663)}] \times V/1000\ W$$

$$\text{Carotenoids} = [(\text{OD 480}) + 0.114 \text{ (OD 663)} - 0.638 \text{ (OD645)}]$$

$$\text{Total chl. (mg/g)} = [20.2(\text{OD645}) + 8.02 \text{ (0D663)}] \times V/1000 \times W$$

where
V= Volume of the concentrate (mL)
W = Weight of the new leaf tissue (g)
E 100% Cm = 2500
Carotenoids (g mL^{-1}) = A car/Em100% × 100

Enzymatic antioxidants: Fresh green leaves (0.25 g) were ground with the help of a cooled pestle and mortar in potassium phosphate buffer (5 mL) with a pH of 7.8. The resulting extract was transferred to a 2 mL Eppendorf tube and centrifuged at 12000 rpm for a period of 15 min. Supernatant, devoid of impurities, was kept safe at -20°C for future use.

Superoxide dismutase (SOD): Superoxide dismutase content was determined with a method proposed by Giannopolitis and Ries (1977). A mixture of 0.05 mL plant extract, 0.05 mL NBT, 0.05 mL riboflavin, 0.1 mL L-methionine, 0.1 mL Triton-X, and 1 mL phosphate buffer having pH 7.8 was thoroughly prepared. This reaction solution was exposed against fluorescent lights (75 µM photons m^2s^{-1}), and absorbance at 560 nm was measured by a spectrophotometer UV-1100 Hitachi 220 (Japan). Superoxide dismutase activity was defined as the enzyme quantity required inhibiting 50% photoreduction of NBT.

Peroxidase (POD): Peroxidase activity was determined as per the procedure proposed by Chance & Maehly (1955). A mixture was prepared for determining POD activity by mixing 100 µL of guaiacol, 100 µL of hydrogen peroxide, 50 µL of the sample extract, and 750 µL of phosphate buffer. The progression of the reaction was stopped by mixing 0.5 mL of 5% (v/v) H_2SO_4 following incubation for 15 min at 25°C. The POD activity was measured at 470 nm after 0, 30, and 60 sec intervals using a spectrophotometer UV-1100, Hitachi 220 (Japan).

Catalase (CAT): Catalase activity was determined by the method outlined by Chance & Maehly (1955). The reaction blend consisted of 1 mL of 5.9 mM H_2O_2, 1.9 mL of 50 mM phosphate buffer having pH 7, and 0.1 mL of enzyme extract within a 3 mL reaction solution. Reduction in absorbance was monitored every 30 sec at 240 nm with a spectrophotometer UV-1100 Hitachi 220 (Japan). A catalase activity unit was characterized as an alteration in absorbance of 0.01 units per minute.

Determination of organic osmolytes

Total soluble proteins: The Bradford (1976) method was employed for calculating total soluble proteins. Newly harvested leaves (0.25 g) were ground in a mortar and pestle by using 5 mL of phosphate buffer having pH 7.8. The resulting homogenous mixture was centrifuged at 12000 rpm for 15 min at 4°C. After the centrifugation process, the clear liquid above, known as the supernatant, was isolated from any contaminants. In test tubes, the extract (0.1 mL) was mixed with 5 mL of the Bradford reagent. The mixture was vortexed, and the optical density of the solution was observed at 595 nm with a Spectrophotometer UV-1100 Hitachi 220 (Japan).

Total soluble sugar: Total soluble sugar concentration was calculated by a method proposed by Yoshida *et al*., (1976). The newly harvested leaves (0.1 g) were subjected to boiling in 5 mL of distilled water at a temperature of 90°C for duration of 1 h. Subsequently, the resulting extract was passed through a filter, and its volume was then brought to 10 mL using distilled water. A mixture was prepared by adding 5 mL of an acidic anthrone reagent to 1 mL of the obtained extract. Following a 3 min vortexing process, the solution was placed in a water bath at a temperature of 90°C for 20 min for incubation. The level of absorbance was measured at 620 nm with a Spectrophotometer UV-1100 Hitachi 220 (Japan).

Total free amino acids: The Hamilton (1973) technique was employed to measure the overall content of free amino acids. In this procedure, 0.5 mL of plant extract was combined with 0.5 mL of 10% pyridine and a solution containing 2% ninhydrin in 25 mL test tubes. These tubes were then exposed to a water bath at a temperature of 100°C for duration of 40 min. Following this, the volume was adjusted to 25 mL using distilled water, and the optical density at 570 nm was gauged with a Spectrophotometer UV-1100 Hitachi 220 (Japan).

Determination of inorganic osmolytes

Inorganic ionic content: The method proposed by Allen *et al*., (1986) was employed to determine inorganic ionic content. The plant sample (0.1 g) was dried and crushed was placed in a digestion flask. Each flask was filled with 2 mL of 95% pure H_2SO_4, covered with aluminum foil, and left at room temperature overnight. The flasks were then gradually heated on a hot plate, maintaining a temperature between 70°C and 200°C. After heating, H_2O_2 was added to each flask, and the reaction was accelerated until neutrality was achieved. Once the flasks had cooled down, their contents were subjected to filtration. The resulting filtrate was then appropriately diluted with distilled water, ultimately reaching a total volume of 50 mL. Using standard values, this filtrate was used to measure distinct cations Na^+, K^+, and Ca^{2+} of the root and shoot using a flame photometer.

Statistical analysis: The study was carried out following a completely randomized design (CRD) and was replicated three times. The data obtained was subjected to statistical analysis using the Analysis of Variance (ANOVA) method within the Co Stat software (Steel *et al*., 1997). Data was graphically represented using Microsoft Excel.

Results

Growth parameters: The results demonstrated that exposure to nickel (Ni) stress led to adverse morpho-physiological changes in the plants, resulting in reduced plant length, fresh and dry weights of roots and shoots, and fewer roots per plant in comparison to control group in both studied cultivars. A significant interaction between variables V (cultivar) and Ni was observed for root length and shoot dry weight (Table 1). When compared to the control treatment, the growth parameters (shoot and root length), biomass (fresh and dry weight), and root and leaf per plant indices of barley seedlings were reduced by 43.18%, 26.1%, 50.57%, and 58.25%, respectively, under Ni exposure alone in the Ni-sensitive cultivar (Jou-17). However, the application of thiourea (TU) improved the length, fresh and dry weights of shoots and roots, as well as the number of roots and leaves per plant under Ni stress conditions. Both levels of TU (50 and 100 mM) enhanced the growth parameters, with the maximum improvement observed at the 100 mM TU level (Fig. 1). Tiourea application ameliorated the toxic effects of Ni stress and enhanced all studied growth parameters of barley. A statistically significant Ni × TU interaction was observed for shoot and root length, whereas this interaction was not significant for other growth attributes (Table 1). Furthermore, the application of TU (100 mM) mitigated the toxic effects of Ni, by increasing shoot length and root length by 37.73% and 42.36%, shoot and root fresh weight by 20.26% and 19.4%, and shoot and root dry weight by 37.55% and 16.6%, respectively, under Ni-induced toxicity in the Ni-sensitive cultivar (Fig. 1). Among both cultivars, Sultan-17 exhibited greater tolerance to Ni stress compared to the Jou-17 cultivar, as it showed more improvement with TU application under Ni stress conditions (Fig. 1). Overall, the results suggest that TU application can alleviate Ni-induced toxicity and promote plant growth by increasing barley plant biomass.

Photosynthetic attributes: Nickel stress led to a reduction in all studied photosynthetic attributes in both barley cultivars (Fig. 2, Table 2). Relative to control plants, Ni stress caused significant decreases in chlorophyll a, chlorophyll b, and carotenoid contents by 32.65%, 93.09%, and 97.89%, respectively. However, the application of TU improved all photosynthetic contents under Ni stress conditions. While both levels of TU (50 and 100 mM) led to improvements, the maximum enhancement was observed at the 100 mM TU level (Fig. 2). Notably, at the 100 mM TU level, chlorophyll a, chlorophyll b, and carotenoid contents were significantly improved by 12.89%, 21.03%, and 88.62%, respectively. The data indicate a positive correlation between TU treatments and enhanced photosynthetic characteristics in both cultivars under Ni stress. Among both cultivars, Sultan-17 exhibited higher tolerance to Ni stress than the Jou-17 cultivar (Fig. 2).

Table 1. Mean squares from analysis of variance (ANOVA) of the data for growth parameters of barley subjected to different levels of thiourea under nickel stress conditions.

Source of variance	df	SL	RL	SFW	RFW
Cultivar (C)	1	91.52 ***	185.41 ***	8.54 ***	0.019 ***
Nickel (Ni)	1	336.11 ***	21.31 ***	110.20 ***	0.0092 ***
Thiourea (TU)	2	327.54 ***	165.91 ***	30.49 ***	0.04 ***
C × Ni	1	0.44 ns	15.34 **	0.10 ns	0.0012 ns
C × TU	2	4.17 ns	11.03 **	0.35 ns	5.38 ns
Ni × TU	2	39.20 ***	10.01 **	0.72 ns	0.0017 *
C × Ni × TU	2	0.86 ns	1.46 ns	0.18 ns	1.79 ns
Error	24	3.59	1.308	0.51	4.18
Source of variance	**df**	**SDW**	**RDW**	**RPP**	**LPP**
Cultivar (C)	1	0.012 ***	0.0014 ***	28.44 ***	0.053 **
Nickel (Ni)	1	0.057 ***	0.001 ***	40.11 ***	0.065 **
Thiourea (TU)	2	0.011 ***	0.002 ***	31.75 ***	0.13 ***
C × Ni	1	9.51 *	2.10 ns	1.77 ns	4.012 ns
C × TU	2	1.31 ns	5.52 ns	0.52 ns	0.002 ns
Ni × TU	2	9.65 ns	4.22 ns	0.36 ns	0.0069 ns
C × Ni × TU	2	9.65 ns	2.61 ns	0.36 ns	2.72 ns
Error	24	1.77	5.56	1.36	0.0056

*, **, *** = Significant at 0.05, 0.01 and 0.001 levels, respectively. ns = Non-significant

Abbreviations*:* Exponent (e), Shoot length (SL), Root length (RL), Shoot fresh weight (SFW), Root fresh weight (RFW), Shoot dry weight (SDW), Root dry weight (RDW), Root per plant (RPP), Leaf per plant (LPP)

Table 2. Mean squares from analysis of variance (ANOVA) of the data for photosynthetic parameters, antioxidants and organic osmolytes parameters of barley subjected to different levels of thiourea under nickel stress conditions.

Source of variance	df	Chl *a*	Chl *b*	Total Chl	Carotenoids	SOD
Cultivar (C)	1	0.35 ***	0.71 ***	0.038 ***	3.27 **	0.13 ***
Nickel (Ni)	1	2.28 ns	0.0012 ns	0.021 ***	17.07 ***	0.73 ***
Thiourea (TU)	2	0.27 ***	0.73 ***	0.085 ***	13.29 ***	0.39 ***
C × Ni	1	0.0019 ns	0.029 ns	3.59 ns	0.11 ns	3.18 ns
C × TU	2	0.03 *	0.029 ns	7.27 ns	0.092 ns	0.012 ns
Ni × TU	2	0.0013 ns	0.042 ns	7.81 ns	0.95 ns	0.021 *
C × Ni × TU	2	0.022 ns	0.006 ns	9.74 ns	0.059 ns	0.0016 ns
Error	24	0.0083	0.019	0.0011	0.36	0.0062
Source of variance	**df**	**POD**	**CAT**	**TSP**	**TSS**	**TFAA**
Cultivar (C)	1	0.25 *	4.73 **	4.73 **	0.003 ***	0.0016 ***
Nickel (Ni)	1	1.67 ***	4.48 **	4.48 **	0.01 ***	0.0093 ***
Thiourea (TU)	2	2.13 ***	15.42 ***	15.42 ***	0.0059 ***	0.0050 ***
C × Ni	1	0.039 ns	0.0069 ns	0.0069 ns	0.001 *	1.77 ns
C × TU	2	0.029 ns	0.92 ns	0.92 ns	1.027 ns	1.08 ns
Ni × TU	2	0.244 *	0.10 ns	0.103 ns	0.001 **	1.94 ns
C × Ni × TU	2	0.030 ns	0.34 ns	0.34 ns	1.19 ns	5.27 ns
Error	24	0.049	0.41	0.41	1.64	8.33

*, **, *** = Significant at 0.05, 0.01 and 0.001 levels, respectively. ns = Non-significant

Abbreviations: Exponent (e), Chlorophyll (Chl), Superoxide dismutase (SOD), Peroxidase (POD), Catalase (CAT), Total soluble proteins (TSP), Total soluble sugar (TSS), Total free amino acid (TFAA)

Table 3. Mean squares from analysis of variance (ANOVA) of the data for inorganic osmolytes of barley subjected to different levels of thiourea under nickel stress conditions.

Source of variance	df	Na (S)	Na (R)	K (S)	K(R)	Ca (S)	Ca (R)
Cultivar (C)	1	841 ***	66.25 ***	667.36 **	33.44 ***	119.17 ***	200.69 ***
Nickel (Ni)	1	1936 ***	1190.25 ***	294.08 *	28.44 *	73.67 ***	220.69 ***
Thiourea (TU)	2	760.86 ***	2177.19 ***	1886.08 ***	351.36 ***	365.51 ***	590.25 ***
C × Ni	1	32.11 ns	84.02 ns	0.25 ns	1 ns	9.50 ns	4.69 ns
C × TU	2	11.58 ns	75.25 ns	57.02 ns	37.19 **	9.84 ns	6.86 ns
Ni × TU	2	11.58 ns	47.25 ns	35.36 ns	1.36 ns	17.01 *	63.86 **
C × Ni × TU	2	19.19 ns	30.52 ns	4.08 ns	1.75 ns	3.84 ns	5.02 ns
Error	24	24.13	29.5	47.72	5.88	4.28	8.61

*, **, *** = Significant at 0.05, 0.01 and 0.001 levels, respectively. ns = Non-significant

Abbreviations: Exponent (e), Sodium (Na), Potassium (K), Calcium (Ca), S (Shoot), R (Root)

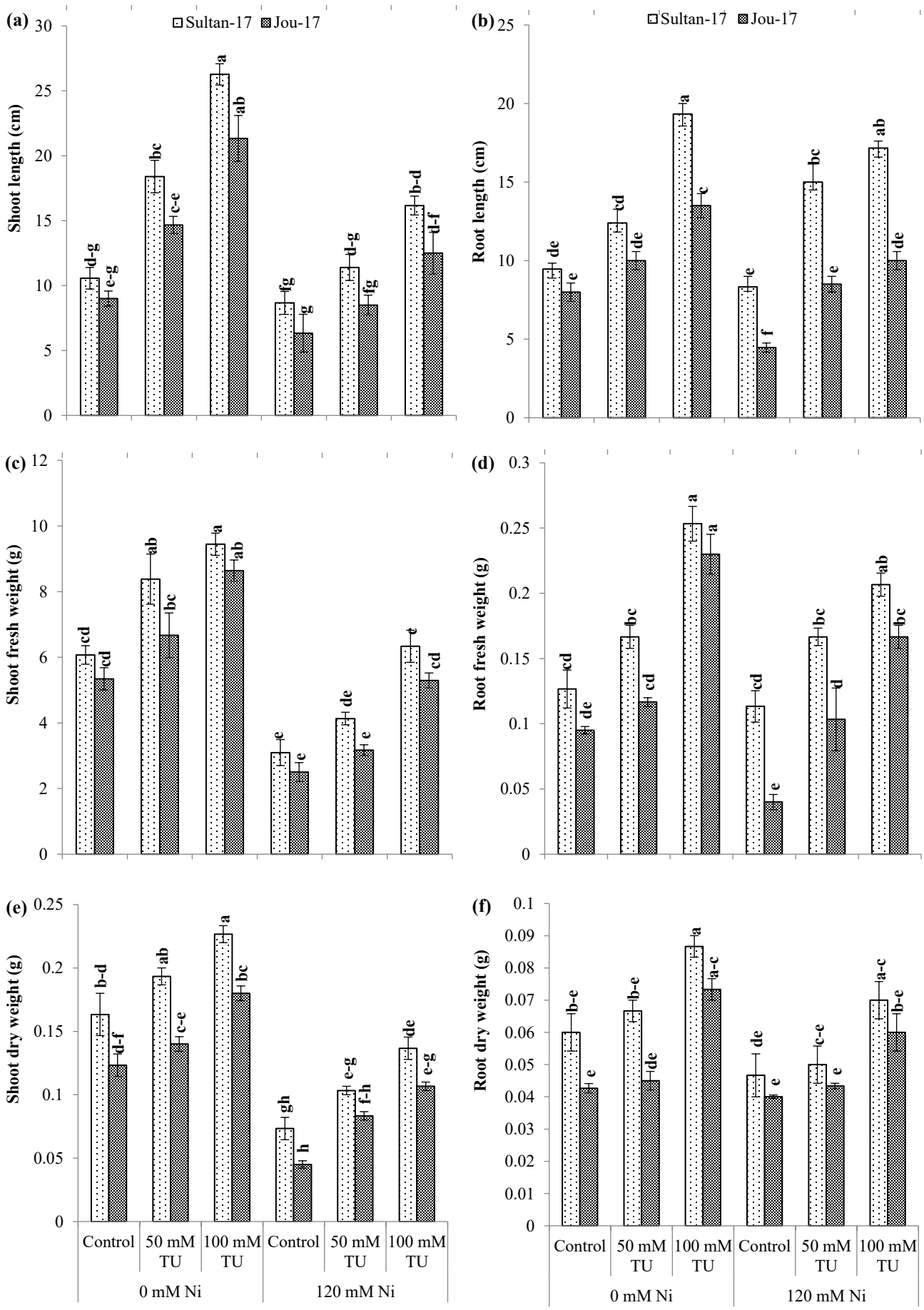

Fig. 1. Impact of TU on shoot length (cm) (a), root length (cm) (b), shoot fresh weight (g) (c), root fresh weight (g) (d), shoot dry weight (g) (e) and root dry weight (g) (f) of barley (*Hordeum vulgare* L.) cultivars under Ni stress.

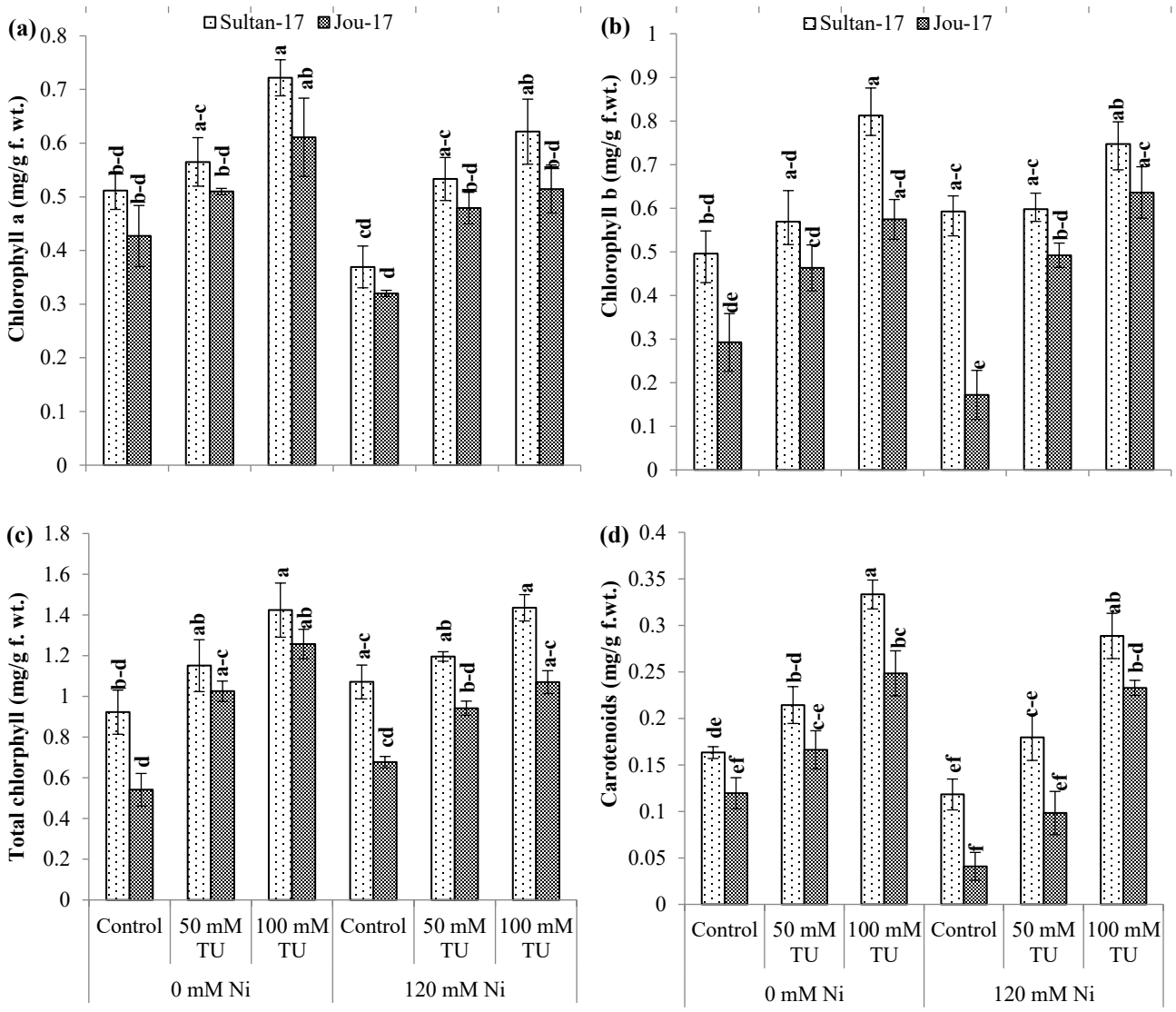

Fig. 2. Impact of TU on chlorophyll a (mg/g fresh weight), (a) chlorophyll *b* (mg/g fresh weight), (b) chla/chl*b* (c), total chlorophyll (mg/g fresh weight) (d) and carotenoids (mg/g fresh weight) € of barley (*Hordeum vulgare* L.) cultivars under Ni stress.

Enzymatic antioxidant activities: To better understand how TU reduces oxidative damage caused by Ni, the activities of three essential enzymes (SOD, POD, and CAT) that reduce reactive oxygen species (ROS) in plants were examined. Under Ni stress alone, a considerable elevation was noted in SOD activity (45.08%), while POD and CAT activities decreased by 27.08% and 82.57%, respectively. Application of thiourea (100 mM) significantly increased these enzyme activities by 109.8%, 60.83%, and 19.20%, respectively, under Ni stress conditions (Fig. 3). These findings suggest that TU might mitigate the effects of Ni-induced oxidative stress.

Organic osmolytes: Among untreated plants, total soluble protein (TSP) and total free amino acid (TFAA) values did not significantly differ from those under control conditions. However, in the Ni-sensitive barley cultivar (Jou-17), Ni-induced toxicity resulted in a significant increase in TSP and total soluble sugar (TSS) values by 62.92% and 100%, respectively, compared to control plants. Exogenous application of TU further elevated TSP and TSS levels by 93.25% and 140%, respectively, in Ni-treated plants (Fig. 4). On the other hand, Ni exposure alone significantly decreased TFAA content by 2.11% compared to untreated plants. Nevertheless, the application of TU led to a modest increase in TFAA levels, raising the value by 1.13% compared to Ni stress alone (Fig. 4). Overall, these results indicate that TU application plays a role in adjusting osmolyte contents within barley plants, thereby promoting their growth.

Inorganic osmolytes: The Ni exposure in this study significantly reduced the inorganic osmolyte characteristics of potassium (K^+) and calcium (Ca^{2+}) content by 5.87%, 15% in shoot and 27% and 18.18% in root respectively of barley plants. While a rise in sodium (Na^+) content was observed in the shoot and root at 36.6% and 33.07% respectively under Ni stress in Ni sensitive cultivar (Jou-17). However, the use of TU significantly improved the shoot and root K^+ and Ca^{2+} content by 25.4%, 13.54%, 50% and 100% under Ni stress (Fig. 5). However, there was also significant variation between the control groups TU enhanced the ionic content in the control state as well (Table 3). Among both cultivars, Sultan-17 accumulated high K^+ and Ca^{2+} content and lower Na^+ content, hence, proved Ni tolerant and Juo-17 was proved Ni sensitive cultivar (Fig. 6).

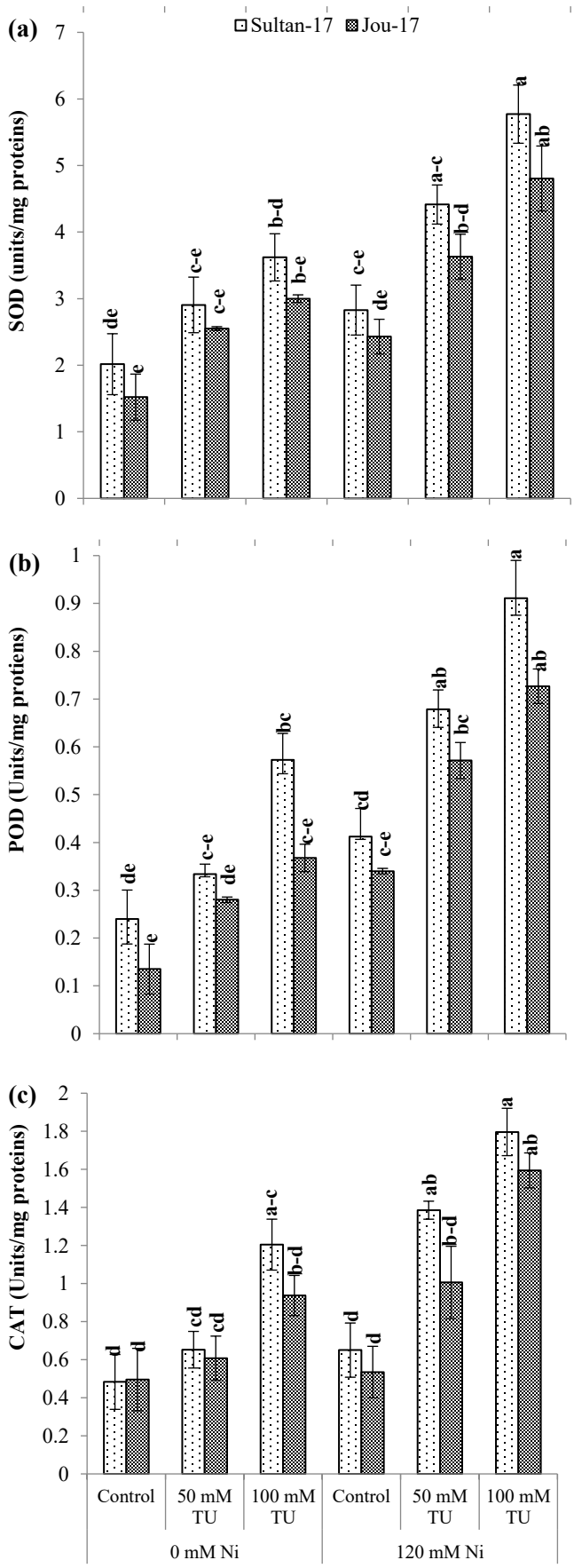

Fig. 3. Impact of TU on superoxide dismutase (SOD) (units/mg proteins) (a), peroxidase (POD) (units/mg proteins), (b) and catalase (CAT) (units/mg proteins) (c) of barley (Hordeum vulgare L.) Cultivars under Ni stress.

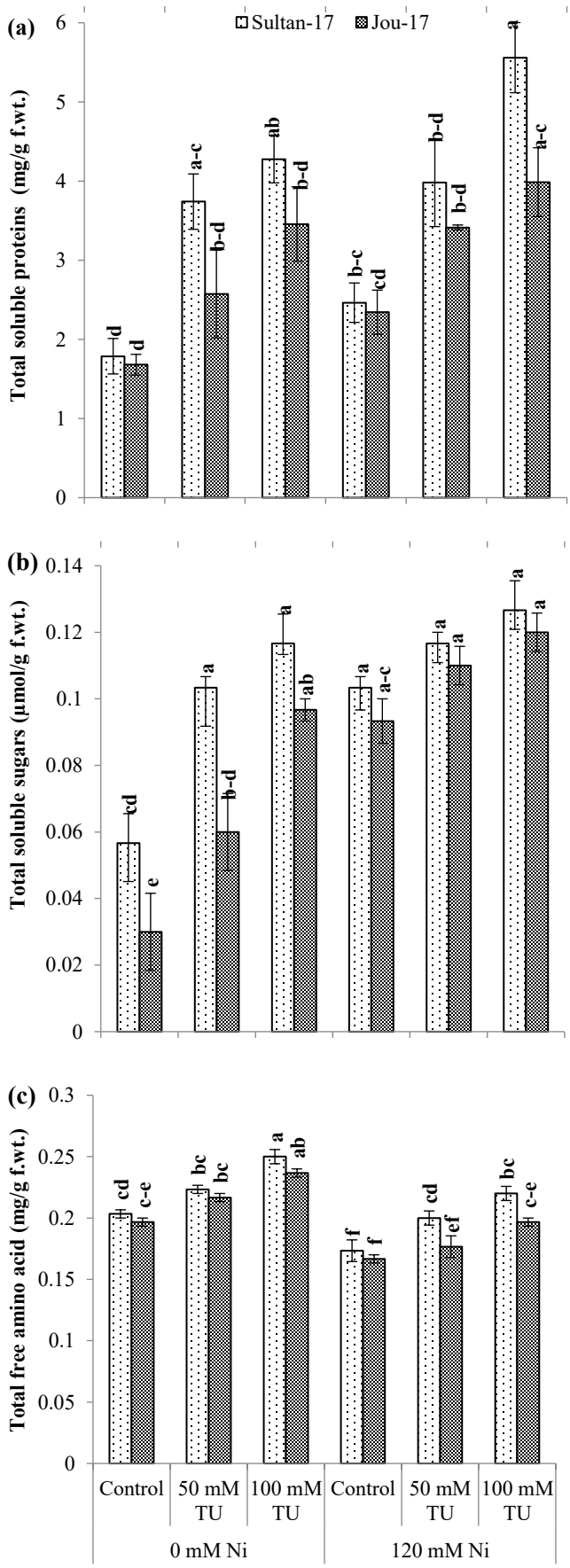

Fig. 4. Impact of TU on total soluble protein (mg/g fresh weight) (a) and total soluble sugars (μmol/g fresh weight) (b) and total free amino acid (mg/g fresh weight) of barley (*Hordeum vulgare* L.) cultivars under Ni stress.

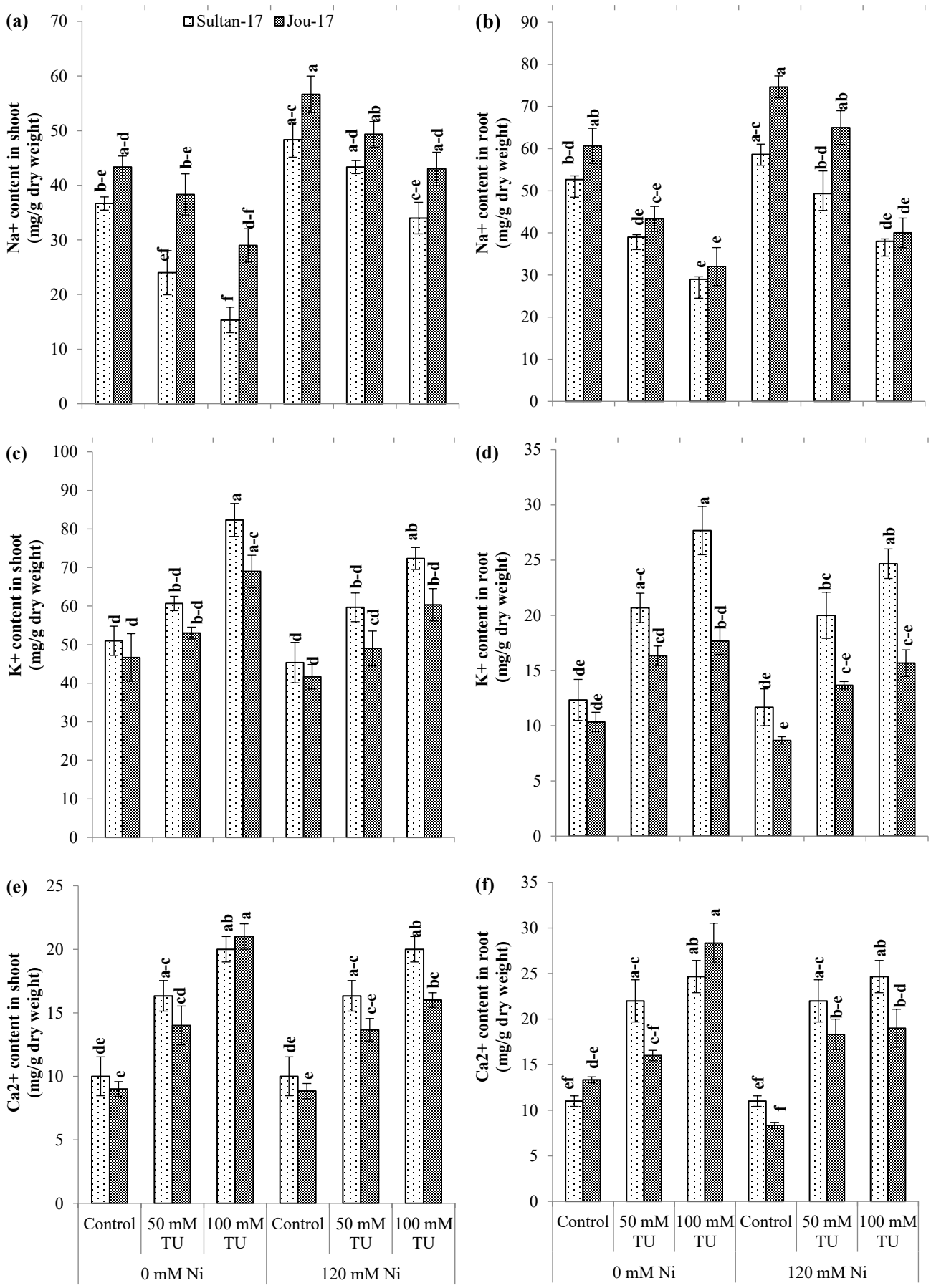

Fig. 5. Impact of TU on sodium (Na^+) content in shoot (mg/g dry weight) (a) sodium (Na^+) content in root (mg/g dry weight) (b), potassium (K^+) content in shoot (mg/g dry weight) (c), Potassium (K^+) content in root (mg/g dry weight) (d), calcium (Ca^{2+}) content in root (mg/g dry weight) (g) (e) and calcium (Ca^{2+}) content in root (f) of barley (*Hordeum vulgare* L.) cultivars under Ni stress.

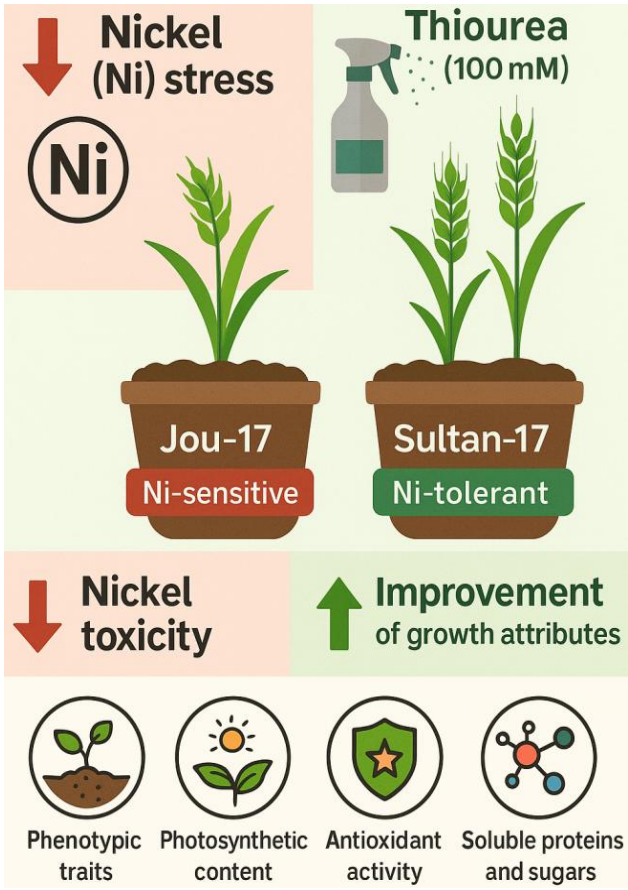

Fig. 6. Proposed mechanism of thiourea induced growth enhancement.

Discussion

Nickel is recognized as a crucial micronutrient for plant growth and development across various plant species (Fabiano *et al*., 2015). However, excessive Ni concentration can exert toxic effects on plant metabolism, growth, and development (Khan *et al*., 2023). The present study's findings unveil that Ni toxicity significantly impacted growth parameters, including plant length, fresh and dry biomass of roots and shoots, and the number of leaves per plant. This aligns with previous research indicating that Ni stress led to decreased shoot and root length in barley plants. Similar reductions in vascular bundles, shoot diameter and cell size in plant storage tissues have also been observed under Ni stress conditions. Nickel exposure disrupts epidermal and root cortical cells, leading to structural changes in cell walls, which consequently affects shoot and root length (Ahmad & Ashraf, 2012). Reductions in fresh and dry root and shoot weights have been reported in other plants due to Ni toxicity, attributed to the disruption of cell proliferation and division mechanisms and alterations in nuclear membrane structure. Notably, Ni-induced stress can ultimately retard vegetative growth and reduce biomass (Ahmad & Ashraf, 2012). Additionally, the present study demonstrated a reduced number of leaves in response to Ni toxicity, which is in accordance with earlier studies (Ameen *et al*., 2019). Interestingly, the exogenous application of TU significantly enhanced root and shoot length in barley plants, indicating a potential role of TU-induced metabolites in improving growth parameters under stress conditions. This is in line with prior studies, including those by Zain *et al*., (2017) and Ahmad *et al*., (2022b), who found increase in growth parameters following TU application in wheat and canola plants, respectively. The positive effects of thiourea on mineral nutrition, metabolism, and chlorophyll content may contribute to its growth-promoting effects, ultimately leading to increased biomass (Wahid *et al*., 2017; Naz & Perveen, 2021).

The study findings indicated a decrease in photosynthetic traits under Ni stress. High Ni levels can lead to leaf chlorosis and necrosis, impacting photosynthetic pigment concentrations such as carotenoids, chlorophyll a, and chlorophyll b (Ahmad & Ashraf, 2012). Correspondingly, previous research has shown decreases in photosynthetic pigments, including chlorophyll a, b, total chlorophyll, and carotenoids, under Ni toxicity in wheat plants (Yusuf *et al*., 2011). However, the treatment of TU improved photosynthetic pigments in barley plants. Previous studies have similarly demonstrated that TU application leads to enhanced photosynthetic pigments i-e. chlorophyll and carotenoid content, compared to controls (Ahmad *et al*., 2021). This enhancement is attributed to TU's ability to mitigate oxidative stress on photosynthetic pigments and improve their concentration (Vineeth *et al*., 2016). Moreover, TU's impact on sucrose translocation and sink-source strength has been linked to enhanced photosynthesis rates and oil biosynthesis in plants (Pandey *et al*., 2013).

The imposition of Ni toxicity was found to elevate enzymatic antioxidant activities, including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT). This aligns with previous studies, such as the findings of Aqeel *et al*., (2022), Rehman *et al*., (2016), and Zahra *et al*., (2022), indicating increased antioxidant activities under Ni stress. Interestingly, TU application significantly increased SOD, POD, and CAT activities in barley plants under Ni stress conditions. This enhancement in antioxidant activity by TU amendment has been observed in previous researches (Hameed *et al*., 2013; Khanna *et al*., 2017), suggesting that TU can boost antioxidant capacity and photosynthesis, consequently increasing POD activity. It is important to note that the antioxidant effects of TU might involve mechanisms related to its interactions with reactive oxygen species (ROS), ultimately enhancing plant defence responses (Akladious, 2014). Additionally, TU's reported ability to increase SOD activity in response to stress might further contribute to its antioxidant effects (Akladious, 2014; Ahmad *et al*., 2022).

The present study demonstrated a significant increase in organic osmolyte content, including total soluble sugars, total soluble proteins, and total free amino acids, under Ni stress conditions. This is consistent with previous studies indicating elevated soluble protein content under Ni stress (Ahmad & Ashraf, 2012; Maheshwari & Dubey, 2007). Elevated levels of total soluble sugar content attributed to Ni stress have been

documented in research involving spinach and soybean plants (Younis *et al*., 2015; Mishra & Dubey, 2013). Interestingly, TU application significantly increased the production of these osmolytes, suggesting its role in enhancing barley plants' protection under Ni stress. Comparable results have been documented in the case of mung bean and cluster bean plants, where the application of TU demonstrated a positive influence on osmolyte content, thereby enhancing stress tolerance (Perveen *et al*., 2016; Burman *et al*., 2007). The potential of thiourea to stimulate enzymatic antioxidant systems and mitigate membrane damage, along with its effect on nitrogen assimilation and protein synthesis, might contribute to the observed increase in osmolyte content (Garg *et al*., 2006; Patade *et al*., 2020).

The study also revealed changes in the uptake of inorganic osmolytes, specifically Na^+, K^+, and Ca^{2+}. Nickel stress elevated Na^+ ion uptake and a reduced K^+ and Ca^{2+} ion content. This corresponds to previous findings that Ni ions can interfere with the transport of Na^+ ions across cell membranes, leading to alterations in cellular ion homeostasis (Jamil *et al*., 2014). The reduction in K^+ uptake under Ni stress might be attributed to the competition between Ni and K^+ for uptake mechanisms (Ishtiaq *et al*., 2014). Interestingly, TU application improved the uptake of these inorganic ions under Ni stress conditions. The beneficial effects of TU on ion homeostasis have been reported in other studies, emphasizing TU's role in regulating cellular ion balance and combating stress effects (Kaya *et al*., 2015).

Conclusion

The exposure to Ni stress exerts a severe influence on the physiological traits of plants, resulting in reduced growth and compromised physiological as well as biochemical attributes. This stress condition further disrupts nutrient uptake, thereby disturbing the overall plant metabolism. In order to restore the normal metabolic processes and bolster the defense mechanisms, the application of TU was employed as a foliar spray. This application strategy effectively mitigated the adverse impacts of Ni stress, primarily by inducing improvements in growth parameters, photosynthetic contents, antioxidant activities, and the presence of both organic and inorganic osmolytes. Among the tested concentrations, the application of TU at a concentration of 100 mM exhibited the highest efficacy in alleviating the toxic effects of nickel stress. The cultivar that demonstrated greater tolerance to Ni stress (Sultan-17), outperformed than (Jou-17) cultivar in terms of growth and other relevant characteristics. This enhanced performance in the face of stress suggests that Sultan-17 could potentially thrive in soils with Ni contamination. Consequently, it is recommended that the application of TU at a concentration of 100 mM can effectively enhance the resilience of barley cultivars to Ni toxicity. This approach yields improvements across various physiological and biochemical attributes, thus offering a promising means of counteracting the detrimental effects of Ni stress.

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