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Alleviation of *Zea mays* L. Nickel Toxicity by Triacontanol Foliar Spray

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ABSTRACT

Nickel toxicity is a crucial ecological issue as it affects most soils utilized for crop cultivation. This work aimed to evaluate the effect of triacontanol (TRIA) foliar spray on photosynthesis, and physiological responses of maize (*Zea mays* L.) seedling under nickel stress. Ten-day-old maize seedlings grown in pots were divided into: (1) control untreated seedlings (irrigated with full nutrient solution), (2) seedlings treated with $NiCl_2.6\ H_2O$ (100 µM), (3) Ni-treated seedlings (100 µM) sprayed with 25 µM TRIA, (4) Ni-treated seedlings (100 µM) sprayed with 50 µM TRIA. Ni treatment reduced the growth of the 21-day-old seedlings, photosynthetic performance index (PI_{abs}) and the contents of photosynthetic pigments, total soluble sugars (TSS), essential elements in roots, relative water content (RWC), as well as the levels of auxins and salicylic acid (SA). Nevertheless, it increased the electrolyte leakage (EL), Ni^{2+} uptake, plant Ni transfer rate ($Ni_{transfer\ rate}$), root transfer rate factor (TF_{root}), and the contents of gibberellic acid (GA_3) and abscisic acid (ABA). Both Rubisco small subunit (*ZmRBCS*) and abscisic acid- stress- ripening (*ZmASR1*) genes were up-regulated under Ni stress. TRIA foliar spray (with a preference for 50 µM) significantly improved growth attributes, PI_{abs}, pigments content, proline content of shoots, the contents of K^+, Mg^{2+}, and Ca^{2+}, and the expression of both *ZmRBCS* and *ZmASR1* genes of Ni-stressed plants. TRIA also reduced membrane damage expressed as EL, Ni_{plant} uptake, and TF_{root} and hence alleviated the toxic effects of Ni stress. TRIA modulated the hormonal balance under Ni stress, particularly SA and auxins.

1. Introduction

Heavy metal stress (HMs) has become one of the major concerns affecting plant growth and crop productivity for the last couple of decades. Nickel (Ni) pollution is a major environmental issue in the Egyptian soils and waters [1,2], as its concentration is increasing day by day in the environment due to industrialization and anthropic activities [3]. Though Ni is an essential microelement required for proper plant growth and enzyme activity, excess Ni limits crop productivity and inhibits seed germination and seedling growth [4, 5]. Moreover, Ni disrupts different physiological processes like photosynthesis, nutrient uptake, plant water relations, and membrane permeability [6, 7], besides it induces osmolytes accumulation like proline [8, 9]. Under the pressure of the world's increasing population, the negative impacts of Ni stress on plant growth and yield offer further constraints on global food crop production. Therefore, there is a dire need to develop eco-friendly applicable treatments to increase crop resistance and yield under the existing polluted environment.

Maize (*Zea mays* L.) is one of the main cereals cultivated globally, coming with the highest consumption for purposes of food, feed, and other uses [10]. Besides the harmful effect of planting maize

in heavy metal polluted soils on its productivity and grain quality, it has hazardous effects on human health as a final consumer [11].

Numerous protectant compounds of diverse functions were found to relieve toxic effects of HMs, such as osmolytes, plant growth hormones, low molecular mass signaling molecules, and trace elements [12]. Triacontanol (TRIA) has been identified as one of the most potent eco-friendly health-safe bio-stimulant growth regulators [13, 14]. TRIA is a natural long-chain (C- 30) primary alcohol present in epicuticular waxes of plant leaves [13, 15]. TRIA was reported to stimulate plant growth and increase stress resistance against a variety of stresses like salinity, drought, and HMs [16 - 18]. Exogenous application of TRIA to plants under abiotic stresses has been shown to prevent oxidative stress, reduce lipid peroxidation of membranes, and regulate genes involved in the modulation of various physiological and biochemical functions, especially stress-related ones [16]. TRIA was also recorded to improve the nutrient uptake, photosynthetic pigments, as well as osmolyte accumulation in response to abiotic stresses [19, 20]. Most studies conducted on alleviating HMs by exogenous application of TRIA focused on photosynthesis and antioxidant defense, but little is known about how TRIA influences mineral nutrition, hormonal balance, and HM absorption and distribution. Therefore, the current study aims to elucidate whether TRIA performs its ameliorative effect on Ni toxicity via enhanced photosynthesis only, or hormonal balance and osmolyte accumulation are involved as well. Another objective is to investigate how TRIA influences the absorption and distribution of both inorganic nutrients and Ni in different parts of maize seedlings under Ni stress.

2. Materials ad Methods

2.1 Plant material and growth conditions

A homogenous lot of viable maize (*Zea mays* L.) grains (hybrid three-way cross 321) was obtained from the Agricultural Research Centre, Giza, Egypt, and kept in the dark at 4 °C. The grains were surface sterilized by 10% sodium hypochlorite (NaClO) solution for 5-10 min, then washed thoroughly with distilled water. The grains were then cultivated in sand-containing plastic pots (diameter 15 cm and height 30 cm). The sand was rinsed with distilled water and then dried in an oven (70 °C for 48 h, then 200 °C for 2 h) before being used. Plants were grown under natural environmental conditions (14-h photoperiod, light/dark temperature 27/15 °C, and relative humidity 60-70%). During the first five days of sowing, all seedlings were irrigated with distilled water, then they were irrigated with a full nutrient solution for another 5 days [21]. On the tenth day, maize seedlings were divided into four groups: (1) control untreated seedlings, where they were irrigated with the nutrient solution [21], (2) Ni-stressed seedlings, which were irrigated with the full nutrient solution [21] with 100 µM Ni ($NiCl_2.6\ H_2O$) added, and (3& 4) Ni-stressed seedlings treated with TRIA (25 or 50 µM, respectively), as Ni was added to the nutrient solution and one of the two concentrations of TRIA (25 and 50 µM) was applied separately as a foliar spray treatment twice (at the first and fifth days of Ni treatment). The irrigation was conducted at a rate of 50 mL per pot, three times a week over eleven days of treatments. For each treatment, three independent biological replications were conducted (10 plants each). After harvest, five plants per treatment were taken to measure the growth criteria. Plant leaves were randomly selected from each treatment to measure pigments content, relative water content (RWC), and electrolyte leakage (EL). The remaining seedlings were immediately uprooted, separated, frozen in liquid nitrogen, and stored at −80 °C for other analyses.

2.2 Determination of growth criteria, toxicity level, and shoot, root, and heavy metal tolerance indices

By the end of the experiment, fresh biomass, shoot and root dry weights (DW) (g per seedling), plant height (cm), and leaf area (cm^2) of the seedlings were determined. The root/shoot height ratio was calculated by dividing the length of the root by the length of the shoot of the fresh seedling. The toxicity level (%) of roots was calculated using the method of **Valivand *et al.*** [22] by the following formula:

Toxicity level (%) = (Root length of control - Root length of treatment/Root length of control) × 100.

The shoot tolerance index (STI) was calculated according to **Alam *et al.*** [23] by the following formulae:

STI (%) = Shoot length of Ni treated plants /Shoot length of control plants × 100.

The root tolerance index (RTI) was calculated using the method of **Amooaghaie *et al.*** [24] by the following formulae:

RTI (%) = Root length of Ni treated plants/ Root length of control plants × 100.

Ni tolerance index (Ni-TI) was calculated based on **Bálint *et al.*** [25] using the formula given below:

% (Ni-TI) = DW of treated plants/DW of control plants × 100.

2.3 Determination of photosynthetic parameters

A portable pulse amplitude modulation portable fluorometer (Handy PEA, Hansatech, Norfolk, UK) was used to measure the photosynthetic performance index (PI_{abs}) of the youngest fully expanded leaves of dark-adapted 21-day-old seedlings [26]. Chlorophylls a (Chl a) and b (Chl b), and carotenoids (Car) were extracted in 80% (v/v) acetone and measured spectrophotometrically (UNICAM Helios α, Unicam, Cambridge, UK) according to **Metzner *et al.*** [27]**.** The pigment concentration was calculated and expressed in μg g^{-1} DW.

2.4 RNA Extraction and quantitative RT-PCR analysis

Total RNA was extracted from 30 mg of fresh leaves of each treatment using Gene JET^{TM} RNA purification Kit (Thermo Fisher Scientific, MA, USA). One μg of total RNA was reverse transcribed into cDNA using Revert Aid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, MA, USA). Ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) small subunit (*RBCS*) and ABA stress and ripening induced protein (*ASR*) genes were previously searched from NBCI to perform qRT-PCR. Primer sequences for the reactions were *ZmRBCS1*; D00170 FP: 5'- GATACCCTGCCTCGNITTCA-3'; RP: 5'- CTCCTGCNICTCCTTGTACA-3', and *ZmASR1*; AF139814.1 FP: 5'- TTCCACCACANIANIGACGA- 3'; RP: 5'-GATCGGATCGGACGGACTAC-3. *Zea mays* actin (GenBank accession No. J01238; FP: 5'-GATGATGCGCCAAGAGCTG -3'; RP:5'-GCCTCATCACCTACGTAGGCAT -3) was used as a reference gene to normalize the relative transcription and to minimize different copy numbers of cDNA templates. PCR amplification specificity was verified using melting curve analysis and data were analyzedfrom three independent samples based on the $2^{-\Delta\Delta Ct}$ method [28] after normalized to the expression of each actin gene.

2.5 Determination of relative water content (RWC)

RWC of plant leaves was measured by the method of **Pan *et al.*** [29]**.** Leaves from selected plants were detached and weighed to obtain their fresh weight (fw), and then soaked in distilled water at room temperature for 24 h, weighed to obtain turgid weight (tw). The leaves were then dried in an oven at 80 °C for 48 h to have dry weight (dw). The RWC was then calculated using the following formula:

$$RWC\ (\%) = (fw - dw)/ (tw - dw) \times 100$$

2.6 Determination of electrolyte leakage (EL)

Membrane damage caused by lipid peroxidation was determined by recording EL as described by **Valentovic *et al.*** [30] using HANNA conductivity meter (HI8733). The EL was determined according to the following equation:

$$EL\ \% = (L_1/L_2) \times 100$$

Where L_1 refers to the electric conductivity of the outer de-ionized water where the leaves were soaked for 24 h at 25°C, and L_2 is the electric conductivity of the outer de-ionized water after autoclaving at 120°C for 20 minutes.

2.7 Determination of soluble sugars and proline contents

Total soluble sugar (TSS) and proline contents were determined using a spectrophotometer (UNICAM Helios α, Unicam, Cambridge, UK) according to **McCready *et al.*** [31] and **Bates *et al.*** [32]**,** respectively.

2.8 Plant tissues Ni and nutrient elemental analysis

The separated roots and shoots of maize plants were oven-dried at 75 °C for 72 h to have the dry weight. The uptake of Ni and elemental nutrients were quantified in the dry samples. The Ni uptake $[Ni]_{uptake}$ (μg g^{-1}) of maize plant was calculated according to the method of **Xiong *et al.*** [33] from the concentrations of the element in roots, shoots, and DW of the respective plant part by using equation no.1:

$$[Ni]_{uptake} = [Ni]_{roots} * DW_{roots} + [Ni]_{shoots} * DW_{shoots}/DW_{roots} + DW_{shoots} \quad (1)$$

The Ni transfer rate (μg day^{-1}) was calculated according to **Xiong *et al.*** [33]**,** by dividing Ni accumulation dose in plants on the exposure duration (11 days) as shown in equation no. 2:

$$Ni\ transfer\ rate = [Ni]_{roots} * DW_{roots} + [Ni]_{shoots} * DW_{shoots}/Exposure\ time\ (days) \quad (2)$$

Root transfer factor (TF_{root}) indicates Ni translocation from exposure medium (soil) to roots of plants. Similarly, the shoot transfer factor (TF_{shoot}) denotes Ni translocation from the roots to the shoots. These transfer factors were estimated using equations 3 and 4 according to **Abbas *et al.*** [34] and **Xiong *et al.*** [33], respectively:

$$TF_{root} = [Ni]_{roots}/[Ni]_{exposure\ medium} \quad (3)$$

$$TF_{shoot} = [Ni]_{shoots}/[Ni]_{roots} \quad (4)$$

Where, $[Ni]_{roots}$, $[Ni]_{shoots}$, and $[Ni]_{exposure\ medium}$ are the concentrations of Ni in maize roots, shoots, and soil, respectively. DW_{roots} is the dry weight of roots, and DW_{shoots} is the shoot dry weight. The concentration of macro- **(**K^+, Ca^{2+}, Mg^{2+}) and micro-nutrients (Fe^{3+}, Zn^{2+}, Mn^{2+}) were determined by atomic absorption spectroscopy (Savant AA, GBC, Australia), and the results were expressed as mg of the metal per Kg DW of the sample.

2.9 Determination of endogenous phytohormones

Plant phytohormones were extracted using freshly collected samples. The quantity of each hormone was determined using high performance liquid chromatography (Agilent 1260 Infinity HPLC Series (Agilent®, USA), equipped with Quaternary pump, a Zorbax Eclipse Plus C18 column (100 mm x 4.6 mm i.d., (Agilent®, USA)) operated at 35°C. The separation was achieved using gradient elution with (A) 5 mM Ammonium acetate/ 0.05 % formic acid in water, (B) Acetonitrile. The injected volume was 20 µL for detection, a Variable Wavelength Detector was set at 254 nm. The concentrations of indole acetic acid (IAA), indole butyric acid (IBA), abscisic acid (ABA), gibberellic acid (GA_3), and salicylic acid (SA) in the samples were assayed against internal standards.

2.10 Statistical analysis

Analysis of variance (ANOVA) and Pearson's correlation analyses of the data were performed using SPSS v. 20.0 (SPSS, ChicNio, USA) software. The means were compared to calculate the statistical significance by Duncan's test at $P \leq 0.05$.

3. RESULTS

3.1 Growth, toxicity level, and Ni tolerance indices

Exposure of maize seedlings to Ni stress reduced its growth significantly in terms of plant height, fresh biomass, leaf area, root/shoot length ratio, and shoot dry weight as compared withtheir controls Fig. 1 & 2. TRIA treatment (25 µM) significantly enhanced the plant height, fresh biomass, leaf area, and shoot dry weight under Ni stress and restored its growth to the level of the control untreated seedlings Fig. 1a,b &c & Fig. 2a. Though a significant enhancement in the root/shoot length ratio was observed when both concentrations of TRIA (25 and 50 µM) were applied to Ni-stressed seedlings, yet it failed to restore its value to comparable levels of the control Fig. 1d. The toxicity level in roots of Ni-stressed plants reached about 40.1% higher than unstressed controls, while this toxicity decreased by 36.4% and 60% at 25 and 50 µM TRIA treatments, respectively Fig. 1e. In response to Ni stress, a significant decrease was recorded in Ni tolerance index (Ni-TI), shoot tolerance index (STI), and root tolerance index (RTI) percentages to 70%, 59.9 %, and 91.9% of the values of the control maize plants, respectively, Fig. 1f & Fig. 2b. However, treatment of Ni-stressed seedlings with TRIA (25 µM) significantly restored both Ni-TI RTI percentages to the control level Fig. 1f & Fig. 2b. The results showed that treatment with 50 µM TRIA was more efficient in reducing Ni toxicity and enhancing shoot and root tolerance indices and growth of maize seedlings under stress.

3.2 Photosynthetic traits

Ni-stressed plants showed significant lower carotenoids and total pigments contents, relative to the controls Fig. 3a. Ni-Stressed plants treated with both concentrations of TRIA contained a significant amount of Chl a, Chl b, Car and total pigments contents (in a concentration-dependent pattern) that exceeded its corresponding levels not only in Ni-stressed plants but also in control plants Fig. 3a. For instance, Ni-stressed plants treated by 50 µM TRIA accumulated about 8, 4.5, 1.6, and 4.5 folds of Chl a, Chl b, Car, and total pigments higher than the control untreated plants, respectively Fig. 3a. Ni-stressed plants exhibited about half the value of PI_{abs} recorded in the controls, whereas treatment with 25 and 50 µM TRIA resulted in higher PI_{abs} by 61.86% and 89.83%, respectively than the Ni-stressed plants' values Fig. 3b, to reach comparable levels to the control. The expression of *ZmRBCS1* was up-regulated in response to Ni stress by 21.4% higher than the controls Fig. 3c, with further increase in the expression levels of rubisco

small subunit gene (*ZmRBCS1*) by 5.5% in 50 µM TRIA treated plants, while a decrease by 6.6% in its expression was recognized in the plant that received 25 µM TRIA Fig. 3c.

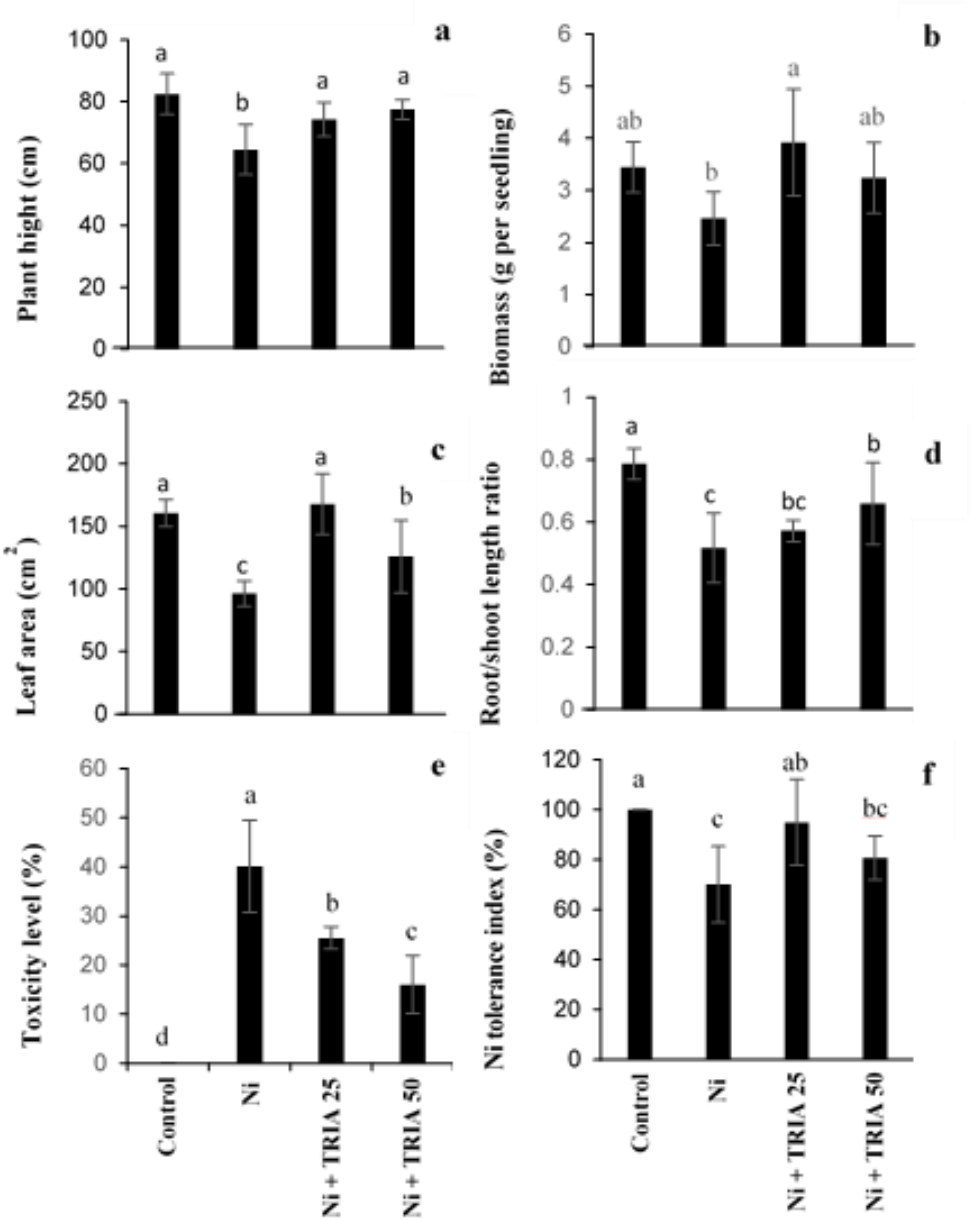

Fig. 1. Effect of nickel (Ni) alone or combined with different concentrations of triacontanol (TRIA; 25 and 50 µM) on **a)** plant height, **b)** plant fresh biomass **c)** leaf area, **d)** root/shoot length ratio, **e)** the percentage of Ni toxicity, and **f)** the percentage of Ni tolerance index as compared with the control untreated maize seedling. Data are means ± SD (n=5), bars with different letters are significantly different at $P \leq 0.05$

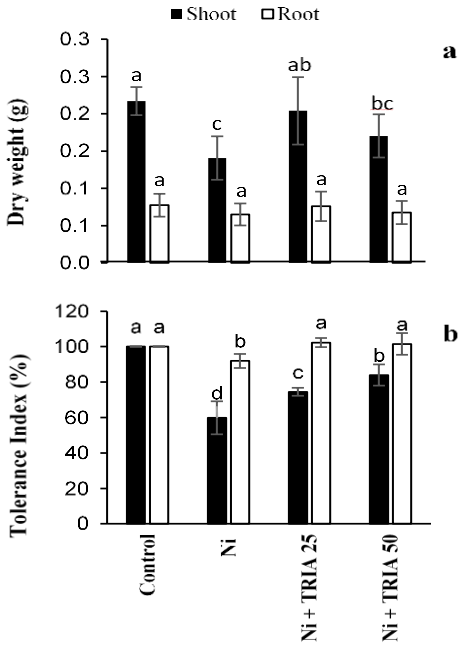

Fig. 2. Effect of Nickel (Ni) alone or combined with different concentrations of triacontanol (TRIA; 25 and 50 µM) on **a)** shoot and root dry weights, and **b)** percentage of the shoot and root Ni tolerance indices as compared with the control untreated maize seedling. Data are means ± SD (n=5), bars with different letters are significantly different at $P \leq 0.05$

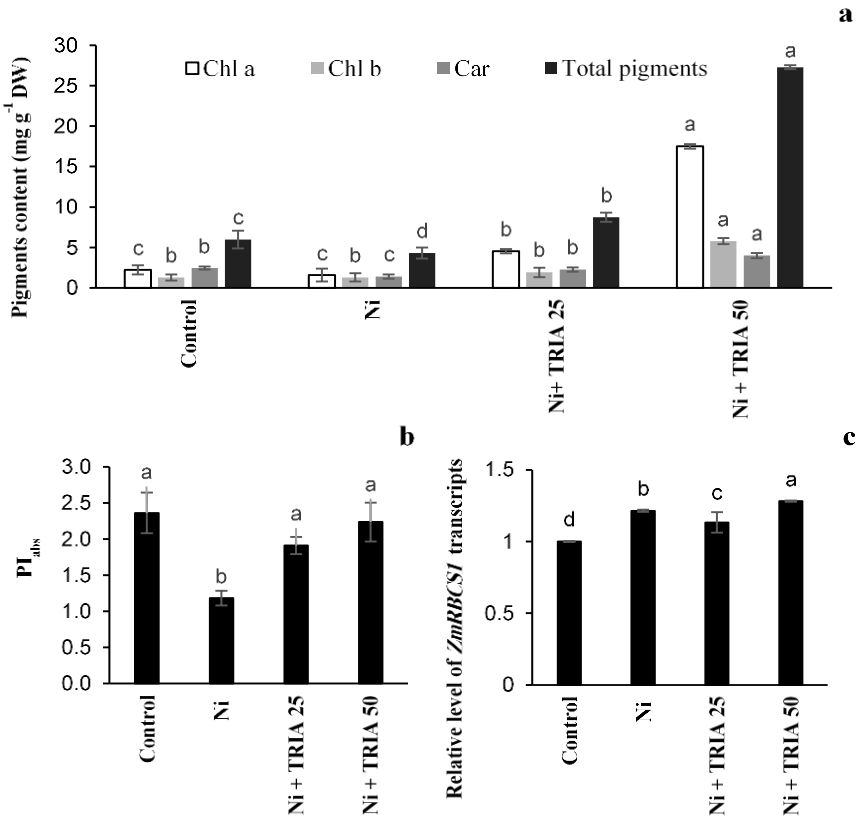

Fig. 3. Effect of nickel (Ni) alone or combined with different concentrations of triacontanol (TRIA; 25 and 50 µM) on **a)** pigments content (mg g-1 DW), **b)** photosynthetic performance index (PIabs), and **c)** ZmRBCS1 (Rubisco small subunit gene) expression of maize leaves. Data are means ± SD, n = 3, bars with different letters indicate significant differences at $P \leq 0.05$

3.3 Relative water content (RWC), the electrolyte leakage (EL), total soluble sugar (TSS), and proline contents

Ni treatment reduced the RWC of both shoot and root by 7.72% and 31.9%, respectively, relative to the controls, while TRIA application to the stressed seedlings had no mitigated impact on the declined RWC, except for TRIA (25 µM) on shoot RWC Fig. 4a. The EL increased in shoots and roots of Ni-stressed plants as compared with the controls, while TRIA treatments counteracted the injurious impact of Ni on the EL level in both the shoots and roots, as EL% was restored to control level in Ni-stressed maize shoots treated with 50 µM TRIA Fig. 4a.

Ni treatment did not have any significant effect on the proline content of both shoot and root as compared to the control untreated sample, whereas both TRIA treatments resulted in a significant decrease in the proline content of the roots. In shoots, the highest proline content was measured in Ni-stressed seedlings treated with 50 µM TRIA, which was 151% and 136 % higher than the proline contents of Ni-stressed and control shoots, respectively. Ni-stressed seedlings treated with 25 µM TRIA accumulated the lowest concentrations of proline, as it was about half the value recorded in those treated with 50 µM TRIA Fig. 3c & 4c.

No significant difference was observed between the TSS contents of roots of all plants under different treatments Fig. 4d. The highest TSS content was measured in the shoot of control plants, which was decreased significantly under Ni stress by16%. The TSS level of shoots fell dramatically (half its value in the Ni-stressed seedlings) upon TRIA treatment combined with Ni stress Fig. 4d.

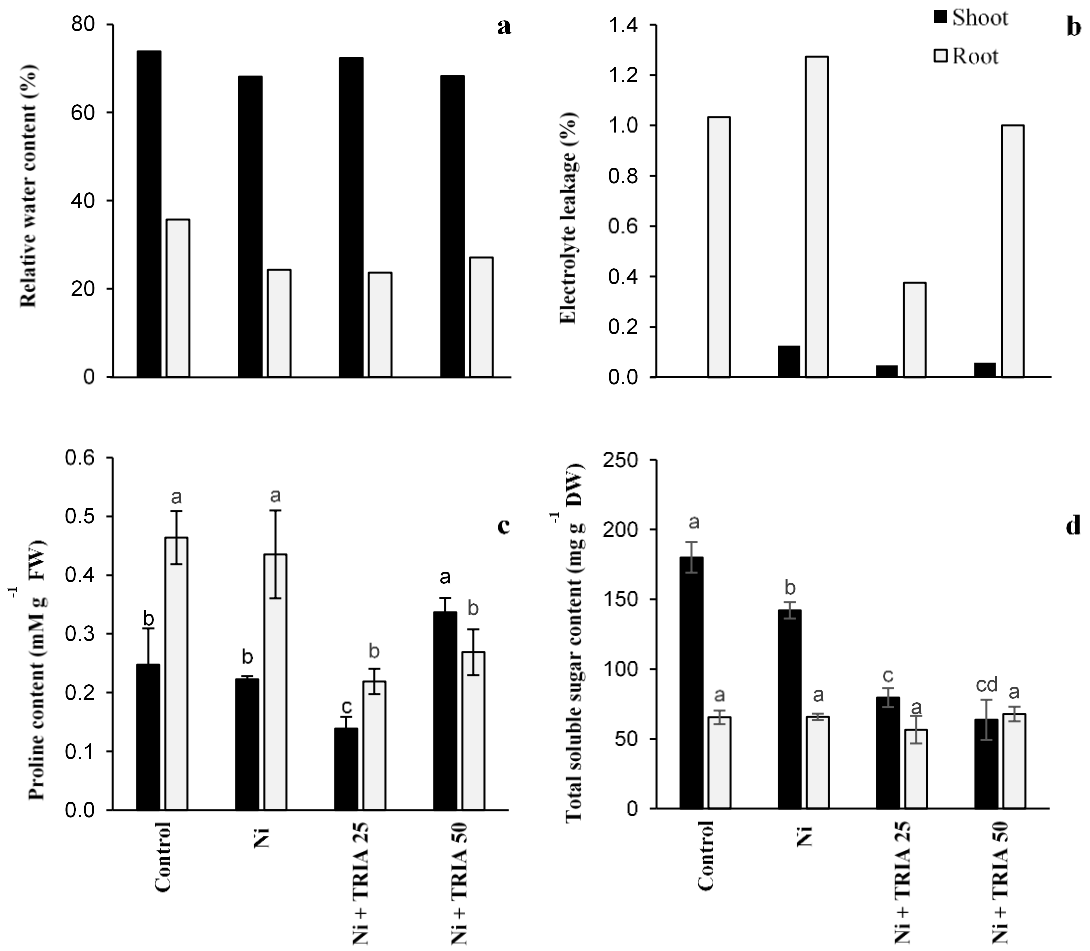

Fig. 4. Effect of nickel (Ni) alone or combined with different concentrations of triacontanol (TRIA; 25 and 50 µM) on **a)** relative water content (RWC), **b)** electrolyte leakage (EL), **c)** proline content, and **d)** total soluble sugar content of maize seedlings. Data with statistical analysis are means ± SD, n = 3, bars with different letters indicate significant differences at $P \leq 0.05$

3.4 Ni uptake, transfer rate, and translocation factor of Ni in shoots and roots

The Ni-treated maize seedlings recorded the greatest Ni_{plant} uptake and transfer rates ($Ni_{transfer\ rate}$) by 27.3% and 35.7% more than the controls, respectively. Ni-Stressed plants showed the highest root transfer factor (TF_{root}) and the lowest Shoot transfer factor (TF_{shoot}), while control plants manifested the maximum TF_{shoot} and the minimum TF_{root}. As the concentration of TRIA treatment to Ni-stressed seedlings increased, TF_{shoot} increased and TF_{root} decreased Table 1. A negative strong correlation was observed between TF_{shoot} and TF_{root} (R^2=0.99) Table 3.

3.5 Plant nutrients uptake

Under Ni stress, K^+, Mg^{2+}, Ca^{2+}, Zn^{2+}, $Mn^{2+,}$ and Fe^{2+} levels in the root tissues were reduced as compared with their corresponding in the controls Table 2. Shoots showed an obvious accumulation of K^+, Mg^{2+}, Ca^{2+}, and Zn^{2+}contents under Ni stress, while $Mn^{2+}$and Fe^{2+} contents were reduced compared to the controls Table 2. Ca^{2+} and Zn^{2+}were the only elements thataccumulated in whole Ni-stressed seedlings more than the control, while the other ions were decreased. Roots of Ni-stressed seedlings treated with TRIA (25 µM) accumulated higher K^+, Mg^{2+}, and Ca^{2+} in the roots by 58.6%, 94.7%, and 60.5% than Ni- stressed roots, and those treated with TRIA 50 µM accumulated the same ions by 67.2%, 121%, and 75%, respectively Table 2. The content of these three ions in the whole plant increased also upon both TRIA treatments under stress Table 2. While treatment with 50 µM TRIA reduced K^+, Mg^{2+}, Ca^{2+}, and Zn^{2+} contents in the shoots, 25 µM TRIA elevated the levels of K^+, $Mn^{2+,}$ and Fe^{2+} in the shoots under Ni stress Table 2.

3.6 Endogenous phytohormone profile

Ni treatment caused a decline in IAA content in the shoots by 39%, meanwhile, it resulted in 171% and 118% increase in its content in the roots and whole seedling, compared with the controls. The levels of IBA decreased and consequently total auxins were decreased in maize shoots and roots under Ni stress relative to control plants Fig. 5b & c. A similar trend was observed in SA content Fig. 5e. A four-fold IAA/IBA ratio was observed in Ni-stressed maize roots, higher than the control ones Fig. 5d. Maize roots and shoots exposed to Ni showed GA_3 contents about five folds higher than that of the controls Fig. 5f. Though no ABA was detected in Ni-stressed maize shoots, a high level was accumulated in the roots Fig. 5g. Application of 50 µM TRIA to Ni-stressed roots resulted in an increase in the IAA, IAA/IBA ratio, and SA levels by 47.3%, 361.1%, and 115%, respectively Fig. 5a, d &e, while it decreased IBA, GA_3, and ABA contents by 59%, 38%, and 20%, respectively, as compared with the untreated Ni-stressed plants Fig. 5b, f &g. Ni treatment caused a marked upregulation of *ZmASR1* expression in shoots but not in roots, which showed further significant upregulation by TRIA treatments in a concentration-dependent manner Fig. 5h. The expression level in 50 µM TRIA-treated plants reached about 5.6-folds in shoots and roots in comparison with the untreated Ni-stressed seedlings Fig. 5h.

Table 1 The Plant Ni uptake (µg g^{-1}), Ni transfer rate (µg day^{-1}), and root and shoot transfer factor (Ni TF) of maize seedlings after 15 days of Nickel (Ni) exposure alone or combined with different concentrations of triacontanol (TRIA; 25 and 50 µM)

Treatment	Plant Ni uptake	Plant Ni transfer rate	Ni TF	
			Shoot	Root
Control	0.0022	0.00014	0.49	0.98
Ni	0.0028	0.00019	0.30	1.51
Ni + TRIA 25	0.0027	0.00018	0.35	1.43
Ni + TRIA 50	0.0026	0.00017	0.36	1.32

Table 2. Effect of nickel (Ni) alone or combined with different concentrations of triacontanol (TRIA; 25 and 50 µM) on the macro and micro-nutrient contents of maize root, shoot, and whole seedling tissues

Nutrients		Organ	Treatment			
			Control	Ni	Ni + TRIA 25	Ni + TRIA 50
Macronutriments contents (mg Kg^{-1} DW)	K	Shoot	47,900.00	49,400.00	52,800.00	43,700.00
		Root	13,400.00	5,800.00	9,200.00	9,700.00
		Whole seedling	61,300.00	55,200.00	62,000.00	53,400.00
		Shoot/root ratio	3.575	8.517	5.739	4.505
	Mg	Shoot	3,000.00	3,700.00	3,500.00	2,600.00
		Root	2,800.00	1,900.00	3,700.00	4,200.00
		Whole seedling	5,800.00	5,600.00	7,200.00	6,800.00
		Shoot/root ratio	1.071	1.947	0.946	0.619
	Ca	Shoot	4,100.00	7,100.00	6,100.00	3,600.00
		Root	10,500.00	7,600.00	12,200.00	13,300.00
		Whole seedling	14,600.00	14,700.00	18,300.00	16,900.00
		Shoot/root ratio	0.390	0.934	0.500	0.271
	Ca/Mg ratio	Shoot	1.37	1.92	1.74	1.38
		Root	3.75	4.00	3.30	3.17
		Whole seedling	2.52	2.63	2.54	2.49
Micronutrients contents (mg Kg^{-1} DW)	Zn	Shoot	55.57	84.67	51.19	71.47
		Root	82.85	82.20	77.07	83.52
		Whole seedling	138.42	166.87	128.26	154.99
		Shoot/root ratio	0.671	1.030	0.664	0.856
	Mn	Shoot	78.93	62.72	70.69	55.59
		Root	168.60	120.97	102.76	86.30
		Whole seedling	247.53	183.69	173.45	141.89
		Shoot/root ratio	0.468	0.518	0.688	0.644
	Fe	Shoot	254.51	168.55	220.18	194.57
		Root	1,861.86	1,816.04	2,087.35	1,670.38
		Whole seedling	2,071.37	2,029.59	2,307.53	1,864.95
		Shoot/root ratio	0.140	0.091	0.105	0.116

3.7 Correlation of traits

Correlation among morpho-physiological traits Table 3 revealed a highly significant ($P < 0.01$) positive correlation between fresh biomass and all the plant height, leaf area, Ni-TI and shoot DW. Similarly, Ni-TI was also significantly positively correlated with plant height, root/shoot ratio, leaf area, RTI, and shoot DW, while it was significantly negatively correlated with the Ni toxicity level.

The contents of Chl a, b and carotenoids exhibited a significant strong positive correlation to each other and proline content of the shoots, but they were significantly negatively correlated with the shoot's TSS. The proline content of the shoots and TSS of the roots were significantly positively correlated, whereas proline content of the roots and TSS of the shoots showed a strong significant positive correlation Table 3.

Table 3. Pearson-Correlation coefficients comparisons for various plant traits under various treatments

	Biomass	Plant height	Root /Shoot	Leaf area	Toxicity level	RTI	STI	Ni-Ti	Shoot DW	Root DW	Chl a	Chl b	Car	Shoot TSS	Root TSS	Shoot proline	Root proline
Biomass	1																
Plant height	.604**	1															
Root/shoot	0.164	.748**	1														
Leaf area	.755**	.605**	0.347	1													
Toxicity level	-0.389	-.815**	-.758**	-.507*	1												
RTI	0.389	.815**	.758**	.507*	-1.000**	1											
STI	0.362	.529*	.461*	0.419	-0.442	0.442	1										
Ni-Ti	.798**	.742**	.452*	.793**	-.643**	.643**	.496*	1									
Shoot DW	.871**	.752**	0.393	.812**	-.633**	.633**	0.335	.927**	1								
Root DW	0.432	0.369	0.350	0.352	-0.294	0.294	.493*	.566**	0.359	1							
Chl a	0.116	0.209	-0.070	-0.248	-0.247	0.247	0.180	-0.146	-0.062	-0.219	1						
Chl b	0.112	0.303	0.037	-0.230	-0.311	0.311	0.347	-0.069	-0.039	-0.175	.967**	1					
Car	0.152	0.364	0.094	-0.047	-0.548	0.548	0.257	-0.006	0.104	-0.271	.916**	.896**	1				
Shoot TSS	-0.340	0.055	0.570	0.014	-0.247	0.247	-0.122	0.120	0.008	0.272	-.735**	-.686*	-0.575	1			
Root TSS	-0.451	0.142	0.264	-0.536	-0.233	0.233	0.133	-0.158	-0.349	0.153	0.227	0.351	0.242	0.197	1		
Shoot proline	-0.354	0.283	0.366	-0.506	-0.414	0.414	0.019	-0.301	-0.263	-0.235	.669*	.691*	.639*	-0.071	.644*	1	
Root proline	-0.468	0.090	0.496	-0.224	-0.210	0.210	-0.123	0.005	-0.172	0.262	-0.536	-0.465	-0.480	.876**	0.402	0.197	1

4. DISCUSSION

In the present investigation, the growth, Ni-TI, RTI, and STI of maize seedlings were negatively affected by Ni treatment. Such impacts of Ni stress were previously reported in different plant species [35, 36]. It was recorded that roots were more sensitive to Ni stress than shoots [37], which was displayed in this study as reduced root/shoot length ratio and RTI % under Ni stress. The Ni-induced decline in maize seedling height, biomass, leaf area, and shoot DW might be attributed to retarded mitotic and metabolic activities, inhibition of cell division, and/or elongation, distorted root hairs and suppressed the emergence of new ones, disturbed mineral uptake, and altered water and hormonal balances [38, 39]. TRIA application to Ni-stressed maize seedlings alleviated the toxic effects of Ni on the growth attributes, Ni-Ti, RTI, STI, and reduced toxicity of Ni tested in this work. Similar ameliorating effects of TRIA on plant growth have been also demonstrated by other workers [15, 37, 38], which is probably due to TRIA-mediated activation of L (+)-adenosin (a second messenger in the signal transduction mechanism of action of TRIA), which leads to cell enlargement and proliferation [42].

The present results also showed that PI_{abs}, Car, and total photosynthetic pigments contents of maize seedlings were greatly reduced by Ni stress, which was mostly related to pigment degradation and inhibited biosynthesis, obstructed structure and function of chloroplasts, and interrupted electron transport, and reduced leaf area as shown by **Younis & Ismail** [43] and **Bhalerao *et al.*** [37]. Though published works demonstrated the reduced activity of Rubisco under Ni stress [44], our data indicated that *RBCS* expression of Rubisco small subunit increased under Ni stress, perhaps acting as a reparation mechanism to compensate for reduced leaf area and PI_{abs}. In the current work, foliar application of TRIA (particularly 50 µM) to Ni-stressed maize seedling resulted in a great leap in the contents of all photosynthetic pigments, PI_{abs}, and *RBCS* expression, which agrees with those of other researchers [13, 17].

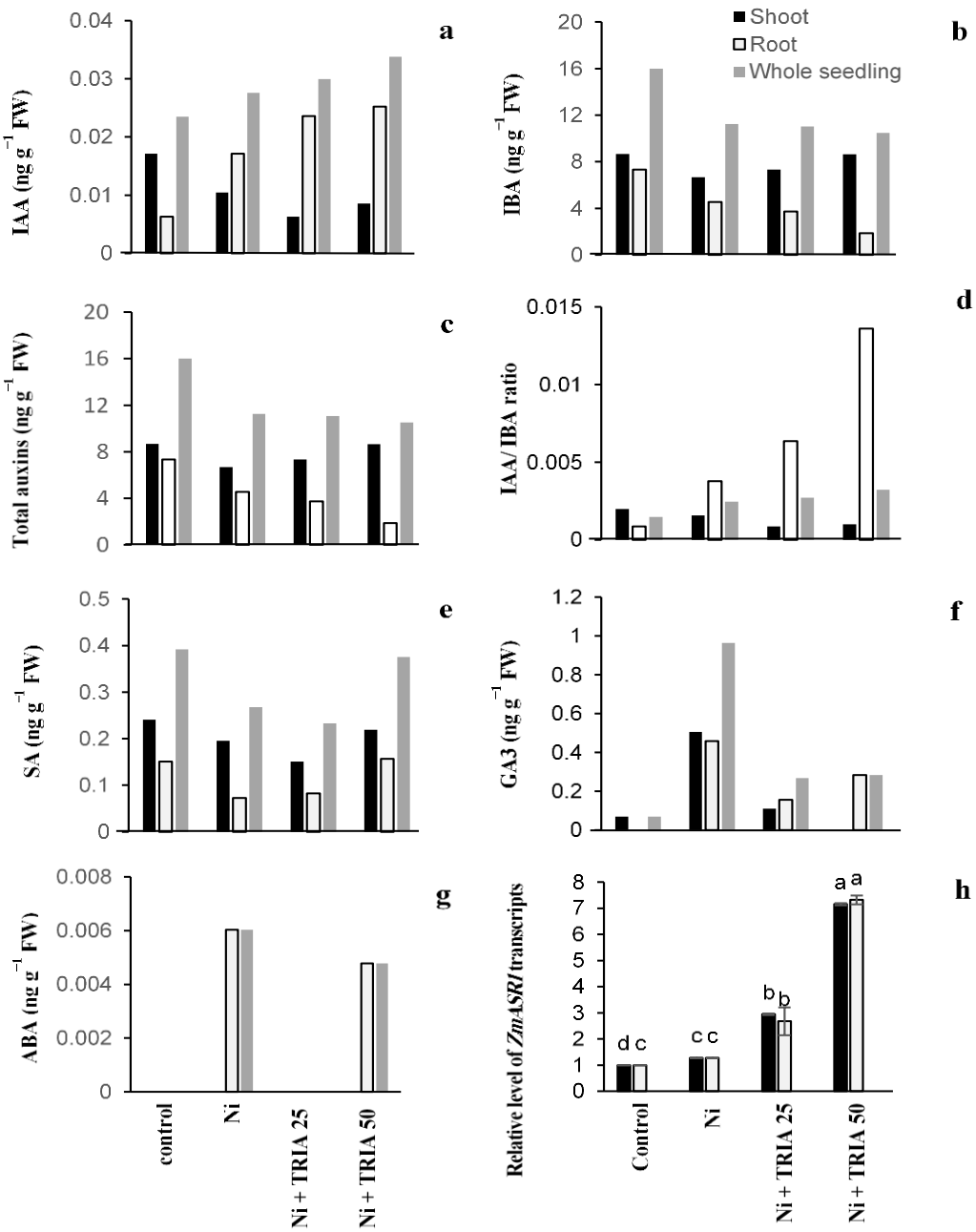

Fig. 5. Effect of nickel (Ni) alone or combined with different concentrations of triacontanol (TRIA; 25 and 50 µM) on plant hormones content **a)** IAA, **b)** IBA, **c)** total auxins, **d)** IAA/IBA ratio **e)** SA, **f)** GA3, **g)** ABA, and **h)** abscisic acid- stress- ripening (ZmASR1) gene expression of maize seedlings. Data of gene expression are means ± SD, n = 3, bars with different letters indicate significant differences at $P \leq 0.05$

Those effects of TRIA on photosynthesis could be presumably attributed to the elevated number and size of chloroplasts, enhanced biosynthesis of chlorophyll, and enhanced photosystem II activity, which were recorded by **Chandra & Roychoudhury** [45] and **Ramos-Zambrano *et al*** [46]. TRIA has also been reported to hamper chlorophyllase activity, modulate the fluidity of chloroplast membrane and its associated bound enzymes, and enhance the progression of thylakoid grana [47].

Relative Water Content (RWC) is an important determinant of a plant's growth under environmental stresses [48]. In this study, Ni reduced the RWC of the shoot and to a higher extent of the root of maize seedling. Reduced root length and small leaf area obtained in the present work are possible reasons for the lower RWC of maize seedlings. TRIA treatment caused a slight retrieval of shoot RWC, which is probably induced by modulating photosynthesis and membrane fluidity [47]. The elevated percentage of EL of Ni-stressed shoots and roots, recorded in this work, is a direct indicator of membrane damage and lipid peroxidation of cell membranes [43, 49], and might also be a result of impaired activity of the plasma membrane transporters [50]. TRIA application mitigated the Ni effect on the EL of both maize shoots and roots, which agrees with the results obtained by **Ali and Perveen** [41] and **Nabi *et al.*** [17] who reported similar ameliorative effects of TRIA on membranes' stability under HMs. TRIA may execute these effects *via* altered fatty acid composition and lipid-protein interaction of cellular membranes [47].

In the current study, the proline content of both shoots and roots was not affected by Ni stress, however, the TSS content of maize shoots has been significantly decreased by Ni treatment, which agrees with the adverse impact of HMs on sugar metabolism in plants [51]. HMs-induced oxidative stress might alter primary carbohydrate metabolism and change sugar source-sink partitioning which eventually inhibits plant growth [52]. On the other hand, the maximum value of proline content was recorded in Ni-stressed maize shoots treated with TRIA (50µM) that exceeded even the control level at the expense of TSS content as the proline biosynthesis is an energy-demanding process [53]. These results suggest that TRIA treatment favors proline accumulation in the shoot to protect the photosynthetic apparatus which is the most important machinery in the plant [38]. This was confirmed by the significant positive correlation (*P< 0.01*) between the proline content of shoots and all photosynthetic pigments.

In the current work, maize plants exposed to 100 mM Ni^{2+} exhibited higher Ni^{2+} uptake, $Ni_{transfer\ rate}$, and TF_{root}, which was probably related to the immobilization of Ni^{2+} ions in the root cortex and its chelation to organic acids in root tissues as a potential tolerance mechanism [54].

Ni^{2+}localizationin the roots recorded in the present investigation might be an adaptive response of plants to prevent the transport of toxic amounts of Ni to the photosynthetic tissues [55]. TRIA application to Ni-treated plants led to lower Ni_{plant} uptake, and TF_{root} in a concentration-dependent manner, implying that TRIA might block the entrance of Ni to the plant, mainly into roots. This might be related to the TRIA lipophilic nature that may enable its interaction with cellular membranes affecting its permeability for heavy metals [47]. Ni fixation in the cell wall, subcellular compartmentalization in roots, and its chelation and sequestration in vacuoles are all potential mechanisms of Ni detoxification [56].

Ni^{2+} stress triggered a reduction in K^+, Mg^{2+}, Ca^{2+}, $Mn^{2+,}$ and Fe^{2+} levels in both roots and whole seedlings of maize. Those essential nutrients participate in many physiological and biochemical processes in plants [57]. Decreased K^+ content under Ni stress may be due to K^+ efflux through potassium outward rectifying channels activated by hydroxyl radicals produced by HMs [58]. Further, Ni stress can inhibit ZIP/NRAMP root transporters linked to the uptake of other divalent cations, such as Ca^{2+}, Mg^{2+}, Fe^{2+}, and Zn^{2+} [59, 60]. Additionally, Ni was reported to compete with Mg^{2+}, $Fe^{2+,}$ and Zn^{2+} (of similar ion size) at their transporters [38]. On the other hand, enhanced contents of K^+, $Mg^{2+,}$ and Ca^{2+} in Ni^{2+}-stressed maize shoot observed in this work may be explained by their enhanced loading in the shoot as a technique to maintain the photosynthetic process under stress, though the whole seedling content of these ions was less than that of the control [61]. Our data showed a similar pattern of Ni uptake and Ca^{2+} content in shoots of maize seedlings, which might be explained as a strategy for sequestration of Ni, as heavy metals were found to be chelated in leaves of some plants in the form of Ca oxalate crystals [62]. In the present work, treatment of Ni-stressed maize seedling with either concentration of TRIA managed to replenish the contents of K^+, $Mg^{2+,}$ and Ca^{2+}in roots and the whole seedling, whereas Fe^{2+} content in shoots and the whole plant was increased by treatment with 25 µM TRIA. A comparable ameliorative effect of TRIA on mineral nutrition balance was documented by **Romero-Martínez *et al.*** [47].

Our data showed that Ni-stressed roots accumulated IAA meanwhile it contained low levels of IBA compared with the control. On the other hand, Ni stress reduced the contents of IAA, IBA, and total auxins of shoots. Our results suggest that Ni stress activated IBA conversion to IAA in roots as well as IAA basipetal transport as compensation for the stunted growth, which was supported by the work of **Frick and Strader** [63]. The effect of TRIA on auxins metabolism, transport, conjugation, and signaling is under investigation, yet our results showed that TRIA increased IAA content in roots, while it reduced its accumulation in shoots of Ni-stressed seedlings. Those results corresponded with the fully revived RTI and partially restored STI of TRIA-treated plants. Moreover, **Soundararajan *et al.*** [64] reported simultaneous enhancement of both IAA content and root emergence upon TRIA treatment of tomato *in vitro* cultures,annotating its role in enhanced root growth. Our results revealed that both shoots and roots of Ni-stressed seedlings exhibited a reduced level of SA compared with the unstressed control, which might be supported by the findings of **Freeman *et al.*** [65], who reported blocked SA signaling by Ni stress. TRIA application (50µM) restored SA levels in both shoots and roots to comparable levels of the control plants. SA is known to repress heavy metals absorption, repair the stress-induced photosynthetic impairment, regulate ROS scavenging, and prevent membrane damage by HMs [66]. The ability of plants to adapt to HMs was found to be related to the low contents of GA_3, while higher concentrations of GA_3 were related to increased oxidative damage caused by HMs [66]. Similarly, in this work, elevated levels of GA_3 were detected in shoots and roots of Ni-exposed maize seedlings accompanied by membrane damage that was displayed as a high level of EL%. Ni-induced accumulation of ABA together with the reduced RWC, recorded in this work, supported the potential involvement of ABA in the inhibition of Ni translocation through the xylem stream to the shoots [67].ABA was reported to stimulate metallothioneins (metal-binding protein responsible for HM sequestration in roots) expression in roots under heavy metal stress [68].In the present work, ABA was not detected in Ni-stressed roots treated with the low concentration of TRIA (25µM), but it was retrieved in those treated with 50 µM TRIA, which agrees with the comparable findings of **El-Shafey *et al.*** [69]. On the contrary, **Chen *et al.*** [70] reported a reduction of ABA content and its related genes in response to TRIA treatment in rice. These contradictory results might be attributed to the complex processes involved in ABA synthesis, perception, and signaling [71]. Additionally, the conditions at which the experiments were conducted, plant species, and growth stage are all factors that must be taken into consideration when dealing with those conflicting results. Given that TRIA treatment, in this work, enhanced the expression of *ZmASR1* (a gene involved in ABA signal transduction) in a concentration-dependent manner under Ni stress treatment. Such enhancement of *ZmASR1* expression by TRIA treatment may be improved roots tolerance to Ni, which was evidenced by high RTI and reduced level of toxicity in roots found in this study.

5. CONCLUSION

In conclusion, Ni stress reduced the growth and developmental processes of the *Zea mays* plants. This work confirmed that foliar spray of maize seedlings with TRIA was effective in mitigating the detrimental effects of Ni on growth and photosynthesis, more so with 50 µM TRIA. TRIA-induced photosynthesis recuperation under Ni-stress might be triggered by enhanced photosynthetic pigments, photosynthetic performance, and *ZmRBCS* expression. This paper aimed to address the interaction between TRIA and both osmolytes and hormonalbalance under Ni stress. Our results showed that TRIA altered osmolyte accumulation, balancing between sugar and proline accumulation in an organ-specific manner, which might be intended for energy-saving purposes. Hormonal balance was greatly transformed by TRIA application (especially 50 µM) under Ni stress, by increasing IAA/ IBA ratio in roots exposed to Ni and restoring SA in both shoots and roots. All those changes caused by TRIA ultimately cast on the better exclusion of Ni from maize seedlings, basically from roots. The interconnection between hormonal signaling and ion transporters under TRIA application and its role in alleviating Ni toxicity will be a prospective point to study.

6. ABBREVIATIONS

ABA: abscisic acid; **Car:** carotenoids; **Chl a:** chlorophyll a; **Chl b:** chlorophyll b; **DW:** dry weights; **EL:** electrolyte leakage; **FW:** fresh weight; **GA_3:** gibberellic acid; **HMs:** Heavy metal stress; **IAA:** indole acetic acid; **IBA:** indole butyric acid; **Ni:** nickel; **Ni-TI:** Ni tolerance index; **$Ni_{transfer\ rate}$:** plant Ni transfer rate; **PI_{abs}:** photosynthetic performance index; **RTI:** root tolerance index; **Rubisco:** Ribulose-1,5-biphosphate carboxylase/oxygenase; **RWC:** relative water content; **SA:** salicylic acid; **STI:** shoot tolerance index; **TF_{root}:** root transfer factor; **TF_{shoot}:** shoot transfer factor; **TRIA:** triacontanol; **TSS:** total soluble sugar; ***ZmASR1***: abscisic acid- stress- ripening gene; ***ZmRBCS***: Rubisco small subunit gene.

7. References

1. **Shaheen, S. M., Abdelrazek, M. A. S., Elthoth, M., Moghanm, F. S., Mohamed, R., Hamza, A., El-Habashi, N., Wang, J. and Rinklebe, J. (2019).** Potentially toxic elements in saltmarsh sediments and common reed (*Phragmites australis*) of Burullus coastal lagoon at North Nile Delta, Egypt: a survey and risk assessment. Sci. Total Environ., **649:** 1237 – 1249.
2. **El-Alfy, M. A., El-Amier, Y. A. and El-Eraky, T. E. (2020).** Land use/cover and eco-toxicity indices for identifying metal contamination in sediments of drains, Manzala Lake, Egypt. Heliyon., **6:** e03177.
3. **Kabata-Pendias, A. and Mukherjee, A. B. (2007).** Trace elements from soil to human. Springer Science & Business Media.
4. **Czajka, K. M., Michael, P. and Nkongolo, K. (2019).** Differential effects of nickel dosages on *in vitro* and *in vivo* seed germination and expression of a high affinity nickel-transport family protein (AT2G16800) in trembling aspen (*Populus tremuloides*). Ecotoxicology, **28:** 92 – 102.
5. **Moreira, I. N., Martins, L. L. and Mourato, M. P. (2020).** Effect of Cd, Cr, Cu, Mn, Ni, Pb and Zn on seed germination and seedling growth of two lettuce cultivars (*Lactuca sativa* L.). Plant Physiol. Reports, **25:** 347 – 358.
6. **Seregin, I. V. and Kozhevnikova, A. D. (2006).** Physiological role of nickel and its toxic effects on higher plants. Russ. J. Plant Physiol., **53:** 257 – 277.
7. **Llamas, A., Ullrich, CI. and Sanz, A. (2008).** Ni^{2+} toxicity in rice: effect on membrane functionality and plant water content. Plant Physiol. Biochem., **46:** 905 – 910.
8. **Rucińska-Sobkowiak, R. (2016).** Water relations in plants subjected to heavy metal stresses. Acta Physiol. Plant, **38:** 1 – 13.
9. **Hassan, M. U., Chattha, M. U., Khan, I., Chattha, M. B., Aamer, M., Nawaz, M., Ali, A., Khan, M. A. U. and Khan, T. A. (2019).** Nickel toxicity in plants: reasons, toxic effects, tolerance mechanisms, and remediation possibilities—a review. Environ. Sci. Pollut. Res., **26:** 12673 – 12688.
10. **FAOSTAT (2020)**. Crop Yields.
11. **Okereafor, U., Makhatha, M., Mekuto, L., Uche-Okereafor, N., Sebola, T. and Mavumengwana, V. (2020).** Toxic metal implications on agricultural soils, plants, animals, aquatic life and human health. Int. J. Environ. Res. Public Health, **17:** 2204.
12. **Hasanuzzaman, M., Nahar, K,. Alam, M. M., Roychowdhury, R. and Fujita, M. (2013).** Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. Int. J. Mol. Sci., **14:** 9643 – 9684.
13. **Chen, X., Yuan, H., Chen, R., Zhu, L. and He, G. (2003).** Biochemical and photochemical changes in response to triacontanol in rice (*Oryza saliva* L.). Plant Growth Regul., **40:** 249 – 256.
14. **Ertani, A., Schiavon, M., Muscolo, A. and Nardi, S. (2013).** Alfalfa plant-derived biostimulant stimulate short-term growth of salt stressed *Zea mays* L. plants. Plant Soil, **364:** 145 – 158.
15. **Chen, X., Goodwi,n S. M., Liu, X., Chen, X., Bressan, R. A. and Jenks, M. A. (2005).** Mutation of the resurrection1 locus of Arabidopsis reveals an association of cuticular wax with embryo development. Plant Physiol., **139:** 909 – 919.
16. **Naeem, M., Khan, M. M. A. and Moinuddin, (2012).** Triacontanol: a potent plant growth regulator in agriculture. J. Plant Interact., **7:** 129 – 142.

17. **Nabi, A., Parwez, R., Aftab, T., Khan, M. M. A. and Naeem, M. (2020).** Triacontanol Protects Mentha arvensis L. from Nickel-Instigated Repercussions by Escalating Antioxidant Machinery, Photosynthetic Efficiency and Maintaining Leaf Ultrastructure and Root Morphology. J. Plant Growth Regul., 1–19.
18. **Heba, M. H. (2013).** The potential role of triacontanol in certain physiological aspects of *Zea mays* L. single cross Giza 310 grown under normal and environmental stress conditions. PhD., Fac. Sci. Ain Shams Univ.
19. **Perveen, S., Shahbaz, M. and Ashraf, M. (2014).** Triacontanol-induced changes in growth, yield, leaf water relations, oxidative defense system, minerals, and some key osmoprotectants in Triticum aestivum under saline conditions. Turk. J. Botany, **38:** 896 – 913.
20. **Islam, S., Zaidm A. and Mohammad, F. (2020).** Role of Triacontanol in Counteracting the Ill Effects of Salinity in Plants: A Review. J. Plant Growth Regul., **40:** 1 – 10.
21. **Smith, G. S., Johnston, C. M. and Cornforth, I. S. (1983).** comparison of nutrient solutions for growth of plants in sand culture. New Phytol., **94:** 537 – 548.
22. **Valivand, M., Amooaghaie, R. and Ahadi, A. (2019).** Seed priming with H_2S and Ca^{2+} trigger signal memory that induces cross-adaptation against nickel stress in zucchini seedlings. Plant Physiol. Biochem., **143:** 286 – 298.
23. **Alam, P., Kaur Kohli, S., Al Balawi, T., Altalayan, F. H., Alam, P., Ashraf, M., Bhardwaj, R. and Ahmad, P. (2020).** Foliar application of 24-Epibrassinolide improves growth, ascorbate-glutathione cycle, and glyoxalase system in brown mustard (*Brassica juncea* (L.) *Czern*.) under cadmium toxicity. Plants, **9:** 1487.
24. **Amooaghaie, R., Tabatabaei, F. and Ahadi, A. (2015).** Role of hematin and sodium nitroprusside in regulating Brassica nigra seed germination under nanosilver and silver nitrate stresses. Ecotoxicol. Environ. Saf. **113:** 259 – 270.
25. **Bálint, A. F., Kovács, G. and Sutka, J. (2002).** Copper tolerance of *Aegilops*, *Triticum*, *Secale* and *triticale* seedlings and copper and iron content in their shoots. Acta. Biol. Szeged., **46:** 77 – 78.
26. **Maxwell, K. and Johnson, G. N. (2000).** Chlorophyll fluorescence—a practical guide. J. Exp. Bot., **51:** 659 – 668.
27. **Metzner, H., Rau, H. and Senger, H. (1965).** Untersuchungen zur Synchronisierbarkeit einzelner Pigmentmangel-Mutanten von Chlorella. Planta, **65:** 186 – 194.
28. **Livak, K. J. and Schmittgen, T. D. (2001).** Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods, **25:** 402 – 408.
29. **Pan, Y., Seymour, G. B., Lu, C., Hu, Z., Chen, X. and Chen, G. (2012).** An ethylene response factor (ERF5) promoting adaptation to drought and salt tolerance in tomato. Plant Cell Rep., **31:** 349 – 360.
30. **Valentovic, P., Luxova, M., Kolarovic, L. and Gasparikova, O. (2006).** Effect of osmotic stress on compatible solutes content, membrane stability and water relations in two maize cultivars. Plant Soil Environ., **52:** 184.
31. **McCready, R. M., Guggolz, J., Silviera, V. and Owens, H. S. (1950)**. Determination of Starch and Amylose in Vegetables. Anal. Chem., **22:** 1156 – 1158.
32. **Bates, L. S., Waldren, R. P. and Teare, I. D. (1973).** Rapid determination of free proline for water-stress studies. Plant Soil, **39:** 205 – 207.
33. **Xiong, T., Dumat, C., Dappe, V., Vezin, H., Schreck, E., Shahid, M., Pierart, A. and Sobanska, S. (2017).** Copper oxide nanoparticle foliar uptake, phytotoxicity, and consequences for sustainable urban agriculture. Environ. Sci. Technol., **51:** 5242 – 5251.
34. **Abbas, Q., Yousaf, B., Liu, G., Zia-ur-Rehman, M., Ali, M. U., Munir, M. A. M. and Hussain, S. A. (2017).** Evaluating the health risks of potentially toxic elements through wheat consumption in multi-industrial metropolis of Faisalabad, Pakistan. Environ. Sci. Pollut. Res., **24:** 26646 – 26657.

35. **Zaid, A., Mohammad, F., Wani, S. H., Siddique, K. M. H. (2019).** Salicylic acid enhances nickel stress tolerance by up-regulating antioxidant defense and glyoxalase systems in mustard plants. Ecotoxicol. Environ. Saf. **180:** 575 – 587.
36. **Aqeel, M., Khalid, N., Tufail, A., Ahmad, R. Z., Akhter, M. S., Luqman, M., Javed, M. T., Irshad, M. K., Alamri, S. and Hashem, M. (2021).** Elucidating the distinct interactive impact of cadmium and nickel on growth, photosynthesis, metal-homeostasis, and yield responses of mung bean (*Vigna radiata* L.) varieties. Environ. Sci. Pollut. Res., 1 – 15.
37. **Bhalerao, S. A., Sharma, A. S. and Poojari, A. C. (2015).** Toxicity of nickel in plants. Int. J. Pure Appl. Biosci., **3:** 345 – 355.
38. **Ahmad, M. S. A. and Ashraf, M. (2012).** Essential roles and hazardous effects of nickel in plants. Rev. Environ. Contam. Toxicol., 125 – 167.
39. **Batool, S. (2018)**. Effect of nickel toxicity on growth, photosynthetic pigments and dry matter yield of *Cicer arietinum* L. varieties. Asian J. Agri. Biol., **6:** 143 – 148.
40. **Naeem, M., Ansari, A. A., Aftab, T., Shabbir, A., Alam, M. M., Khan, M. M. A. and Uddin, M. (2019).** Application of triacontanol modulates plant growth and physiological activities of *Catharanthus roseus* (L.). Int. J. Bot. Stud., **4:** 131 – 135.
41. **Ali, H. M. M. and Perveen, S. (2020).** Effect of foliar applied triacontanol on wheat (*Triticum aestivum* L.) under arsenic stress: a study of changes in growth, yield and photosynthetic characteristics. Physiol. Mol. Biol. Plants, **26:** 1215 – 1224.
42. **Khan, Z. H., Mohammad, F. and Khan M. M. A. (2014**). Enhancing the growth, yield and production of essential oil and citral in lemongrass by the application of triacontanol. Int. J. Agric. Res., **4:** 113 – 122.
43. **Younis, A. A. and Ismail, H. A. (2019).** Triacontanol Alleviated Nickel Toxicity in Maize Seedling by Controlling Its Uptake and Enhancing Antioxidant System. J. Adv. Plant Biol., **1:** 1 – 15.
44. **Hussain, M. B., Ali, S., Azam, A., Hina, S., Farooq, M. A., Ali, B., Bharwana, S. A. and Gill, M. B. (2013**). Morphological, physiological and biochemical responses of plants to nickel stress: A review. African J. Agric. Res., **8:** 1596 – 1602.
45. **Chandra, S. and Roychoudhury, A. (2020).** Penconazole, Paclobutrazol, and Triacontanol in Overcoming Environmental Stress in Plants. Prot. Chem. Agents Amelior. Plant Abiotic Stress Biochem. Mol. Perspect, 510 – 534.
46. **Ramos-Zambrano, E., Juárez-Yáñez, T. E., Tapia-Maruri, D., Camacho-Díaz, B. H., Jiménez-Aparicio, A. R. and Martínez-Ayala, A. L. (2020).** Effects of Triacontanol and Light on Stomatal and Photochemical Responses in *Solanum lycopersicum* L. J. Plant Growth Regul., 1 – 13.
47. **Romero-Martínez, N., Ramos-Zambrano, E., Osorio-Ruiz, A. and Martínez-Ayala, A. L. (2021).** Main Mechanisms of Action of Policosanol in Animal and Plant Cells. Int. J. Pharm. Res. Allied Sci., **10**(2): 10 – 20.
48. **Onyekachi, O. G., Boniface, O. O., Gemlack, N. F. and Nicholas, N. (2019).** The effect of climate change on abiotic plant stress: a review. Abiotic Biot. Stress Plants.
49. **Gajewska, E., Niewiadomska, E., Tokarz, K., Słaba, M. and Skłodowska, M. (2013).** Nickel-induced changes in carbon metabolism in wheat shoots. J. Plant Physiol., **170:** 369 – 377.
50. **Nazir, F., Hussain, A. and Fariduddin, Q. (2019)**. Interactive role of epibrassinolide and hydrogen peroxide in regulating stomatal physiology, root morphology, photosynthetic and growth traits in *Solanum lycopersicum* L. under nickel stress. Env. Exp. Bot., **162:** 479 – 495.
51. **Rosa, M., Prado, C., Podazza, G., Interdonato, R., González, J. A., Hilal, M. and Prado, F. E. (2009)**. Soluble sugars: Metabolism, sensing and abiotic stress: A complex network in the life of plants. Plant Signal Behav., **4**: 388 – 393.
52. **Gill, M. (2014).** Heavy metal stress in plants: a review. Int. J. Adv. Res., **2:** 1043 – 1055.

53. **Zhang, L. and Becker, D. (2015)**. Connecting proline metabolism and signaling pathways in plant senescence. Front Plant Sci., **6:** 552.
54. **Page, V. and Feller, U. (2015).** Heavy metals in crop plants: Transport and redistribution processes on the whole plant level. Agronomy, **5:** 447 – 463.
55. **Mallick, S., Sinam, G., Mishra, R. K. and Sinha, S. (2010).** Interactive effects of Cr and Fe treatments on plants growth, nutrition and oxidative status in *Zea mays* L. Ecotoxicol. Environ. Saf., **73:** 987 – 995.
56. **Shahzad, B., Tanveer, M., Rehman, A., Cheema, S. A., Fahad, S., Rehman, S. and Sharma, A. (2018).** Nickel; whether toxic or essential for plants and environment-A review. Plant Physiol. Biochem., **132:** 641 – 651.
57. **White, P. J. and Brown, P. H. (2010).** Plant nutrition for sustainable development and global health. Ann. Bot., **105:** 1073 – 1080.
58. **Palm, E., Nissim, W. G., Giordano, C., Mancuso, S. and Azzarello, E. (2017).** Root potassium and hydrogen flux rates as potential indicators of plant response to zinc, copper and nickel stress. Environ. Exp. Bot., **143:** 38 – 50.
59. **Asha, S. and Anju, D. (2013).** Nickel and cadmium toxicity in plants. J. Pharm. Sci. Innov., **2:** 20 – 24.
60. **Deng, T. (2016).** Nickel uptake and transport in the hyperaccumulator Noccaea Caerulescens.
61. **Hasanuzzaman, M., Bhuyan, M. H. M., Nahar, K., Hossain, M., Mahmud, J. Al., Hossen, M., Masud, A. A. C. and Fujita, M. (2018).** Potassium: a vital regulator of plant responses and tolerance to abiotic stresses. Agronomy, **8:** 31.
62. **Osmolovskaya, N., Dung, V. V. and Kuchaeva, L. (2018)** The role of organic acids in heavy metal tolerance in plants. Biol. Commun., **63**(1): 9 – 16.
63. **Frick, E. M. and Strader, L. C. (2018).** Roles for IBA-derived auxin in plant development. J. Exp. Bot., **69:** 169 – 177.
64. **Soundararajan, M., Swamy, G. S., Gaonkar, S. K. and Deshmukh, S. (2018).** Influence of triacontanol and jasmonic acid on metabolomics during early stages of root induction in cultured tissue of tomato (*Lycopersicon esculentum*). Plant Cell, Tissue Organ Cult., **133:** 147 – 157.
65. **Freeman, J. L., Garcia, D., Kim, D., Hopf, A. and Salt, D. E. (2005).** Constitutively elevated salicylic acid signals glutathione-mediated nickel tolerance in *Thlaspi* nickel hyperaccumulators. Plant Physiol., **137:** 1082 – 1091.
66. **Saini, S., Kaur, N. and Pati, P. K. (2021).** Phytohormones: Key players in the modulation of heavy metal stress tolerance in plants. Ecotoxicol. Environ. Saf. **223:** 112578.
67. **Bücker-Neto, L., Paiva, A. L. S., Machado, R. D., Arenhart, R. A. and Margis-Pinheiro, M. (2017**). Interactions between plant hormones and heavy metals responses. Genet. Mol. Biol., **40:** 373 – 386.
68. **Leszczyszyn, O. I., Imam, H. T. and Blindauer, C. A. (2013).** Diversity and distribution of plant metallothioneins: a review of structure, properties and functions. Metallomics, **5:** 1146 – 1169.
69. **El-Shafey, S. A., Hassan, H. M. and Ahmad, H. F. S. (2018).** Involvement of Triacontanol in Regulation of Seed Germination and Seedling Growth of *Zea mays* L. J. Exp. Biol., **14:** 375 –383.
70. **Chen, X., Yuan, H., Chen, R., Zhu, L., Du, B. and Weng, Q. He. G. (2002).** Isolation and Characterization of Triacontanol-Regulated Genes in Rice (*Oryza sativa* L.): Possible Role of Triacontanol as a Plant Growth Stimulator. Plant Cell Physiol., **43:** 869 – 876.
71. **Rehman, A., Azhar, M. T., Hinze, L., Qayyum, A., Li, H., Peng, Z., Qin, G., Jia, Y., Pan, Z. and He, S. (2021).** Insight into abscisic acid perception and signaling to increase plant tolerance to abiotic stress. J. Plant Interact., **16:** 222 – 237.