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Histological and ionomics assessment to elucidate tolerance mechanisms of nickel-tolerant and sensitive cultivars of bread wheat (*Triticum aestivum* L.)☆

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ABSTRACT

Recent literature has raised concerns over crop safety due to nickel (Ni) contamination of irrigation water and agricultural soil in Pakistan. Consequently, wheat crop has suffered in terms of nutrient disbalance and yield reduction. Therefore, it is important to screen tolerant wheat cultivars against Ni toxicity with the ability to accumulate low concentration in grains. In this regard, two wheat cultivars (SKD-1 and Borlaug-16) were exposed to Ni stress (100 mg/L) in a pot experiment for 21 days. In the present study, trace elements (As, Cr, Cu, Cd, Mn, Hg, Ni, Pb, and Zn) and mineral nutrients (Ca, Fe, K, Mg, Na, and P) were tested by ICP-OES. To understand tolerance mechanisms, wheat tissues were tested for morphological parameters, anatomical alteration, oxidative stress, and antioxidant capacity. The translocation of Ni was higher in SKD-1 (0.62) compared to Borlaug-16 (0.45) due to low Ni accumulation in roots of Borlaug-16. In the roots of Borlaug-16, trace elements (Cr, Cu, Mn, Pb, and Zn) were comparatively higher than SKD-1. On the contrary, nutrients (Ca, Fe, Mg, Na, and P) were higher in leaves of SKD-1. Under Ni stress, the root anatomy of Borlaug-16 exhibited a marked increase in the cellular thickness of the cortex by 130.38% and stele by 46.2%. Conversely, the bundle sheath cell thickness rose by 68.36% in the SKD-1. In terms of leaf anatomy, Borlaug-16 showed enhanced thickness in the xylem by 13.01% and bundle sheath cells by 84.95%. Meanwhile, the upper epidermis and phloem of SKD-1 registered thickness increases of 21.80% and 24.47% under Ni stress. Oxidative stress (MDA and H_2O_2) was comparatively highly induced in SKD-1 tissues compared to Borlaug-16. On the other hand, antioxidants (SOD, CAT, and APX) were comparatively higher in root and leaf tissues of Borlaug-16. The lower Ni uptake, less cellular damage, and high antioxidant capacity of Borlaug-16 makes it more tolerant to Ni stress. Such findings will pave the way towards sustainable crop production.

1. Introduction

The increasing concentration of nickel (Ni) in agricultural soils is causing a threat to the sustainable crop production (Kayode et al., 2022). The alarming level of Ni in the soil arises from mining, smelting, automobile emissions, domestic, municipal, industrial waste disposal, and several other reasons (Salt et al., 2020). Ni toxicity can cause non-carcinogenic and carcinogenic risks such as asthma, contact dermatitis, respiratory tract cancer and cardiovascular diseases (Mohammadi et al., 2019). Soil samples showed Ni concentration of 324 mg/Kg in Lahore due to industrial pollution which was significantly higher than the permissible range (30–75 mg/Kg) (Waseem et al., 2014), The excessive accumulation of Ni from soil in crops results into different physiological modifications and many toxicity symptoms like necrosis and chlorosis (Ansari et al., 2015; Chen et al., 2017). Wheat (*Triticum aestivum* L.) is one of the most important staple crops, with an annual production of 770.88 million metric tons worldwide in 2021 (FAO, 2021). The exposure of excessive Ni in wheat results into the reduction

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of seed germination, biomass accumulation, yield reduction, root length, and shoot length (Hassan et al., 2019). Besides, Ni toxicity also has adverse effects on the cellular anatomy of plants. Ni is known to stop cell division in roots, root branching and eventually inhibit transport of nutrient across tissues (Ribeiro et al., 2020). The toxicity of Ni, not only inhibit the nutrient uptake but also hinders the translocation of these nutrients from roots to the upper parts of the plants (Pandey and Sharma, 2002). Amjad et al., (2019) reported the negative impact of Ni on the nutrient acquisition of maize (*Zea mays* L.) hybrids. Therefore, it is crucial to find a sustainable solution for reducing Ni contamination in crops, specifically wheat. Thus, the identification and use of Ni-tolerant cultivars can be a very suitable approach in tackling Ni toxicity, as previous studies have demonstrated promising results (Ge et al., 2020).

Plants tackle environmental stresses by undergoing various physiological, biochemical, and morphological modifications (Raza et al., 2019). These stress-resilient modifications are mainly dependent on the mineral availability and accumulation. In recent studies, essential and non-essential ions have been identified having a significant role in studying the stress physiology of plants (Muszyńska and Labudda, 2019). Therefore, ionomics can play a vital role in understanding the crosstalk of minerals, their transportation, and their role in the stress tolerance of plants. The presence of Ni in wheat induces oxidative stress through the production of reactive oxygen species (ROS). Recent studies have reported the increasing amount of ROS in wheat under the excessive exposure of Ni (Gajewska and Skłodowska, 2007; Gajewska et al., 2006). Plants have developed a complex antioxidant defence mechanism, which consists of enzymatic and non-enzymatic antioxidants, to counter the damaging effects of ROS overproduction (Gajewska and Skłodowska, 2007). Among enzymatic antioxidants, superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione peroxidase (GPX), and ascorbate peroxidase (APX) play most important role in ROS detoxification. Whereas, carotenoids, ascorbic acid (AsA), glutathione, and phenolics are the most important non-enzymatic antioxidants involved in the detoxification of ROS (Mitra et al., 2018). Zaid et al., (2019) and Abd_Allah et al. (2019) both reported the upregulation of the AsA-GSH cycle under Ni stress in mustard plants. In Pakistan, the use of wastewater for irrigation, particularly in peri-urban areas, has led to the accumulation of metals such as Ni in wheat, posing potential health risks to both children and adults (Nawaz et al., 2021).

The concentration of Ni was reportedly above the permissible levels of metal toxicity set by World Health Organization (WHO) in ground and surface water (Khalid et al., 2020; Wahid et al., 2021). The concentration of Ni ranged from 1.62 to 1.82 mg/kg in the soils of multiple regions in Peshawar (Wahid, 2021). Similarly, Ni concentration ranged from 0.57 μg/g in soils and 0.14 μg/g in water of Multan (Afzal et al., 2023), 0.130 mg/kg in soils of Lahore (Ghouri et al., 2022), and 0.0 ± 1.05 mg/L with an average value of 0.09 in groundwater of vihari (Khalid et al., 2020). (Afzal et al., 2023) also reported 0.19 μg/g concentration of Ni in plants of multan. The concentration of Ni reported in different rice varieties of Pakistan showed an average of 139 μg/kg (Wasim et al., 2019). Similarly, Iqbal Khan et al., (2023) reported variable ranges of Ni toxicity in various wheat varieties of Pakistan, i.e., 1.35–2.45 mg and 1.09–2.10 mg/kg. Although previous studies have explored the impact of Ni on wheat, there has been no comprehensive investigation comparing the morpho-physiological, anatomical, and ionomic adaptations of wheat cultivars to discern their tolerance or sensitivity to Ni stress in Pakistan. Therefore, current study aimed to investigate the ionomic variations and oxidative burst caused by Ni toxicity in wheat along with the physiological, anatomical, and antioxidant regulatory response of wheat towards Ni toxicity. The study also aimed to explore the wheat cultivars that are comparatively tolerant to Ni stress. The current study hypothesizes that exposure of Ni can adversely affect the cellular anatomy, nutrient homeostasis, antioxidant capacity, and eventually the overall physiology of wheat. The hypothesis also suggests that tolerant wheat cultivars can mitigate the effects of Ni toxicity by regulating the AsA-GSH cycle and translocating less Ni compared to the sensitive cultivar.

2. Materials and methods

2.1. Experimental design and physiological analysis

Two cultivars of wheat (SKD-1 and Borlaug-16) were selected from the historical bread wheat panel of Pakistan. This selection was carried out on the basis of tolerance and sensitivity against cadmium stress in our previous studies (Maqsood et al., 2023; Safdar et al., 2020). The germplasm of these cultivars were obtained from Crop Science Institute (CSI), National Agricultural Research Centre (NARC), Pakistan. Healthy germplasms of uniform size were first germinated in petri dishes after properly surface sterilizing with 10% hydrogen peroxide (H_2O_2). After germination, seeds were then transferred to the pots, which were arranged in a completely randomized design (CRD) with three replicates for each treatment. Pots of both treated and control conditions were filled with autoclaved soil and peatmoss in an equal ratio. The experiment was laid out in a growth chamber (Percival, Model: 1-35LLVL), with growth conditions 25 ± 2 °C temperature, 50–60% humidity and a day/night cycle of 12/12 h. A fresh solution of 100mg/L Ni was prepared using nickel chloride (Sigma Aldrich) in distilled water (dH_2O) and applied to the 7 days old seedlings of treated group. The concentration applied was kept higher than the permissible limit 5.3 μg/L set by Crommentuijn et al. (1997). Soil plant analysis development (SPAD) values for chlorophyll analysis were done on the 7th, 14th, and 21st day of stress after Ni treatments in both cultivars, whereas various phenotypic traits were also recorded. These phenotypic traits included plant height (PH), root length (RL), shoot length (SL), leaf area (LA), fresh weight (FW), dry weight (DW), turgid weight (TW), and relative water content (RWC). RWC (Fariñas et al., 2019) and LA (Yoshida, 1976) were evaluated using following equations.

$$RWC\% = \frac{FW - DW}{TW - DW} \times 100 \quad (1)$$

$$\mathrm{LA} = Ll \times Lw \times 0.725 \quad (2)$$

In above Eq. (2), Ll = Leaf length and Lw=Leaf width.

Furthermore, root analysis software "Rhizo-Vision Explorer" was used to analyze various root morphological traits including root length (RL), network area of roots (NAR), no. of branch points (NBP), and no. of root tips (NRT).

2.2. Anatomical studies

To study the impact of Ni toxicity on cellular thickness of leaf and root tissues, anatomical studies were performed using standard histological method of Sass, (1958) for both cultivars. A 10μm thick section was sliced using Microm HM 350SV microtome and stained using 0.05% Toluidine blue O. A light microscope (IRMECO microscope) was used for the quantitative measurement of cellular thickness in leaf and root tissues for both cultivars. A Tucsen digital camera (Model ISH1000) was then used to photograph the root at 40X and leaf tissues at 10X magnification. TCapture software was used to perform the quantitative measurements of cellular thickness in μm square.

2.3. Ionomics and metal analysis

Acid digestion method was used to determine the accumulation of mineral nutrients and trace elements (Allen, 1986). Quantitative analysis for various mineral nutrients including calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), phosphorous (P), sodium (Na), and zinc (Zn), along with trace elements such as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), manganese (Mn), and Ni was performed using inductively coupled plasma optical emission (ICP-OES) spectrometer (Thermo scientific iCAP 6000 series). For

quality control (QC), Certified reference solution of multi-element plasma standard solution (Specpure Alfa Aesar) was used. Calibration curves for As, Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, Pb and Zn were prepared using mercury plasma standard solution (Specpure Alfa Aesar), and semi metals plasma standard solution (Specpure Alfa Aesar). P stock solutions were prepared in deionized water by dissolving stoichiometric amounts of sodium dihydrogen phosphate ($NaH_2PO_4.H_2O$; Sigma Aldrich). To overcome the spectral interferences, emission lines were used to determine mineral nutrients and trace elements. The respective emission wavelengths and limit of detection (LOD) used for the trace elements and minerals determination are given in (Table S1).

For the calculation of translocation factor (TF), Marchiol et al., (2004) equation was followed.

$$TF = \frac{Conc.(Leaf)}{Conc.(root)} \quad (3)$$

Where, Conc. (Leaf)= Concentration of elements in leaf, and Conc. (Root)= Concentration of elements in roots

2.4. Oxidative stress markers

For the determination of hydrogen peroxide (H_2O_2) content in the leaves and roots of both cultivars, Velikova et al. (2000) method was followed. In this method, 0.1% trichloroacetic acid (TCA) was used to homogenize fresh tissues of roots and leaves. After centrifugation, 1M potassium iodide and 10mM potassium phosphate buffer (pH 7) were added to the supernatant. The optical density (O.D) was recorded at 390 nm and H_2O_2 amount was expressed as μmol g^{-1} FW.

Heath and Packer (1968) colorimetric method was followed for the measurement of malondialdehyde (MDA) content. Fresh tissues of roots and leaves were homogenized in TCA and centrifuged. The supernatant was mixed with a reaction mixture containing thiobarbituric acid (TBA) and 20% TCA and then heated at 95 °C (30 min). This mixture was then recentrifuged, cooled and analyzed at 600 nm wavelength for MDA concentration. The concentration was expressed as mmol/g of FW.

2.5. SOD, POD, and CAT assay

The enzymatic antioxidants (SOD, POD, and CAT) were determined using Beauchamp and Fridovich (1971), Lundquist and Josefsson (1971), and Aebi (1984) methods respectively. The activity of SOD enzyme (EC 1.15.11.1) was recorded at 560nm wavelength. POD enzyme (EC 1.11.1.7) activity was recorded at 420nm, and CAT enzyme (EC 1.11.1.6) activity was recorded at 240nm O.D by spectrophotometer model WFX-210 (Beijing Beifen-Ruili Analytical Instrument 10 212 Co., Ltd. China). Activities of all three enzymes were expressed as Unit g^{-1} FW.

2.6. Ascorbate-glutathione (AsA-GSH) cycle

AsA content was determined using Fadhel (2012) method. AsA content was recorded at an O.D of 350nm, and the concentrations were expressed as mg/g. APX enzyme activity (EC 1.11.1.11) was recorded using Nakano and Asada (1981) method. APX activity was recorded at 290nm (30 seconds) and enzyme activity was expressed as units of enzyme activity per minute per gram fresh weight (U/min/g FW). For the analysis of glutathione content, oxidized glutathione (GSSG), reduced glutathione (GSH), total glutathione (GSSG+GSH), and GPX (EC 1.11.1.9) was determined. Anderson (1985) method was followed for the analysis of glutathione content and O.D was recorded at 412nm. GSH and GSSG values were given in standard curves. Glutathione peroxidase (GPX) assay was performed by following the Nagalakshmi and Prasad (2001) method. The absorbance was measured at 340nm for 1 min at 30° C. The extinction coefficient was ε= 9.6 mM^{-1} cm^{-1}.

2.7. Proline, carotenoids, phenolics, and total antioxidant capacity assay

For the determination of proline content, Parveen and Siddiqui, (2021) method was followed with slight modifications. Fresh root and leaf tissues were homogenized in a solution of 3% sulphosalicylic acid and left overnight at 5 °C. It was then centrifuged at 3000 rpm (5 min) and the supernatant was then diluted with acidic ninhydrin in test tubes. The solution was then heated at 100°C for 1 hour in water bath. The mixture was then cooled and extracted using toluene and recorded at an O.D of 520nm against toluene as blank. Different concentrations of L-proline including 0, 5, 10, 15, 20, 25, and 30 μg were used to devise a standard curve.

For the determination of carotenoid content, Jensen (1978) method was followed. Fresh tissues were homogenized in acetone and centrifuged for 10 min at 2500rpm. The supernatant obtained was analyzed for carotenoid content at an O.D of 480nm. Following equation was used to determine the final concentration of carotenoid content in leaf and root tissues:

$$Total\ Carotenoids = \frac{(7.6{*}OD\ at\ 480nm) - (1.49{*}OD\ at\ 510nm){*}V}{1000{*}W(g)} \quad (4)$$

Phenolics content was determined by Bray and Thorpe (1954) Folin–Ciocâlteu reagent (FCR) method. The alcoholic extract was prepared and mixed with FCR reagent and dH_2O. 20% sodium carbonate (Na_2Co_3) was then added to the mixture after 3 min and was kept in boiling water-bath for 1 min. The mixture was then cooled at room temperature and absorbance was recorded at an O.D of 650nm.

For the analysis of total antioxidant capacity (TAC), phosphomolybdenum method of Prieto et al. (1999) was followed. AsA was used as a standard and plant tissues were mixed with a reagent containing 4 mM ammonium molybdate, 0.6 M sulfuric acid, and 28 mM sodium phosphate. This was then incubated in water-bath for 90 min at 95 °C. The absorbance was then recorded at 695nm against blank after cooling the solution. The formula used for measuring the TAC concentration is:

$$\text{Totalantioxidantcapacity}(\%) = \text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}} / \text{Abs}_{\text{control}} \text{x} 100 \quad (5)$$

2.8. Statistical analysis

XLStat 2021 was used to analyze the analysis of variance (ANOVA), descriptive statistics, and post-hoc test student–newman–keuls (SNK) for least significance difference (LSD). The graphical construction of heatmaps, Q.Q-scatterplots, and principal component analysis (PCA) was done by RStudio (v 4.0.3). Bivariate correlation (Pearson) analysis between variables was carried out using IBM SPSS (ver. 25). Ionomics network was constructed using Cytoscape (v.3.9.1). Hierarchical clustering analysis (HCA) was performed using MetaboAnalyst 3.0 (web interface) bioinformatic tool.

3. Results

3.1. Evaluation of phenotypic traits

The exposure of Ni stress caused a reduction in PH, and FW of both cultivars, where SKD-1 showed more reduction compared to Borlaug-16 (Table 1). In the case of SL, DW, and RWC, Borlaug-16 showed significant reduction under Ni-stressed conditions. SKD-1, on the other hand, showed an increasing amount of SL, DW, and RWC under Ni toxicity. The chlorophyll activity showed an increasing amount in the leaves of Borlaug-16 cultivar under Ni stress. Similarly, a slight increase in chlorophyll content of Borlaug-16 leaves was also observed (Table 1). SKD-1 cultivar showed a better phenotypic response to Ni stress compared to Borlaug-16. On the contrary, the roots of Borlaug-16 showed better response to Ni stress conditions. In case of root morphology, RL reduced in both cultivars under Ni stress, but SKD-1 showed significantly more reduction compared to Borlaug-16, whereas

Table 1
ANOVA-Table of the phenotypic, root morphological, root and leaf cellular anatomical traits under Ni exposure.

S. No	Variables	Borlaug-16		P*Cv	SKD-1		P*Cv	P*T	% Diff Borlaug-16	% Diff SKD-1
		Control (Mean±SD)	Ni (Mean±SD)		Control (Mean±SD)	Ni (Mean±SD)				
Phenotypic Traits										
1	Chl7 (SPAD Count)	36.8±1.18	41.5±1.31	ns	40.47±1.26	38.33±0.98	**	ns	12.75	-5.29
2	Chl14 (SPAD Count)	32.17±1.19	34.15±1.22	ns	37.55±1.27	35.08±1.31	ns	ns	6.17	-6.57
3	Chl21 (SPAD Count)	31.11±1.15	31.61±1.23	ns	33.41±1.02	32.84±1.23	ns	ns	1.58	-1.69
4	SL (cm)	33.54±1.02	31.76±1.22	ns	32.56±1.4	36.6±1.22	ns	ns	-5.31	12.41
5	LA (cm)	8.65±1.22	7.56±1.22	ns	9.62±1.63	11.61±1.22	*	ns	-2.64	23.58
6	PH (cm^2)	81.92±3.67	69.32±2.86	*	98.96±4.49	72.18±2.86	ns	**	-15.39	-27.07
7	FW (mg)	533.72±3.12	498.58±2.45	*	746.1±3.14	664.92±2.24	ns	ns	-6.58	-10.88
8	DW (mg)	56.92±0.88	12.4±0.98	*	49.88±0.95	69.75±0.78	***	ns	-78.26	39.79
9	TW (mg)	466.44±0.86	705.56±1.63	*	821.16±2.32	421.58±1.63	***	ns	51.26	-48.66
10	RWC (%)	126.25±1.06	67.6±1.02	ns	94.34±0.95	170.09±1.42	**	ns	-44.14	80.81
Root Morphological Traits										
11	NAR (cm^2)	59.28±3.67	60.92±2.86	ns	103.83±3.27	54.69±3.27	ns	ns	2.76	-47.33
12	RL (cm)	48.38±4.08	37.56±4.49	ns	66.4±4.9	35.58±4.49	ns	*	-22.38	-46.42
13	NRT	14±1.63	7±1.63	ns	20±2.45	11±1.63	ns	*	-50.00	-45.00
14	NBP	22±1.63	22±1.63	ns	27±2.45	24±1.63	ns	ns	0.00	-11.11
Root Cellular Anatomy										
15	Ep (μm^2)	3.074±0.216	1.866±0.349	ns	3.417±0.157	2.311±0.177	ns	***	-39.28	-32.36
16	C (μm^2)	1.034±0.099	2.382±0.585	ns	1.651±0.492	2.03±0.178	ns	*	130.38	22.99
17	BS (μm^2)	0.598±0.423	0.65±0.129	ns	0.594±0.151	0.999±0.092	*	ns	8.64	68.36
18	S (μm^2)	17.609±0	25.746±0	***	19.387±0	22.781±0	**	***	46.21	17.51
Leaf Cellular Anatomy										
19	UEp (μm^2)	3.45±0.52	2.43±0.2	ns	3.71±1	4.52±1.31	*	ns	-29.53	21.80
20	LEp (μm^2)	2.84±0.42	2.78±0.41	ns	4.42±1.84	4.3915±0.01	*	ns	-1.98	-0.59
21	Xy (μm^2)	2.46±0.18	2.78±0.3	*	1.59±0.31	0.61±0.01	**	ns	13.01	-61.17
22	Ph (μm^2)	0.39±0.01	0.3671±0.06	**	0.16±0.02	0.199±0.03	*	ns	-5.88	24.47
23	BS (μm^2)	1.59±0.21	2.36±0.42	*	2.99±0.4	0.726±0.15	**	ns	48.95	-75.66
24	C (μm^2)	2.06±0.24	0.997±0.27	ns	1.8±0.06	1.11±0.42	ns	***	-51.57	-37.94

ns = non-significant,
* = $P < 0.05$,
** = $P < 0.01$,
*** = $P < 0.001$
*Chlorophyll on 7th day of Ni exposure (Chl7), Chlorophyll on 14th day of Ni exposure (Chl14), Chlorophyll on 21st day of Ni exposure (Chl21), Shoot length (SL), Leaf area (LA), Root length (RL), Plant height (PH), Relative water content (RWC), Fresh weight (FW), Dry weight (DW), Turgid weight (TW), Network area of root (NAR), Root length (RL), Number of root tips (NRT), and Number of branch points (NBP), Epidermis (Ep), Cortex (C), Bundle Sheath (BS), Stele (S), Upper epidermis (UEp), Lower epidermis (LEp), Xylem (Xy), Phloem (Ph).

NAR increased in Borlaug-16 and reduced in SKD-1 under Ni stress (Table 1). NRT also reduced significantly in both cultivars, i.e., 50% in Borlaug-16 and 45% in SKD-1 under Ni stress.

3.2. Cellular anatomy

Ni stress showed damaging effects on cellular thickness of roots for both cultivars. The cellular thickness of epidermis (Ep) reduced in both cultivars with comparatively less reduction in SKD-1. Thickness of bundle sheath (BS) increased more in SKD-1 than Borlaug-16 (Fig. 1). On contrarily, Borlaug-16 showed increase in cellular thickness of cortex (C) and stele (S) (Table 1).

In case of leaf anatomy, Ni stress caused significant impact on the cellular thickness in both cultivars. Upper epidermis (UEp) and phloem (Ph) cells significantly reduced in cellular thick with increasing thickness in SKD-1 (Fig. 2). Unlike which, xylem (Xy) and BS cells showed significant increase in cellular thickness with reducing thickness in SKD-1. Cellular thickness of C decreased in both cultivars where Borlaug-16 showed significant damage and reduced more compared to SKD-1 (Table 1).

3.3. Ionomic profiling of trace and mineral elements

The concentration of Ni increased significantly in roots and leaf of both cultivars under Ni stress (Fig. 3A and B). In case of roots, SKD-1 showed lesser uptake of Ni (93.38%) compared to Borlaug-16 (94.81%). Whereas in leaf, SKD-1 showed more uptake of Ni (99.87%) compared to Borlaug-16 (82%) (Fig. 3B). Besides Ni, other trace elements and mineral nutrients also showed significant differences under Ni stress. The concentrations of As, Cd, Cr, Cu, and Pb increased in the roots of both cultivars under Ni stress. Borlaug-16 showed higher uptake of As, Cr, Cu, and Pb compared to SKD-1. The concentration of Mn reduced by 10.93% in SKD-1 roots and increased by 2.10% in Borlaug-16 roots. Zn concentration on the other hand, reduced by 2.25% in the roots of Borlaug-16 but 20.71% in the roots of SKD-1 under Ni stress. For leaf, the concentrations of As, Cd, Cr, Cu, and Pb increased for both cultivars under Ni stress. Unlike roots, the concentration of Mn decreased in Borlaug-16 and increased in SKD-1 under Ni stress. Zn concentrations also reduced in leaves of both cultivars, where Borlaug-16 showed more decrease (71.05%) compared to SKD-1 (55.81%). Furthermore, the concentrations of mineral elements including Ca, Fe, K, Mg, Na, and P decreased significantly in roots and leaves of both cultivars. The roots of SKD-1 showed more reduction in Ca, Fe, and Mg compared to Borlaug-16 roots (Fig. 3C). Whereas in leaf, SKD-1 showed more reduction in K and P compared to Borlaug-16 leaf under Ni stress (Fig. 3D).

The translocation of trace elements from roots to leaf varied significantly in both cultivar under Ni stress. Borlaug-16 showed an increase in the TF of As, Cd, Cr, and Cu under Ni stress compared to control (Fig. 3E). Whereas in SKD-1, Ni stress caused an increase in the TF of As, Cd, Cr, Cu, Mn, Ni, and Pb. SKD-1 showed higher TF in case of Cr, Cu, Mn, Ni, Pb, and Zn under Ni stress compared to Borlaug-16. For mineral nutrients, the Ni stress caused a decrease in the concentration of Fe and Mg in Borlaug-16 and the concentration of all mineral elements increased in SKD-1 under Ni stress. Overall, SKD-1 showed more TF of mineral elements including Ca, Fe, K, Mg, and P compared to Borlaug-16 (Fig. 3F).

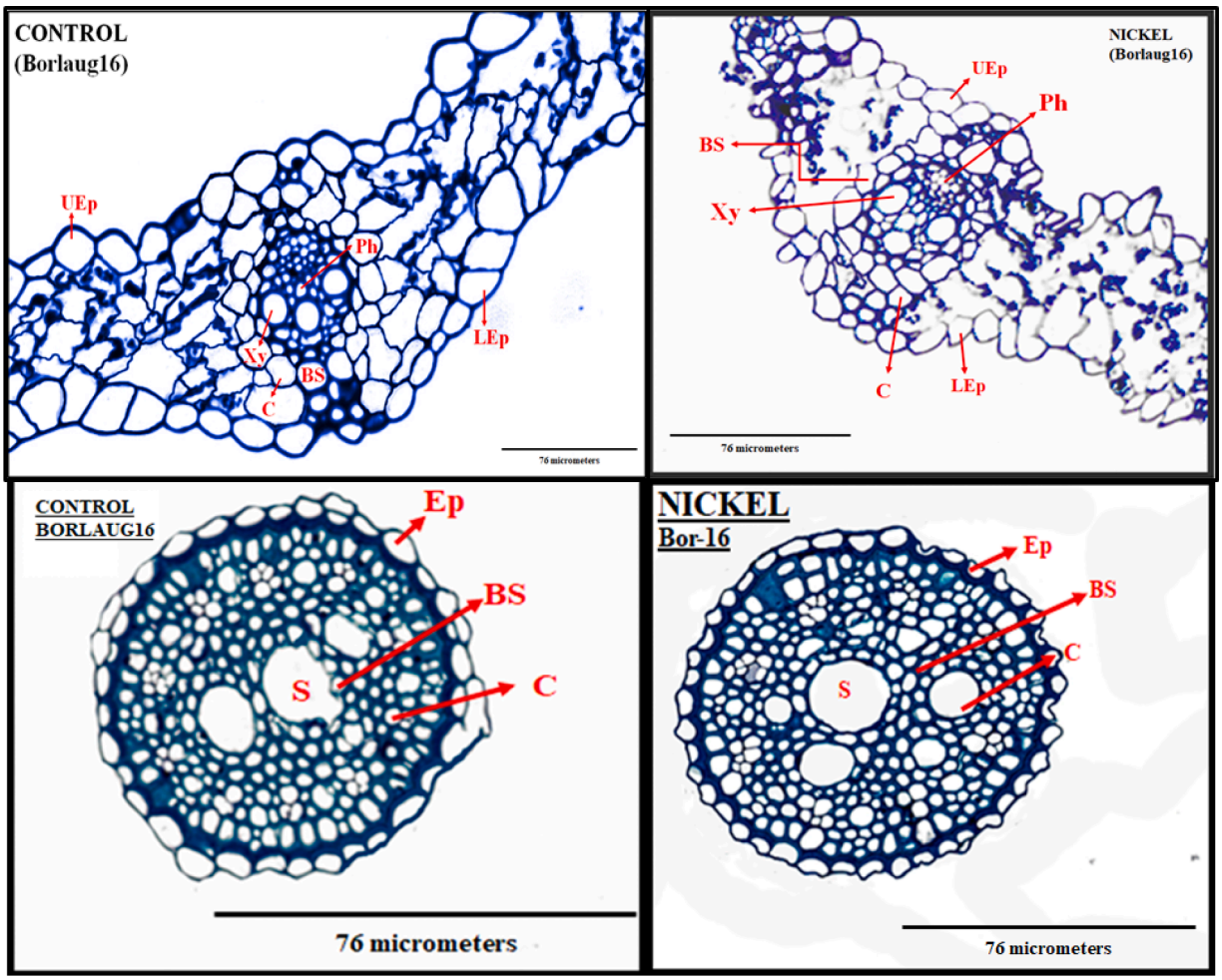

Fig. 1. Impact of Ni on root and leaf anatomical characteristics of Borlaug16 cultivar at 40X. The root cellular thickness was recorded from cells; Ep: epidermis, C: cortex, BS: bundle sheath, and S: stele. The leaf cellular thickness was recorded from cells; UEp: upper epidermis, LEp: lower epidermis, C: cortex, BS: bundle sheath, Xy: Xylem, and Ph: Phloem thickness was recorded.

3.4. Ionomic network of trace and mineral elements

The association between elements in the leaves and roots of both cultivars was presented using ionomic networks (Fig. 4A & B). The positive interactions in ionomic network are shown by the solid lines, whereas negative interactions are shown by the dotted lines. The $P < 0.05$ significance is displayed by the dashed dotted lines whereas dashed lines show a significance of $P < 0.01$. The ionomic network of Nickel is highlighted with red lines, whereas interactions involving all other elements are marked in blue. Correlation analysis (Pearson) was performed for the verification of interactions in leaf and root ionomes of both cultivars. The interaction of trace elements and mineral nutrients was visualized using hierarchical clustering analysis (HCA) (Fig. 5). HCA showed a positive association of Ni with Cd, Cr ($p<0.05$), Cu ($p<0.01$), Mn, and Pb ($p<0.01$) in Borlaug-16. Whereas a negative association of Ni was observed with Ca, Fe, K, Mg ($p<0.01$), Na, P, and Zn. A positive association of Cu was found with Cr and Pb. Fe showed positive interaction with Ca and Mg. K was also associated positively with Mg and Na. Moreover, Zn and Mn both showed a positive association. Zn and Fe were also associated positively with Mn as well. In SKD-1, a positive association was recorded between Ni and Cd, Cr, Cu, and Pb. Whereas Ca, Fe, K, Mg ($p<0.01$), Na, P, Mn, and Zn showed positive association with Ni. A highly significantly positive ($p<p<0.01$) association of Ca was observed with Fe and Na. Similarly, Cd was also significantly positive ($p<0.01$) associated with Cu and Pb. and K was associated with P at $p<0.01$. Zn was also found in positive association with Ca, Fe, and K while in a negative association with Cd, Cu, Mn, Ni, and Pb. No presence of Hg was reported in both cultivars.

3.5. Oxidative Stress Markers

An increase in the MDA content was observed in the root tissues of both cultivars under Ni stress with higher amount in SKD-1. Whereas in leaf tissues, MDA reduced significantly in both cultivars under Ni stress with more reduction in Borlaug-16 (Table 2). Similarly, H_2O_2 levels also increased in the root tissues of both cultivars with higher levels in SKD-1 under Ni stress (Figure S2). The levels of H_2O_2 increased in leaves of Borlaug-16 but reduced in the leaf tissues of SKD-1 under Ni stress (Table 2).

3.6. SOD, POD, and CAT analysis

SOD content was reported to be reduced in the root and leaf tissues of SKD-1 under Ni stress. In contrast, SOD increased significantly in both the leaf and root tissues for Borlaug-16 (Table 2). POD, on the other hand, increased in the roots but reduced in the leaves of SKD-1. In Borlaug-16, POD is reduced in both root and leaf tissues under Ni stress (Fig. S3). Levels of CAT enzyme reduced in the leaf and root tissues of SKD-1 and increased in Borlaug-16 (Table 2).

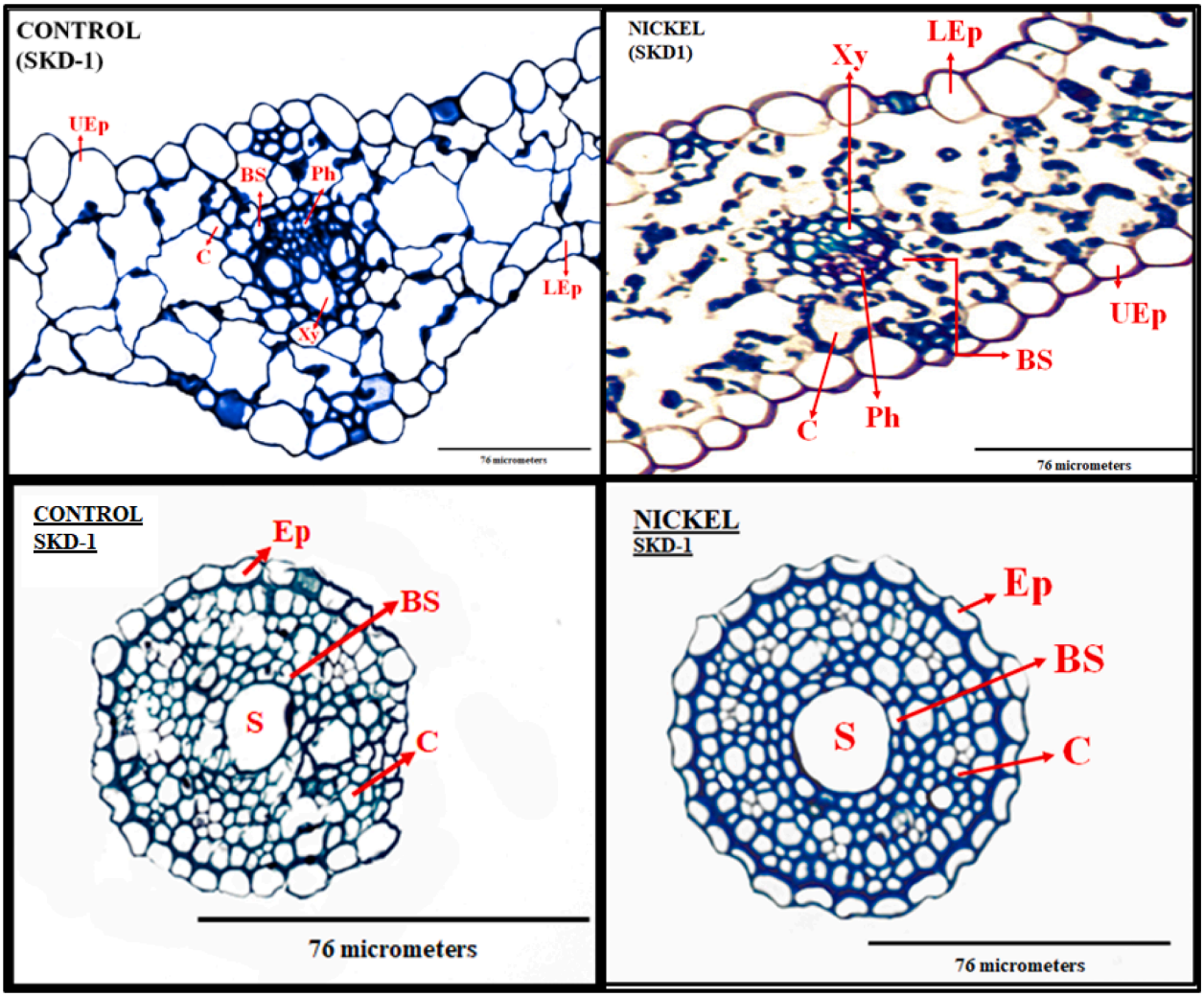

Fig. 2. Impact of Ni on root and leaf anatomical characteristics of SKD-1 cultivar at 40X. The root cellular thickness was recorded from cells; Ep: epidermis, C: cortex, BS: bundle sheath, and S: stele. The leaf cellular thickness was recorded from cells; UEp: upper epidermis, LEp: lower epidermis, C: cortex, BS: bundle sheath, Xy: Xylem, and Ph: Phloem thickness was recorded.

3.7. Ascorbate-glutathione (AsA-GSH) pathway

A reduction in the APX levels was observed in root and leaf tissues for SKD-1 (Fig. S5). On the other hand, APX reduced in roots of Borlaug 16 with a significant increase in leaves under Ni stress. The AsA content increased in the roots but reduced in the leaves of both cultivars under Ni stress, where Borlaug-16 showed more increase in roots and more reduction in leaves compared to SKD-1 (Table 2).

The levels of GPX enzyme showed significant increase in root and leaf tissues of SKD-1 under Ni stress. Whereas in Borlaug-16, GPX reduced in both root and leaf tissues under Ni stress (Table 2). An increase in GSSG content was recorded in the leaves of SKD-1 whereas GSSG reduced in the roots of SKD-1 under Ni stress. GSSG content reduced in leaves of both cultivars under Ni stress. GSH content was found to be decreasing in both the leaves and roots of SKD-1 cultivar under Ni stress. In Borlaug-16, GSH reduced slightly in roots but increased in leaf tissues under Ni tissues. Overall, GSSG+GSH content reduced in both root and leaf tissues for SKD-1. An increase of GSSG+GSH content in the leaf tissues of Borlaug-16 was also observed under Ni stress (Table 2).

3.8. Proline, carotenoids, phenolics, and antioxidant capacity analysis

Both cultivars showed increasing content of proline in root tissues under Ni stress with more increase in Borlaug-16. Proline content decreased in the leaves of both cultivars under Ni stress. The content of phenolics also increased in leaves and reduced in the roots of both cultivars under Ni stress (Table 2). The carotenoid content was also reported to be increasing in leaves of both cultivars with a decrease in the roots under Ni stress. Borlaug-16 showed significantly higher carotenoid content in leaves compared to SKD-1 under Ni stress (Fig. S6). TAC levels were reported to be increasing in leaves of SKD-1 and roots of Borlaug-16 under Ni stress (Table 2).

3.9. Principle component analysis (PCA)

PC analysis showed different variations between both parts (Leaf and Root) of both Wheat cultivars, PC1 explained 52.2% of variation and PC2 explained 15.3% of variation Antioxidants that were present in same axes were termed to be positively correlated with each other whereas those in different axes were negatively correlated with each other. Oxidative stress markers that showed a positive relation with TG, TPC, GSH, TCC, SOD, APX, and GPX. On the contrary, the negative correlation was present with GSSG, H_2O_2, Ascorbic acid, POD, Proline, and MDA. GPx and CAT lied in same axes thus in positive correlation with each other whereas TAC was found in a different axis and in negative correlation with all other antioxidants (Fig. 6).

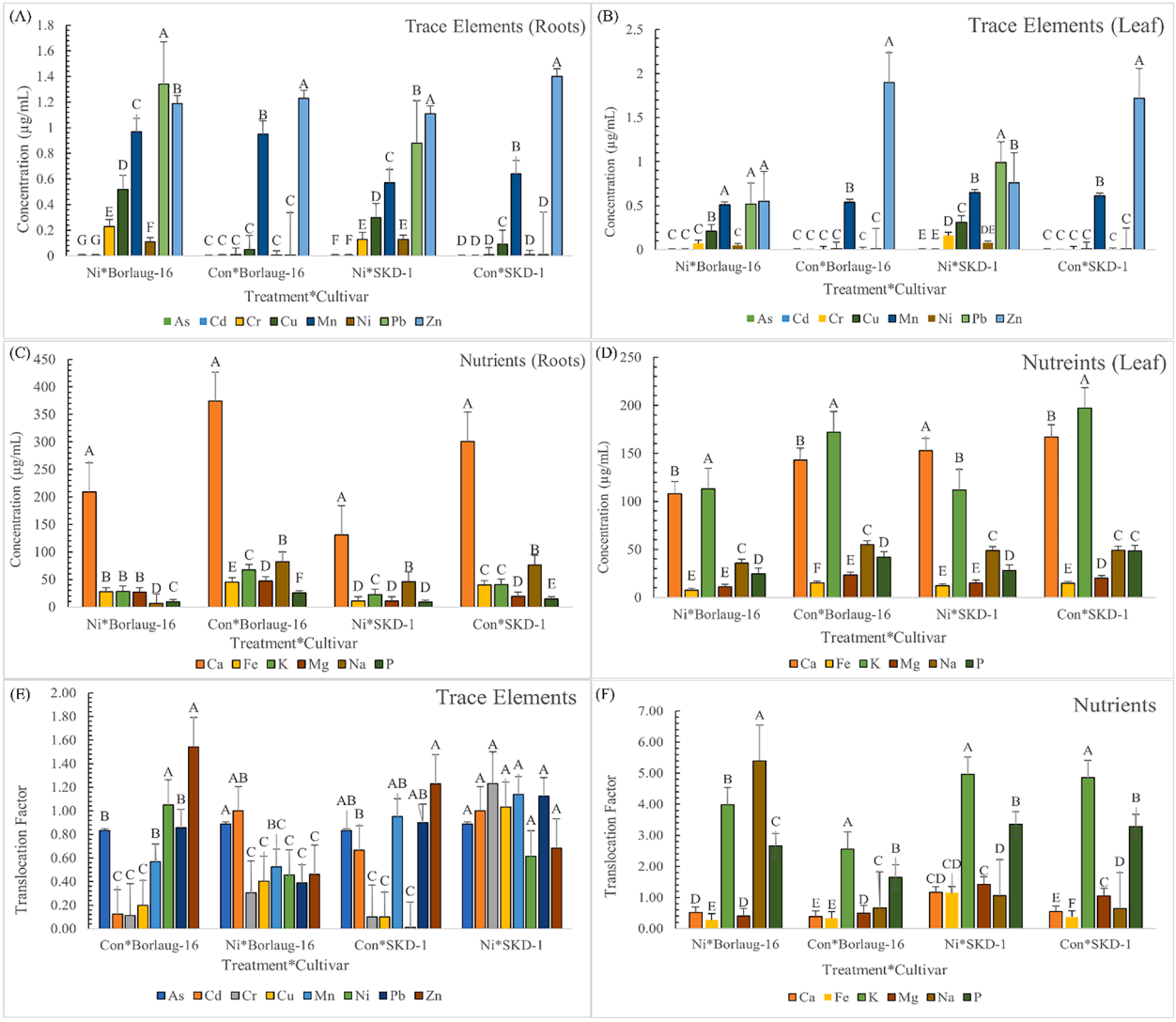

Fig. 3. Trace elements uptake in root (A), leaf (B), Nutrients in root (C), leaf (D), and translocation of trace elements (E), and minerals (F) in both cultivars. As: arsenic, Cd: cadmium, Cr: chromium, Ni: nickel, Pb: lead, Mn: manganese, Zn: zinc, Fe: iron, Ca: calcium, K: potassium, Mg: magnesium, Na: sodium and P: phosphorous.

4. Discussion

The current study demonstrates the impact of elevated nickel concentrations on the ionomic profile, anatomical alteration, oxidative stress, and antioxidant response in the root and leaf tissues of two wheat cultivars, i.e., SKD-1 and Borlaug-16, to understand their tolerance against Ni stress. The concentration of Ni was found to be lower in SKD-1 compared to Borlaug-16 under Ni stress conditions (Fig. 3). Previously, Gajewska et al. (2006) reported higher accumulation of Ni in the shoots of wheat under Ni stress. One of the most useful methods to study the relationship between elements in plants is correlation analysis (Feng et al., 2017). Current study observed many significant correlations between trace elements and minerals in leaf as well as root tissues under Ni stress, providing important ionomic information on the interaction of elements (Fig. 5). The accumulation of Ni showed a positive correlation with Cd, Cu, Cr, and Pb in SKD-1 and negative correlation with Mg**, Mn, Na, and P. Whereas in Borlaug-16 cultivar, Ni accumulation showed a significantly positive correlation with Cr*, and Cu** and a negative correlation with P and Zn. Previously, Hamnér et al. (2013) also reported the positive correlation of Ni with metal concentrations in cereals. The ionomic network variations under stress conditions are controlled primarily by the plant's genome (Jeyasingh et al., 2017). Current study also showed a significantly positive correlation of Cd with Cr*, Cu**, and Pb** in SKD-1 and with Cu, Fe, and Mn in Borlaug-16, Similarly, P showed a positive correlation with K**, Mg, and Mn in SKD-1 and with K, and Na in Borlaug-16 and a negative correlation with Fe in both cultivars (Fig. S1). On contrary, Du et al. (2020) reported a positive relation between P and Fe in cereal grains under as stress. Wang et al. (2019) reported that the deficiency of P is mediated by Fe homeostasis in *Arabidopsis thaliana*. So the negative correlation between K and Fe is because the deficiency of P causes an increase in the availability of Fe (Chutia et al., 2019).

Current study also reported that the concentration of As, Cr, Cu, and Pb uptake increased significantly in the roots of Borlaug-16 compared to SKD-1. Athar and Ahmad (2002) previously reported higher accumulation of metals including Pb, Cu, Cr, Ni, and Cd after the application of metals stress in wheat. In present study, the concentration of mineral elements including Ca, Fe, K, Mg, Na, and P decreased significantly in roots and leaves of both cultivars, where SKD-1 showed more reduction in Ca, Fe, and Mg compared to Borlaug-16 in roots and SKD-1 showed more reduction in K and P compared to Borlaug-16 in leaf (Fig. 3). Amjad et al. (2019) reported a significant decrease in the concentration of mineral nutrients under Ni stress in maize cultivars. Present study also showed that SKD-1 showed higher TF in case of Cr, Cu, Mn, Ni, Pb, and

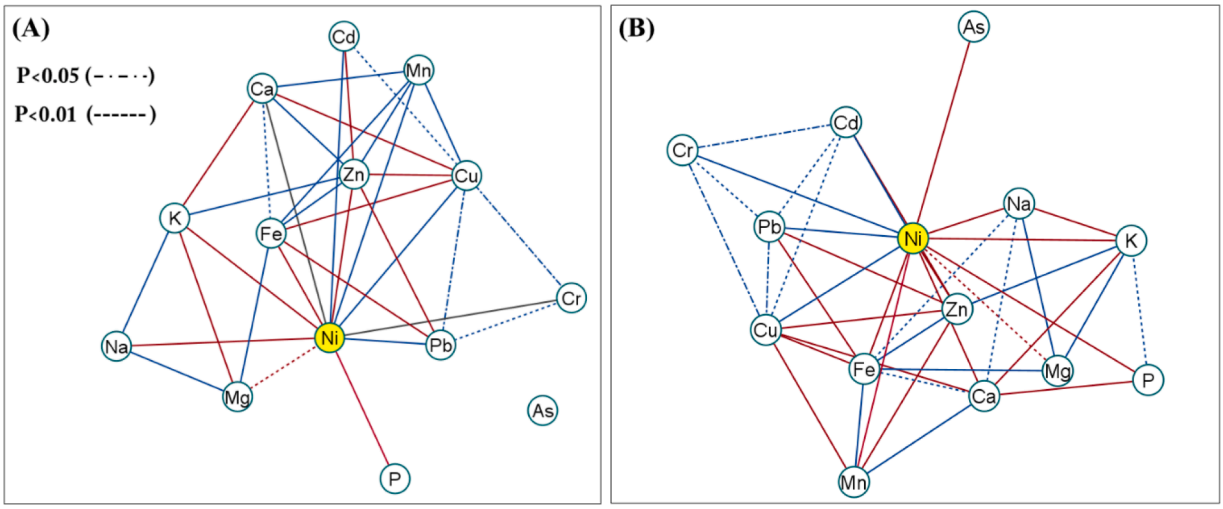

Fig. 4. Ionomic Network Interactions in Borlaug-16 (A) and SKD-1 (B) Cultivars. The ionomic correlations are based on the Pearson coefficient. Positive interactions (correlations) are depicted by solid lines, while negative interactions are denoted by dotted lines. The significance of these interactions is indicated as follows: $P < 0.05$ (dashed-dotted line: -.-.-) and $P < 0.01$ (dashed line: ——). The ionomic network of Nickel is highlighted with red lines, whereas interactions involving all other elements are marked in blue. Ca: Calcium, Cd: Cadmium, Cr: Chromium, Cu: Copper, Fe: Iron, K: Potassium, Mg: Magnesium, Mn: Manganese, Na: Sodium, Ni: Nickel, P: Potassium, Pb: Iron, and Zn: Zinc. Positive interactions: Solid lines, Negative interactions: Dotted lines, Significance levels: $P < 0.05$ (dashed-dotted line: -.-.-), $P < 0.01$ (dashed line: ——), Nickel interactions: Red lines, and All other elements: Blue lines.

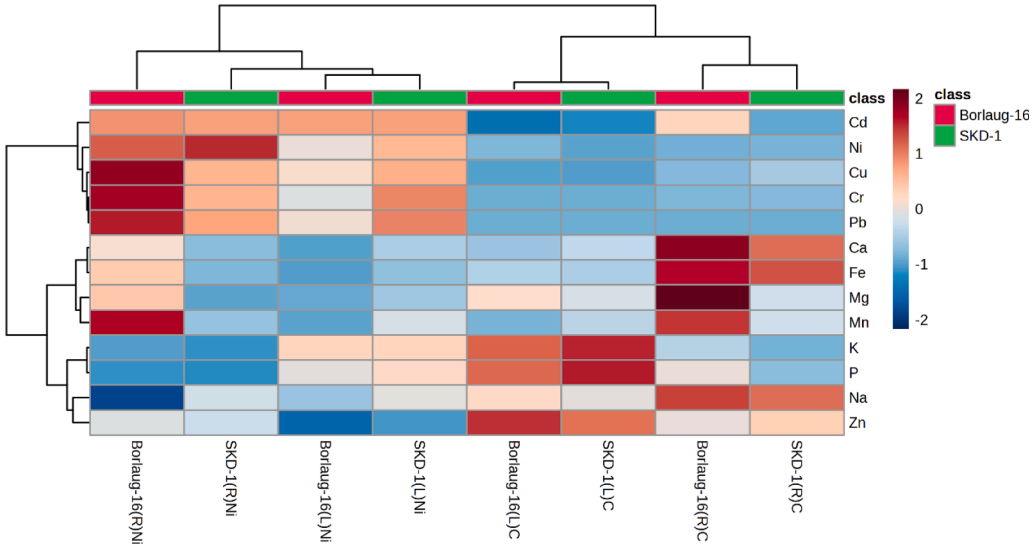

Fig. 5. Hierarchical Clustering Analysis (HCA) of Ionomes in Leaf and Root Tissues of SKD-1 and Borlaug-16 Cultivars. The heatmap represents the ionomic profiles of SKD-1 and Borlaug-16 cultivars in both leaf and root tissues. The color-coded HCA was performed using the Ward method and Euclidean distance metric. Borlaug-16 is represented in red, while SKD-1 is shown in green. The ionomic variations are visualized on a color scale ranging from -2 (blue) to +2 (red), indicating the extent of ionome expression differences between the two cultivars.

Zn under Ni stress compared to Borlaug-16 whereas SKD-1 showed more TF of mineral elements including Ca, Fe, K, Mg, and P compared to Borlaug-16 (Fig. 3). In previous studies, Amjad et al. (2019) also reported higher root to shoot nutrient translocation under Ni stress in tolerant maize cultivar. This is because Ni possesses similar characteristics to both micro and macronutrients, thus competing with these minerals in transpiration and sorption activities of plants (Gajewska et al., 2012). Due to this, elevated Ni concentrations hinder nutrient uptake, translocation, and sorption, thus causing nutrient deficiency in plants (Ahmad et al., 2010).

Nickel toxicity is known to have a severe impact on the development and growth of plants (Hussain et al., 2013). In present study, SKD-1 showed more phenotypic damage under Ni stress compared to Borlaug-16. SKD-1 showed a reduction in PH, RL, FW, and TW whereas Borlaug-16 reduced in terms of PH, and FW. Traits including SL, DW, and RWC increased in SKD-1 after Ni stress (Table 1). Previously, Ahmad et al. (2023) reported less reduction in shoot length of tolerant sunflower cultivar under Ni stress. This is because the toxicity of Ni causes an aberrant uptake and translocation of micro and macro nutrients thus affecting the growth and development of plants (Gajewska et al., 2012; Yusuf et al., 2011). In case of root morphology, Borluag-16 showed better response. NAR increased in Borlaug-16, whereas RL was reduced less compared to SKD-1. On the other hand, SKD-1 showed significant reduction in RL and NAR under Ni stress (Table 1). Previous study showed that the induction of Ni stress caused a decline in the roots of sensitive wheat cultivars, whereas tolerant cultivars showed maximum RL under Ni stress (Kumar and Verma, 2018). This is because roots are directly exposed to excess concentration of Ni that can lead to

Table 2
ANOVA-Table of the oxidative stress markers recorded in roots and leaves under Ni exposure.

S. No	Variables	Borlaug-16		P*Cv	SKD-1		P*Cv	P*T	% Diff Borlaug-16	% Diff SKD-1
		Control (Mean ±SD)	Ni (Mean ±SD)		Control (Mean ±SD)	Ni (Mean ±SD)				
Root Antioxidant Content										
1	H_2O_2 (µmol g/FW)	14.63±0.49	17.53±0.82	ns	14.95±0.78	29.63±1.31	***	ns	19.85	98.17
2	MDA (mmol/g FW)	3.23±0.33	4.52±0.41	ns	3.87±0.29	6.45±0.74	ns	ns	40	66.67
3	SOD (Unit g^{-1} FW)	1±0.41	2±0.41	ns	2.17±0.37	1.21±0.33	ns	ns	100.4	-44.38
4	POD (Unit g^{-1} FW)	3.49±0.41	3.02±0.41	*	3.01±0.41	4.18±0.29	**	ns	-13.63	38.66
5	CAT (Unit g^{-1} FW)	0.37±0.01	0.372±0.01	ns	0.3952±0.01	0.38±0.01	ns	ns	0.59	-3.84
6	Asc. Acid (mg/g)	19.47±0.78	22.02±0.82	ns	22.64±0.9	24.42±1.14	***	*	13.1	7.85
7	APX (U/min/g FW)	54.92±0.82	54.72±0.98	ns	55.38±0.41	54.32±0.65	ns	ns	-0.36	-1.91
8	GSH (mmol g^{-1} FW)	25.11±0.9	24.74±0.98	***	95.3±1.02	17±0.82	***	ns	-1.47	-82.16
9	GSSG (mmol g^{-1} FW)	20.39±0.69	20.76±0.61	**	20.02±0.41	18.35±0.69	***	*	1.82	-8.33
10	GSSG+GSH (mmol g^{-1} FW)	45.5±1.22	45.5±1.22	***	115.32±1.06	35.35±1.1	**	***	0	-69.34
11	GPX (µ mg^{-1} protein)	104.34±1.06	101.61±1.31	***	102.09±0.9	104.02±0.82	***	ns	-2.62	1.89
12	Proline (µg g^{-1} FW)	8.06±0.45	9.29±0.49	ns	8.23±0.57	9.11±0.49	ns	ns	15.22	10.64
13	Carotenoids (mg/g)	11.84±1.02	10.44±0.74	***	16.59±0.86	13.27±0.61	*	ns	-11.85	-20
14	Phenols (mg/g)	45.51±1.22	27.73±1.39	***	37.27±1.02	16.81±1.47	***	***	-39.06	-54.91
15	TAC (µg/mL)	19.97±0.78	20.49±0.78	ns	19.9±0.73	18.4±0.94	**	ns	2.6	-3.72
Leaf Antioxidant Content										
16	H_2O_2 (µmol g/FW)	24.79±1.02	32.37±1.51	***	33.66±0.94	26.4±1.14	***	ns	30.58	-21.56
17	MDA (mmol/g FW)	8.39±0.78	3.87±0.69	ns	7.1±0.49	3.87±0.69	ns	**	-53.85	-45.45
18	SOD (Unit g^{-1} FW)	1.44±0.45	1.57±0.61	**	2.5±0.49	1.57±0.53	ns	ns	8.78	-37.2
19	POD (Unit g^{-1} FW)	5.79±0.61	5.5±0.82	ns	5.17±0.78	4.02±0.82	***	ns	-5.08	-22.11
20	CAT (Unit g^{-1} FW)	0.34±0.01	0.3411±0.01	ns	0.3421±0.01	0.34±0.01	ns	ns	0.33	-0.62
21	Asc. Acid (mg/g)	60.13±1.71	31.99±1.59	**	67.32±1.06	65.31±1.88	***	**	-46.79	-2.99
22	APX (U/min/g FW)	54.86±0.78	55.54±1.1	ns	55.24±0.61	54.8±1.14	ns	ns	1.24	-0.8
23	GSH (mmol g^{-1} FW)	141.15±2.16	278.7±2.2	***	232.47±2	139.45±2	***	ns	97.45	-40.01
24	GSSG (mmol g^{-1} FW)	56.31±1.47	45.76±2.25	***	91.13±1.55	65.02±1.63	**	***	-18.74	-28.65
25	GSSG+GSH (mmol g^{-1} FW)	197.47±2	324.46±2.82	***	323.6±2.12	204.47±2	***	ns	64.3	-36.81
26	GPX (µ mg^{-1} protein)	102.41±1.47	101.29±1.88	ns	101.77±1.35	109±1.63	*	ns	-1.1	7.11
27	Proline (µg g^{-1} FW)	14.54±1.22	11.39±1.1	***	10.51±1.22	8.76±1.27	**	***	-21.69	-16.67
28	Carotenoids (mg/g)	37.08±1.67	265.87±2.33	***	274.71±2.2	337.48±2	***	***	617.09	22.85
29	Phenols (mg/g)	64.49±1.59	70.05±1.67	**	59.68±1.35	69.31±1.88	ns	***	8.61	16.14
30	TAC (µg/mL)	20.33±1.06	19.07±0.86	ns	19.66±0.94	19.81±1.06	ns	ns	-6.2	0.75

ns = non-significant,
* = $P < 0.05$,
** = $P < 0.01$,
*** = $P < 0.001$, p*Cv= P value between cultivar, P*T= P value between treatments.
*Superoxide dismutase (SOD), Peroxidase (POD), Catalase (CAT), Ascorbate peroxidase (APX), Glutathione peroxidase (GPX), Melondihyde (MDA), Total Antioxidant Capacity (TAC), Glutathione oxidised (GSSG), Glutathione reduced (GSH), and Total glutathione (GSSG+GSH).

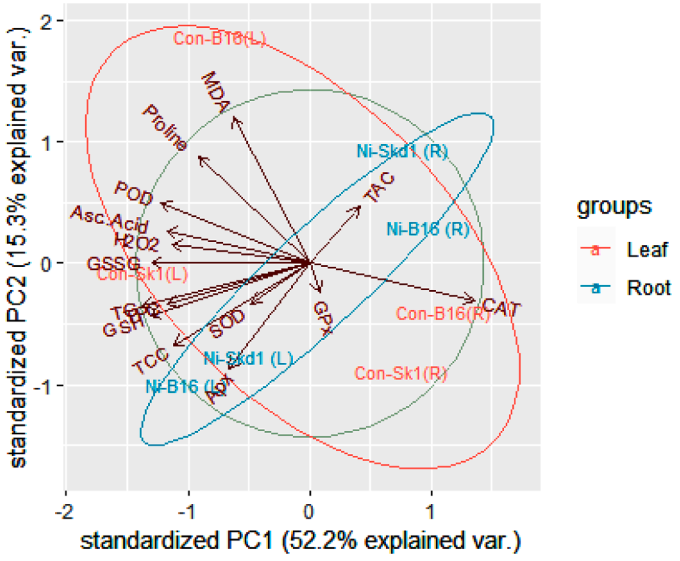

Fig. 6. PCA plot of antioxidants in leaf and root tissues of SKD-1 and Borlaug-16 cultivars. Two groups were made for both tissues. All the traits that appeared in the zone of leaf tissues were enclosed in red circle while all those in root tissues were enclosed in blue circle.

reduction in their growth and proliferation (Demchenko et al., 2010).

The exposure of Ni stress caused damaging effects on the leaf and root tissues of both cultivars. In case of root tissues, Borlaug-16 showed reduction in Ep with increasing thickness of BS, C, and S (Table 1). SKD-1, on the other hand, showed less reduction in Ep and more increase in BS compared to Borlaug-16. C and S cells also increased in thickness for SKD-1 cultivar under Ni stress (Fig. S7). Maksimović et al. (2007) observed an increase in cellular layers of root cortex due to the exposure of Ni to the Maize. Atabayeva et al. (2016) reported a decrease in the thickness of root Ep cells in copper (Cu) tolerant cultivars of wheat under Cu stress. It might be due to the reason that Ni can efficiently penetrate endodermal layer and gather in the pericycle cells, thus affecting cell division and cell multiplication (D'Antò et al., 2012).

In leaf tissues, Borlaug-16 showed reduction in the cellular thickness of UEp, LEp, Ph, and C. On the other hand, SKD-1 showed decrease in LEp, Xy, BS, and C with increasing cellular thickness of UEp and Ph (Table 1). Overall, the impact of Ni stress showed variable effects on leaf cellular thickness of both cultivars (Fig. S8). Current results align with the previous studies where Atabayeva et al. (2016) reported a reduction in the thickness of vascular bundles in Cu tolerant wheat cultivars under Cu stress. The decrease of diameter of the central cylinder is possibly a prerequisite of low growth parameters under the effect of toxic elements in the varieties. Vascular bundles diameter reduction is an indicator of water and minerals conductivity decrease (Céccoli et al., 2011).

A variation in the levels of MDA and H_2O_2 was observed in the root

and leaf tissues of both cultivars under Ni stress. In Present study, MDA levels increased in the roots and decreased in the leaf tissues of both cultivars under Ni stress (Table 2). SKD-1 showed more increase of MDA levels in roots and more reduction of MDA in leaf compared to Borlaug-16. The Ni stress caused an increase in H_2O_2 levels of root tissues in both cultivars with SKD-1 showing significantly more increase. In leaf tissues, H_2O_2 levels increased in Borlaug-16 with decreasing levels in SKD-1 (Table 2). In previous studies, Iqbal et al. (2019) reported higher levels of MDA and H_2O_2 in drought-sensitive cultivar of soybean.

Correlation analysis showed the significant relation between Ni and stress markers (MDA, H_2O_2) in both roots and leaf. In roots, Ni showed a positive relation with MDA and H_2O_2, whereas a negative correlation of Ni was observed between Ni with MDA and H_2O_2 in leaf tissues (Fig. S2). Previously, Chiao et al. (2020) in his study reported a positive correlation of MDA and H_2O_2 with Cd in roots while lesser accumulation of Cd and MDA levels were recorded in leaves of Cd stresses rice. This is because Ni toxicity leads to the formation of free radicals, which lead towards the overproduction of MDA and H_2O_2 in plants (Gaweł et al., 2004; Kumar et al., 2022).

SKD-1 showed a reduction in the SOD levels for both root and leaf tissues. Borlaug-16 on the other hand reported elevated levels of SOD for both leaf and root tissues. POD levels were reported to be reduced in the leaf tissues of both cultivars. The roots of SKD-1 showed an increase in POD levels with decreasing levels in roots of Borlaug-16 under Ni stress (Table 2). The levels of CAT enzyme showed reduction in the root and leaf tissues of SKD-1 under Ni stress. In Borlaug-16, CAT levels increased in both root and leaf tissues. Previously, Yusuf et al. (2012) also reported the increasing levels of SOD, POD, and CAT under Ni stress in Ni-tolerant variety of mung bean. Uruç Parlak (2016) reported no change in the activity of SOD after the exposure of Ni to the Wheat. The need for the superoxide scavenging through SOD may not be required after the reduction in the levels of superoxide radicals (Chen et al., 2008).

In present study, a negative correlation was observed between Ni and the levels of SOD in both root and leaf tissues, whereas Ni showed a positive correlation with POD in roots and a negative correlation with POD in leaf tissues. Previously (Sharma et al., 2008) also reported a positive association of SOD and POD with increasing Ni concentration. The decreasing levels of SOD indicate towards the decreasing superoxide radicals levels (Chen et al., 2008).

The present study observed a reduction of APX levels in the roots of both cultivars under Ni stress. In case of leaf, Borlaug-16 showed increase in APX levels with reducing APX levels in SKD-1 under Ni stress, showing a negative correlation with Ni accumulation (Table 2), which correlates with previous studies where Chakraborty and Pradhan (2012) reported increasing amount of APX in the tolerant wheat varieties under water stress. It shows that at lower load of H_2O_2 in leaf, APX enzyme also decreases due to lesser ROS production.

The roots of both cultivars showed a positive correlation of Ni with AsA content under Ni stress, where Borlaug-16 showed more increase compared to SKD-1 (Table 2). In leaf tissues, AsA showed a negative correlation with Ni accumulation in both cultivars with Borlaug-16 showing more AsA reduction compared to SKD-1. Similarly, high levels of AsA were also reported in tolerant wheat cultivars under water stress in previous studies (Chakraborty and Pradhan, 2012). This is because AsA plays a vital role in maintaining cellular homeostasis by detoxifying ROS and reducing H_2O_2 to water (Ashraf, 2009). The levels of GPX reduced in the roots of Borlaug-16 with increasing GPX levels in SKD-1 under Ni exposure (Table 2). A similar trend was also observed in leaf tissues where SKD-1 showed increasing GPX levels with a reduction in Borlaug-16 under Ni stress. On contrary, Al-issawi et al. (2015) reported higher levels of GPX in sensitive wheat cultivars under cold stress compared to tolerant cultivars. This is because plants enhance GPX expression under adverse environments to deal with excessive ROS production (Al-issawi et al., 2015).

In the present study, overall GSSG+GSH content reduced significantly in the roots of SKD-1 but for Borlaug-16 it showed no variation under Ni stress. In leaf tissues, GSSG+GSH showed significant increase in Borlaug-16 but a decrease was observed in SKD-1 under Ni stress. Overall, GSSG=GSH showed a positive correlation with Ni accumulation in roots. GSSG also reduced in both leaf and root tissues of SKD-1 while it increased in both tissues for Borlaug-16 under Ni stress (Table 2). GSH also showed a similar trend for SKD-1 where it reduced in both tissues (leaf and root). In case of Borlaug-16, GSH only reduced in root tissues under Ni stress. Previously, Hu et al. (2017) reported an increase in the levels of GSH content in As-tolerant cultivar in plants under As stress, suggesting a more prominent ROS detoxification due to higher GSH levels.

Present study showed that proline content increased in roots and decreased in leaves of both cultivars under Ni stress. However, in roots, Borlaug-16 showed more increase compared to SKD-1 and in leaves, SKD-1 showed less reduction compared to Borlaug-16 under Ni stress (Table 2). Overall, a positive correlation was found between Ni and proline content in roots and a negative correlation was observed in leaf tissues. Previously, increasing amount of proline in wheat seedlings under Ni stress was also reported by Uruç Parlak (2016). Yusuf et al. (2012) reported higher levels of proline content in Ni-tolerant mung bean variety compared to Ni-sensitive variety under Ni stress. This is because proline amongst various solutes, is one of the fewest molecules to protect plants against damage caused by free radicals and ROS under stress (Alía et al., 1997).

Ni stress caused a decrease in the root carotenoid content of both cultivars with SKD-1 showing more reduction compared to Borlaug-16 in present study (Table 2). For leaves, both cultivars showed an increase in carotenoid content under Ni stress where Borlaug-16 showed comparatively more increase. On the contrary, Chakraborty and Pradhan (2012) reported decline in the carotenoid content in sensitive-wheat cultivars under water stress. Present study showed a negative correlation of Ni concentration with carotenoid content in roots whereas in leaves a positive correlation was observed between Ni and carotenoids. Previously, Singh and Pandey (2011) also reported a decrease in the levels of carotenoids in water lettuce after the exposure of Nickle. This is because carotenoids have the ability of ROS quenching and playing role in antioxidant defense of cells (Sen and Mukherji, 2009).

Our findings showed that phenols decreased in the roots of both cultivars under Ni stress with SKD-1 showing more reduction compared to Borlaug-16, showing an overall negative correlation with Ni (Table 2). In leaves, phenolics increased in both cultivars with SKD-1 showing more increase compared to Borlaug-16 under Ni stress, showing a positive correlation with Ni accumulation. There results correlate with previous studies where Chakraborty and Pradhan (2012) reported high phenolics content in tolerant wheat cultivars under water stress. This is because plants activate phenylpropanoids secondary metabolic pathway in stress conditions, which produces phenolic compounds as a source of non-enzymatic antioxidant (Feduraev et al., 2020; Huang et al., 2010). We observed a reduction of TAC in the roots and an increase in leaves of SKD-1 under Ni stress. Whereas TAC increased in the roots and decreased in the leaves of Borlaug-16 under Ni stress. Overall, a negative correlation was observed between Ni accumulation and TAC in both leaf and root tissues. Present study correlate with previous studies, where the levels of TAC increased in tolerant maize cultivar under Cd stress (Abbas et al., 2021). According to Caverzan et al. (2016), the antioxidant capacity is higher in tolerant genotypes which help in the reduction of less oxidative damage, which depends most likely on the genetic potential of those genotypes.

5. Conclusion

The presence of Ni caused negative impact on physiological growth and anatomical structure in wheat varieties (Fig. 7). The findings revealed that Ni stress caused more physiological damage and anatomical disruptions in SKD-1 compared to Borlaug-16. A higher uptake of Ni was observed in Borlaug-16, whereas SKD-1 showed

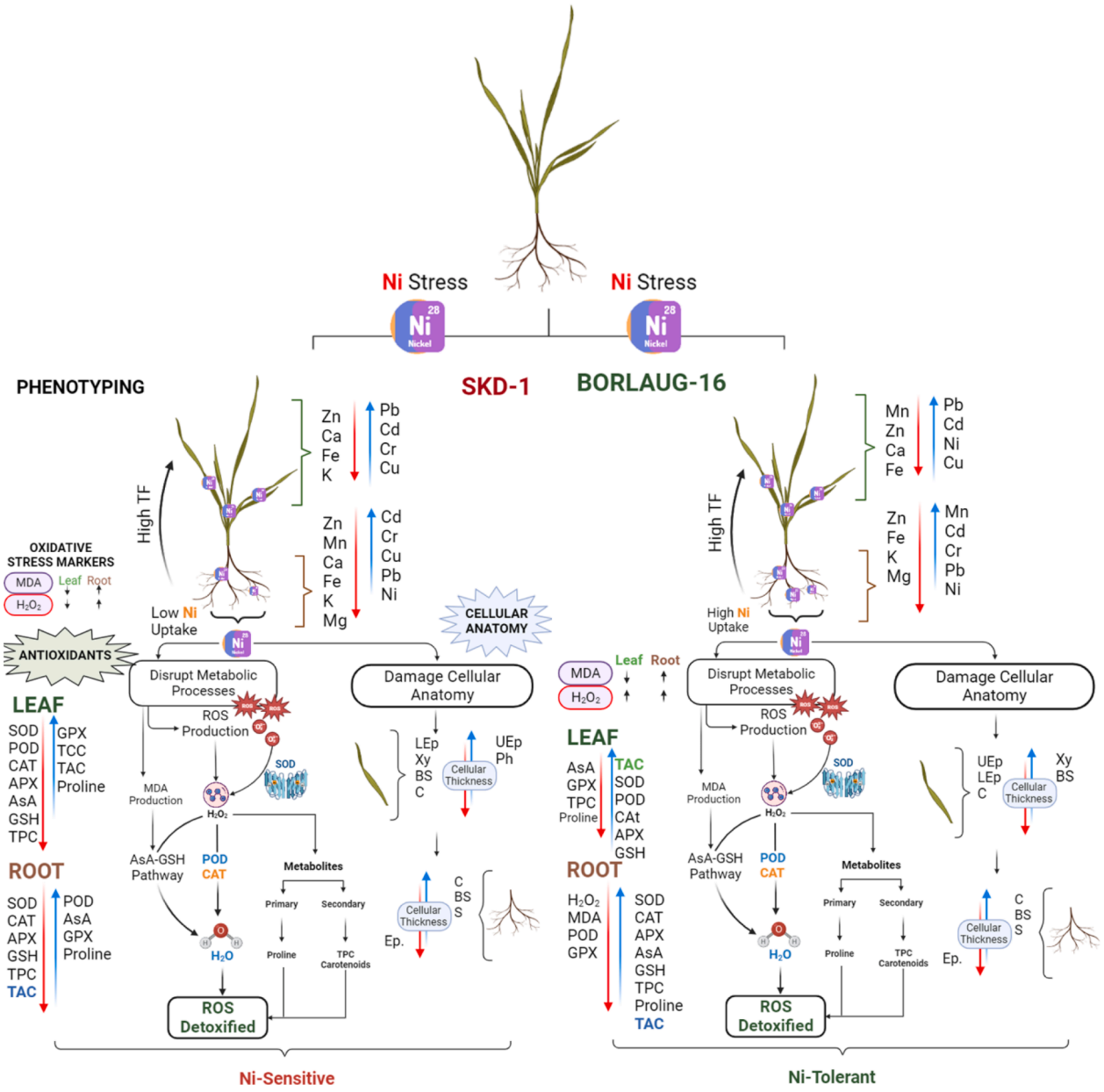

Fig. 7. Overview of the impact of Ni stress on tolerance mechanism of SKD-1 and Borlaug-16 cultivars.

comparatively lower Ni uptake but a comparatively higher translocation from roots to leaf which resulted into the high induction of oxidative stress in the roots and leaf of SKD-1 cultivar compared to Borlaug-16. Furthermore, SKD-1 showed a higher concentration of macronutrients in leaf compared to Borlaug-16 under Ni stress. Overall, Boralug-16 was proved to have higher tolerance against Ni stress having better antioxidant defense system (SOD, POD, CAT, APX, GSH, TPC, Proline, and TAC), and anatomical structure. Given the current findings, it is suggested to utilize tolerant cultivars in areas contaminated with Ni stress in future. Anatomy can also be used as a very useful indicator in studying Ni toxicity in crops. However, the present study is lacking molecular studies of tolerance mechanism due to the lack of resources. So, in future, it is important to target molecular mechanisms underlying Ni toxicity in these cultivars. Furthermore, Present study opens avenue for breeders to develop Ni-tolerant cultivars, which would ultimately ensure food security and meet SDG's.

Author contributions

Muhammad Anas performed research work and written the manuscript, Muhammad Saeed reviewed and helped in writing manuscript, Minhas Elahi helped in experimental and in analysis part, Kashif Naeem and Munib Ahmed Shafique performed ICP-OES analysis, Umar Masood Quraishi has designed and supervised the whole work.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.stress.2023.100277.

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