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Genetic dissection of Ni toxicity in a spring wheat diversity panel by using 90 K SNP array

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ABSTRACT

Excess Ni intake has harmful implications on human health, which include chronic bronchitis, reduced lung function, and cancer of lung and nasal sinuses. Like other toxic metals, higher Ni accumulation in grains leads to excess intake by humans when the contaminated grains are consumed as food. There is little information about the genetic factors that regulate Ni uptake in plants. To investigate genetic architecture of Ni uptake in leaf and translocation to grain, we performed a genome-wide association study with genotyping from 90 K array in a historical bread wheat diversity panel from Pakistan. We observed that Ni toxicity caused more than 50 % reductions in biological yield and grain yield, other agronomic traits were also partly or severely affected. Genetic association study helped identify 23 SNP-trait associations involved in Ni uptake in leaf and translocation to grains. These 23 SNPs covered 15 genomic loci at chromosomes 1A, 2D, 3B, 4A and 4B of wheat. The favorable alleles of these SNPs were randomly distributed in subpopulations indicating no selection pressure for this trait during breeding improvement. These regions had 283 low-confidence and 248 high-confidence protein coding genes. Among these, 156 were annotated using databases of wheat and closely related grass species. Since there is no previous report on genetic information of Ni uptake and translocation, these results provide sufficient grounds for further research of candidate genes and varietal development.

1. Introduction

For decades, scientific community has warned about overpopulation and the damaging effects it may bring on human civilization, food security being the topmost among them. Global population is expected to reach 9.8 billion by 2050 [1], which demands continuous increase in the production of staple crops. Wheat as a staple crop feeds more than one-third of the world population, providing carbohydrates, proteins, vitamins, antioxidants, fibers, and minerals [2]. In 2017-2018, wheat production was estimated at 756.8 million tons [3], significantly higher in proportion compared to previous five years. However, sustainable production is not the only challenge for wheat breeders. The nutritional quality of grains is an equally critical challenge because the excess uptake of toxic metals, such as Cd and Ni, can reduce the uptake of some of the nutritionally essential elements like Fe and Zn, and can also contaminate the grain, ultimately causing health concerns for humans. A large percentage of the population relying on wheat for food suffers from mineral deficiencies like Ca, Cu, Fe, Mg, and Zn [4–6]. Therefore, reducing the excess uptake of toxic metals or maintaining homeostatic conditions in terms of minerals uptake becomes critically important for health safety and proper crop growth.

The presence of excess Ni in soil can cause considerable changes in growth pattern and developmental aspects of plants, ranging from seed germination [7], seedling growth [8], leaf shedding [9], and reduction in overall biomass. Many physiological processes can also be disturbed due to exceeding exposure to Ni such as disturbance in enzymatic

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activities [10], photosynthetic electron transport [11], chlorophyll biosynthesis and membrane damage [12]. All these toxic effects result in yield penalties of agricultural crops, and, in turn, contribute to food insecurity as well as human health concerns. Studies have shown that excessive Ni intake can cause several health conditions like dermatitis, respiratory disorders, fibrosis of lungs and even cancer [13,14]. On the other hand, no reports are available to describe any nutritional value of Ni for human consumption [15] that permit for defining Ni thresholds in staple food crops such as wheat. Hence, the phytotoxic effect of Ni, rather than deficiency, appears to be a greater challenge for future. In Pakistan, Ni toxicity has increased rapidly due to industrial pollution. The soil samples from contaminated sites in the city of Lahore have been reported to have as high as 324 mg/kg Ni concentration, the permissible value in these areas is 30−75 mg/kg [16]. There is, however, limited data about the physiological implications of Ni uptake and toxicity in crops.

Like other nutrients, Ni uptake and translocation in plants are quantitative traits. Because quantitative traits are influenced by multiple genetic factors, they show greater variation in natural populations. These variations can be exploited in quantitative population genetics to identify alleles for desirable phenotype and also for breeding stress resistant cultivars. Two most commonly used approaches for such purposes are QTL mapping and GWAS. The latter is a relatively newer approach emerged with the advent of high throughput genotyping platforms and next generation sequencing technology. It is considered more powerful than QTL mapping for associating phenotype and genotype variations. For the details about limitations and strengths of GWAS, we suggest referring to the review by Arthur Korte and Ashley Farlow [17]. GWAS has successfully identified common variants from natural populations for a number of human diseases and agronomically important plant traits. In our recent reports, we identified genetic factors underlying Cd uptake and translocation [18] and K-use efficiency [19] using GWAS in the same natural population used for the current study. For Ni uptake, there has been only one study in synthetic hexaploid wheat, where Bhatta *et al.* identified eight single nucleotide polymorphisms (SNPs), on chromosomes 1A, 2D, 3A, 4D, 5B, and 6A, associated with Ni variation in grains [20]. Their study was focused on several minerals and did not investigate Ni uptake in leaf. Also, there is a much larger genetic diversity in natural bread wheat populations and historical landraces that remain to be explored for their immense genetic potential.

To address these concerns, we performed GWAS for Ni uptake in leaf and translocation to grains using a historically diverse spring wheat panel from Pakistan. We performed largescale phenotyping under Ni stress. The aim of this study was: a) to evaluate the morphophysiological implications of Ni toxicity on wheat; and b) to identify genetic factors underlying Ni uptake variation so that they can be further explored for gene identification in future studies. We identified 15 potential loci for Ni uptake in leaf and translocation to grain. The phenotypic observations and the identified genetic loci will provide useful information for future research in this area.

2. Materials and methods

2.1. Plant material and experimental design

A bread wheat panel consisting of 120 genetically and historically diverse lines suitable for cultivation in both irrigated and rain-fed areas of Pakistan was selected for performing the study (**Supplementary** Table 1). The panel was selected based on genetic diversity, history, and breeding importance, hence, constituted of 19 landraces, 30 green revolution lines (1965–1989), 32 post green revolution lines (1990–2000) and 39 elite cultivars (post 2000). Phenotypic purity check for the germplasm was performed for three years (2011–2014) at National Agricultural Research Centre [21]. Later, the experiment was planted using an augmented triplicate alpha-lattice design under normal conditions (treatment 1; T_1) and 75 mg/Kg $NiSO_4$ for Ni stress (treatment 2; T_2). Experimental conditions and details of soil sample analysis were explained in our previous report on genetic regulation of Cd uptake for the same germplasm [18]. Thirty seeds were sown in 1 m rows after surface sterilizing with 4% H_2O_2 for 10 min and washing with deionized water.

2.2. Phenotyping

Phenotypic observations were made on 11 morpho-physiological traits and stress tolerance index (STI) was also computed. Data were taken on plant height (PH), spike length (SL), tillers per plant (T/P), thousand kernel weight (TKW), grains per spike (GPS), and grain yield per plot (GY) at ripening stage for each genotype, following the previously established phenotyping protocols [22]. Chlorophyll index was measured at heading ($Chl_{\_head}$) and tillering ($Chl_{\_till}$) stage by using a portable chlorophyll meter (SPAD-502 Minolta, Japan). The formula used for computing stress tolerance index (STI) for all the genotypes was [23]:

$$STI = \frac{GY \text{ in } T1 \text{ x } GY \text{ in } T2}{\text{average } GY \text{ in } T1}$$

Where, GY means the average grain yield of all three replicates of any individual line while the average GY in the denominator is the mean of grain yield of all the lines in normal growing conditions (T_1).

Ni concentrations from flag leaf ($Leaf_{\_Ni}$) after anthesis and seed ($Seed_{\_Ni}$) after harvesting were measured. For T_1, the values for Ni concentration were either zero or negative. The accuracy of analytical equipment was verified by using a certified reference material (CRM) of duck weed BCR® (BCR-670; Sigma-Aldrich). Sample digestion and estimation using atomic absorption spectrometer were described in an earlier report (LBS). The detected and standard values of CRM for Ni were identical, verifying the accuracy of analytical methods.

2.3. Statistical analysis

Differences between treatments were tested using paired sample *t*-test at 95 % confidence interval in XLSTAT software. Pearson correlations between traits were estimated by R software and visualized along with histograms and scatter plots for all the traits by using ggplot2 package in R environment [24].

2.4. Genotyping and population genetic diversity

Genotyping was performed using 90 K wheat SNP array with 81,587 SNP markers across the whole genome. Physical map positions for all the SNP markers were extracted by alignment with IWGSC RefSeq v.1.0. After the quality control steps, 14,960 polymorphic SNP markers were finalized for genome-wide association study. Population stratification analysis, published previously, showed that there were seven subgroups instead of four (based on historical evidence). This indicated rich allelic diversity of the germplasm, which could prove of significant value for identifying rare alleles ignored during artificial selection and breeding history, as this trait has been largely ignored in the previous genomic selection studies of wheat.

2.5. Genome-wide association study and gene predictions

Interaction between genotype and phenotype ($Leaf_{\_Ni}$ and $Seed_{\_Ni}$) was tested using Efficient Mixed Model Association eXpedited (EMMAX), which uses variance components to generate a kinship matrix to be used as a covariate during association tests to account for spurious associations emerging due to cofounder variables [25]. Genome-wide significance of threshold was set as $P \leq 0.001$ and no multiple testing correction was applied because repeated GWAS studies, including our

previous report on this panel [18], have reported that controlling for false positives from type-I error can result in false negatives from type-II error – we chose not to skip any important information because this was the first ever comprehensive investigation for Ni toxicity in wheat. For locus identification, we relied on sub-genomic population LD decay as pairwise LD between neighbouring SNPs had resulted in smaller genomic blocks, in our previous study, due to low density of array dataset. Genes were extracted from RefSeq v.1.0 gff3 for all loci using *location* tab from EnsemblPlants database and annotations were retrieved using *BioMart* function from EnsemblPlants.

3. Results

3.1. Ni stress drastically reduced agronomic performance and grain yield of wheat

Comparison of means between genotypes in two different treatment conditions by paired sample *t*-test revealed significant differences in various plant traits such as PH, $Chl_{\_till}$, BY, T/P, SL, GPS and GY. This indicated toward significant impact of Ni treatment on the overall agronomic performance of various genotypes in the study. Ni stress caused considerable reductions in yield contributing traits: grain yield and biological yield were reduced by 67.42 % and 56.94 %, respectively. Similarly, other traits were also affected in Ni stress treatment: T/P (-37.62 %), SL (-27.49 %), GPS (-21.55 %), PH (-12.46 %), and TKW (-7.21 %). The genotypic variation in quantitative traits was substantial for two treatments, which signified the possible impact of Ni treatment. The min. and max. grain yield per plot for T_1 was found 67.1 g and 351.3 g in comparison to the T_2 min. 9.2 g and max. 132.8 g per plot (Table 1). Eight genotypes (Kohsar-95, Lasanai-08, Sarhad-82, Aas-2011, Seher-06, Manthar, Pirsabak-05 and Imdad-05) were observed as having low seed Ni concentration (below optimal limit <0.39 mg/kg); however, grain yield in all these varieties reduced by 51–84 % and biomass by 30–74 % **(Supplementary** Table 2**)**. On the other hand, AS-2002 was the only cultivar in the panel which had no reduction in grain yield and biomass, despite having sligtly high seed and leaf Ni concentrations. Low Ni uptake varities and sustainable yield maintaining varieties can be combined in future studies for further targeted investigation.

Correlation analysis revealed positive association between all the yield contributing traits regardless of the treatments they were grown in, which suggested the presence of common genetic factors underlying variation. In T_1, GY showed positive correlations with T/P ($r^2 = 0.69$), and TKW ($r^2 = 0.52$); in T_2 (Ni stress), GY showed positive correlations with T/P ($r^2 = 0.51$), and GPS ($r^2 = 0.78$). STI also positively correlated with all yield related traits: PH ($r^2 = 0.41$), T/P ($r^2 = 0.36$), GPS ($r^2 = 0.72$), GY ($r^2 = 0.83$) and BY ($r^2 = 0.31$). Details of trait correlations and phenotypic description are provided in Fig. 1.

3.2. Genotype data identified rich genetic diversity in the wheat panel

Genotype data was published in our earlier report on the same set of genotypes [21]. Of 81,587 SNP markers, quality control steps resulted in the removal of a sizeable portion leaving only 14,960 polymorphic and high-quality SNPs for association analysis. Population stratification indicated the existence of seven subgroups, instead of the four previously categorized on historical basis. For subgroups and stratification details, please refer to the previous study of Ain *et al.* [Ref. 21]. Average sub-genomic LD decay distance, which was used for locus assignment, was observed as 300 kb, 800 kb, and 500 kb for A, B, and D sub-genomes, respectively [19].

3.3. Genome wide association scans for Ni regulation identified 15 novel loci

Genome-wide association tests from EMMAX identified 23 SNPs associated with Ni phenotype in leaf and grains at the genome-wide significant threshold of $P \leq 0.001$ (Fig. 2). These 23 SNPs covered 15 loci on five chromosomes (1A, 2D, 3B, 4A and 4B) based on sub-genomic LD decay distance. Chromosomal distribution of loci was: 1A = 6, 3B = 4, 4A = 2, 4B = 2 and 2D = 1. Nine loci were associated with $Seed_{\_Ni}$ at chromosomes 1A (5), 3B (2) and 4B (2), while six loci were associated with $Leaf_{\_Ni}$ at 1A (1), 2D (1), 3B (2) and 4A (2). Loci were named according to the nomenclature suggested elsewhere [26]. For example, *qNi1* represents a genomic region located on chromosome 1A for $Seed_{\_Ni}$ at 369085792 bp (Table 2).

3.4. Allelic polymorphisms considerably impacted Ni accumulation in grains

Because the ultimate impact of Ni toxicity on grains causally links to health safety and grain mineral quality concerns, we evaluated the impact of alleles from significantly associated MTA with $Seed_{\_Ni}$ on the overall concentration of Ni in grains. Fig. 3 shows that the presence/absence of various alleles of these MTA caused considerable variation in Seed_Ni concentration among panel members. This data can help in the future studies for the identification and characterization of candidate/causal genes (Fig. 3).

Table 1
Descriptive statistics, comparison of treatments and impact of Ni stress on morpho-physiological traits.

Trait*	Normal Conditions			Ni Stress			G × T†	Ni Impact
	Min	Max	Mean	Min	Max	Mean		
PH	53.3	102.6	67.3	36.6	92.6	58.9	<0.0001	−12.46 %
$Chl_{\_till}$	24.3	50.2	39.4	28.6	51.4	36.8	<0.0001	−6.4 %
$Chl_{\_head}$	36.3	60.1	45.5	30	50.6	43.3	0.872	+2.95 %
BY	10.5	57.9	23.5	2.2	28.9	10.1	0.01	−56.94 %
T/P	2.6	7.3	4.3	1	4.3	2.7	0.009	−37.62 %
SL	4.6	13	8.1	3	11.3	5.8	<0.0001	−27.49 %
TKW	18	53.6	37.3	19.2	46	34.6	0.16	−7.21 %
GY	67.1	351.3	136.6	9.2	132.8	44.5	<0.0001	−67.42 %
GpS	8	61	30.6	8	58	24.05	0.003	−21.55 %
$Leaf_{\_Ni}$	–	–	–	1	57.1	3.9		
$Seed_{\_Ni}$	–	–	–	0.06	7.8	28.1		
STI	–	–	–	0.05	1.08	0.3		

* Plant height in cm (PH), Chlorophyll content at tillering ($Chl_{\_till}$), Chlorophyll content at heading ($Chl_{\_head}$), Biological yield per plant in g (BY) Tiller per plant (T/P), Spike length in cm (SL), Thousand kernel weight in g (TKW), Grain yield per plot in g (GY), Grains per spike (GpS), Leaf Ni content in mg/kg ($Leaf_{\_Ni}$), Seed Ni content in mg/kg ($Seed_{\_Ni}$) and Stress tolerance index (STI).

† Comparison of means between genotypes in two treatment conditions by paired sample *t*-test.

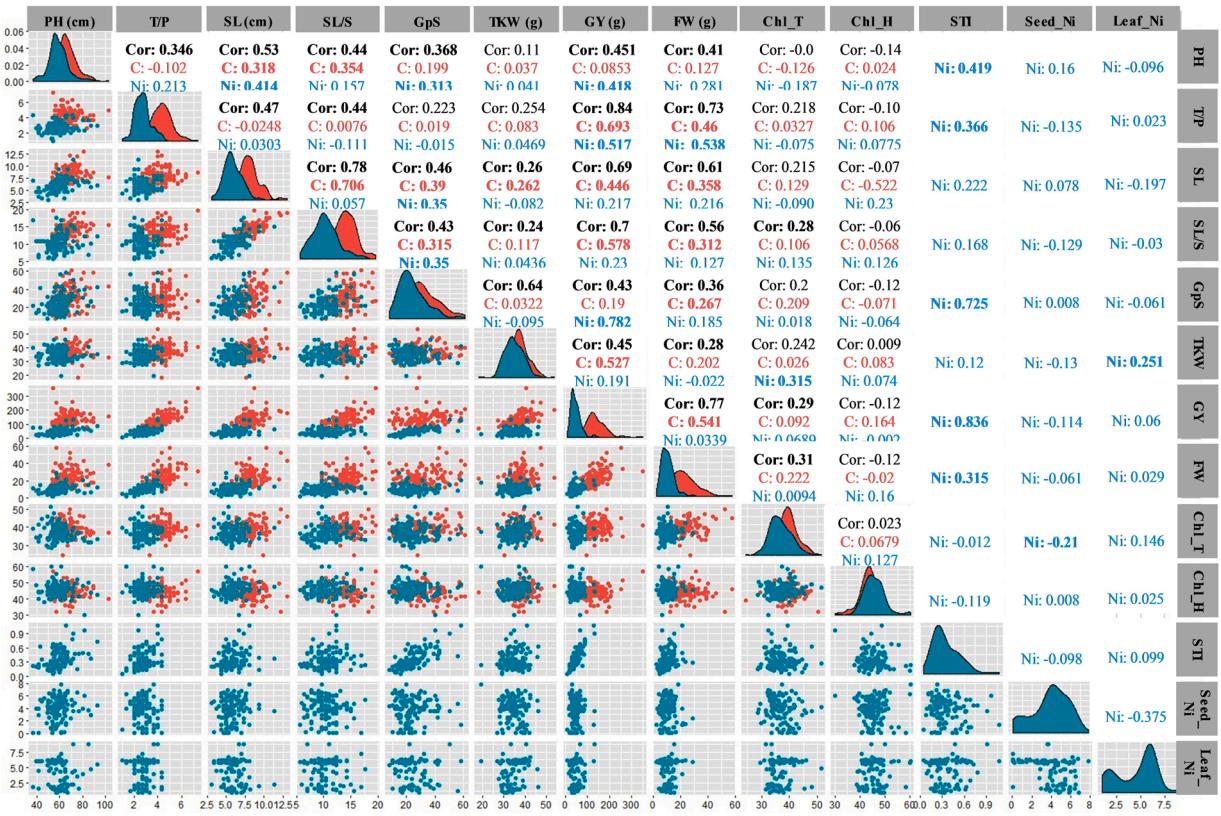

Fig. 1. Scatterplots, histograms, and coefficient of correlation between traits under normal conditions (red color) and Ni stress (blue color). The lower triangle represents scatter plots of traits, the upper triangle represents coefficient of correlation values, and the middle line dissecting the two triangles represents histograms of traits. ***cf.** Trait nomenclature is presented in Table 1 legends (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

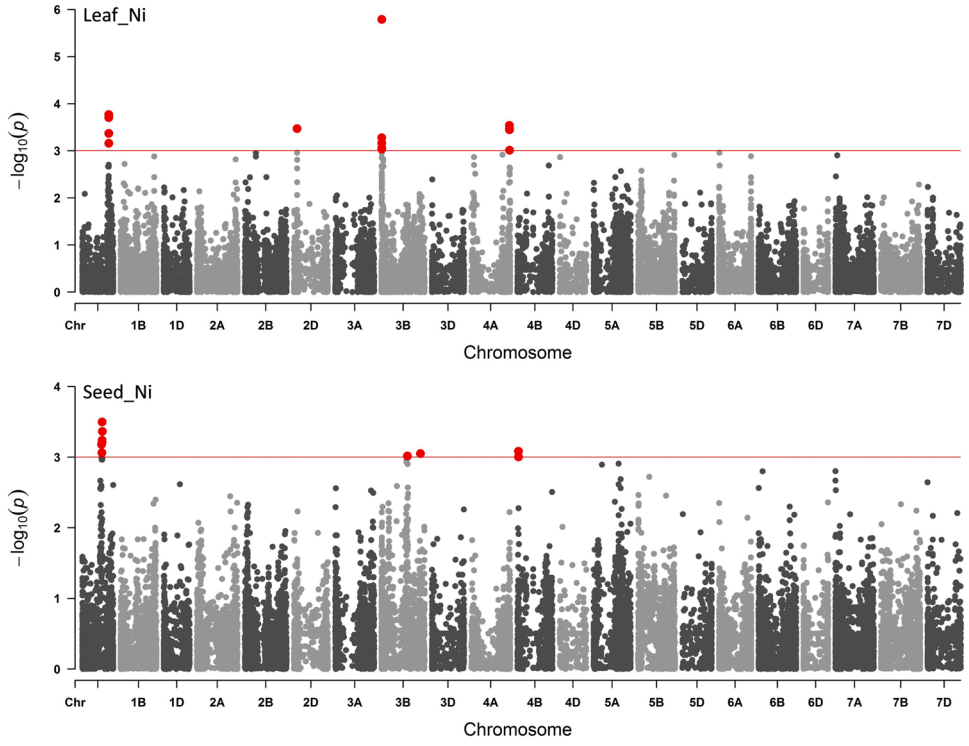

Fig. 2. Manhattan plot of MTA identified by GWAS for Leaf$_{Ni}$ (upper panel) and Seed$_{Ni}$ (lower panel). The *x-axis* represents chromosomal locations of MTA and the *y-axis* represent P-values as -log10. The MTA above the genome-wide significance level at $P \leq 0.001$ are highlighted as red (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

Table 2
Wheat genomic regions associated with Ni uptake in seed and leaf.

Loci	Chr	SNP Pos*	P-value†	-log10p	Association
qNi1	1A	369085792	0.0006	3.178	Seed_Ni
qNi2	1A	377428003	0.0008	3.062	Seed_Ni
qNi3	1A	380973141	0.0006	3.213	Seed_Ni
qNi3	1A	381097037	0.0005	3.239	Seed_Ni
qNi4	1A	382233357	0.0003	3.497	Seed_Ni
qNi5	1A	385355702	0.0004	3.363	Seed_Ni
qNi6	1A	508638177	0.0001	3.767	Leaf_Ni
qNi6	1A	508640911	0.0006	3.158	Leaf_Ni
qNi6	1A	508640934	0.0001	3.704	Leaf_Ni
qNi6	1A	508640961	0.0004	3.369	Leaf_Ni
qNi7	2D	62288723	0.0003	3.469	Leaf_Ni
qNi8	3B	5673670	0.0008	3.052	Leaf_Ni
qNi8	3B	5673703	0.0005	3.276	Leaf_Ni
qNi8	3B	5674447	0.0009	3.035	Leaf_Ni
qNi8	3B	5952324	1.63E-06	5.787	Leaf_Ni
qNi9	3B	7187840	0.0006	3.158	Leaf_Ni
qNi10	3B	499454400	0.0009	3.015	Seed_Ni
qNi11	3B	750355413	0.0008	3.049	Seed_Ni
qNi12	4A	732512367	0.0002	3.539	Leaf_Ni
qNi13	4A	733888036	0.0003	3.469	Leaf_Ni
qNi13	4A	733999817	0.0003	3.445	Leaf_Ni
qNi14	4B	21556672	0.0008	3.082	Seed_Ni
qNi15	4B	22902515	0.0009	3.002	Seed_Ni

* SNP physical position on wheat reference genome IWGSC RefSeq v1.0.
† P-value derived from efficient mixed model association test. SNP were considered significant at $P \leq 0.001$.

3.5. Candidate genes for Ni predicted with comparative genomes and literature data

The sub-genomic LD decay distance around the 15 identified loci was used to extract genes form the wheat reference assembly IWGSC RefSeq v.1.0 database [27] embedded in EnsemblPlant (http://plants.ensembl.org/Triticum_aestivum/Info/Index). The 15 genomic regions had 283 low-confidence and 248 high-confidence protein coding genes (**Supplementary** Table 3). Among these, 156 were annotated using databases of wheat and closely related grass species (**Supplementary Table 4**). These genes were annotated for ABC transporters, plasma membrane ATPases, zinc finger like proteins, Ca-dependant protein kinase, genes related to transmembrane activity and redox homeostasis. Some of these genes/proteins (32 from our list) were recently reviewed for their possible role in metal stress [28], and were listed as candidates (Table 3). This data will pave way for the research of identifying causal genes for Ni uptake and translocation; however, all genes at the 15 loci can be considered using various computational, evolutionary, and comparative genomics approaches.

4. Discussion

In the present study, we describe the effects of excess Ni uptake on agronomic potential of wheat; discuss health safety risks associated with excess Ni concentration in wheat grains; and identify genetic factors underlying Ni uptake and translocation in wheat.

There has been a limited to no research on the impact of Ni toxicity in plants, especially crops of global interest, such as wheat, maize, and rice. This lack of interest in geneticists is perhaps because Ni only contributes in traces to the overall mineral composition of plant, and the normal cultivated soils across the world are not subject to higher Ni concentrations. However, in the recent few decades, industrial advancements are contributing to Ni contamination of water and land resources, especially in the developing countries where the health safety laws are not practiced to their full scale. For example, soils analyses from Pakistan have shown an average 172 mg/kg of Ni in areas prone to contamination, which is far higher than the permissible limits (30−75 mg/kg in case of standard soils) set by the US and EU [16]. This makes Ni uptake by crop plants a genuine concern, especially in polluted areas, in terms of agricultural production and public health. A little is known about Ni uptake by plants and paucity in global research exists in terms of understanding the genetics behind the uptake and accumulation of Ni in wheat and other related grass species.

We observed considerable reductions in all the studied traits in Ni treated plots that indicated the type of physiological disturbances plants can face in Ni contaminated natural soils. The most affected traits by Ni stress were grain yield (reduced by 67 %) and biological yield (reduced by 57 %). Such huge reductions in yield components alarm the future food security for an ever-increasing population especially in potentially vulnerable areas. The major physiological system in plants which contributes to yield components is photosynthesis, which, as generally known, depends on the efficiency of photosystems and chlorophyll. We observed that Ni application reduced the chlorophyll index at tillering stage by 6%. This may have occurred due to the displacement of Mg ions by Ni resulting in an altered structure and/or activity of chlorophyll and ribulose-1,5-bisphosphate in Ni treated plants [29,30]. Finally, the significantly positive correlations between stress tolerance index and yield traits (Fig. 1) evidenced the impact of excess Ni in soil.

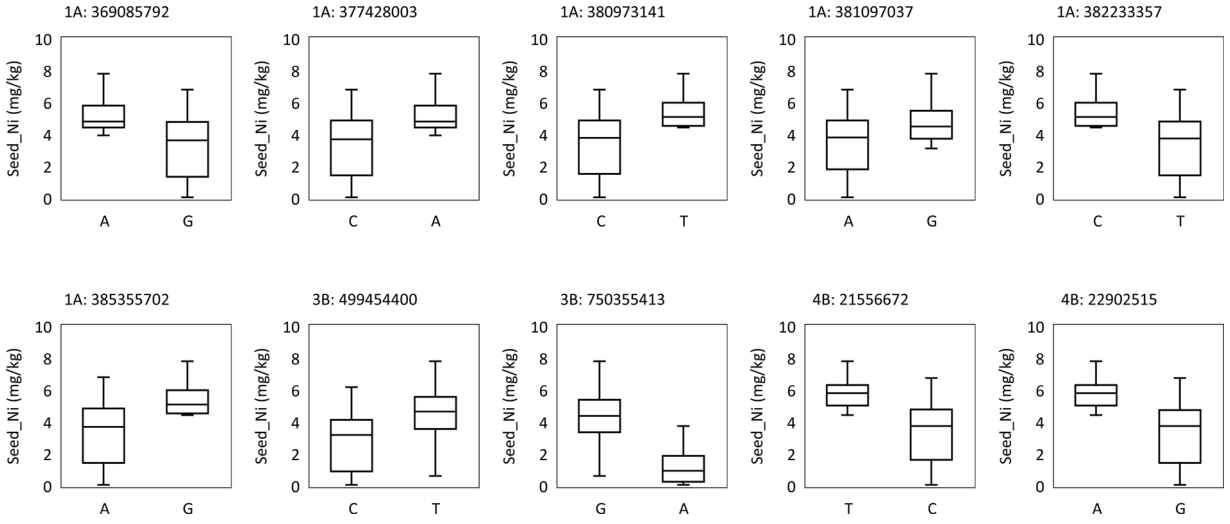

Fig. 3. Box plots of MTA associated with Ni accumulation in seed (mg/kg). Chromosome and position (bp) of SNPs are given on the top of each box-and-whisker. Boxes represent the first quartile, the median, and the third quartile, respectively. The thick horizontal lines correspond to the mean. Whiskers represent variability outside the upper and lower quartiles.

Table 3
A list of 32 genes identified as likely candidates for Ni variation based on literature information of previously characterized genes.

Wheat Gene	Arabidopsis Ortholog	Rice Ortholog	Gene Description*
TraesCS3B02G016600		*Os01g0121600*	ABC transporter-like domain containing protein
TraesCS4B02G030100	*AT1G74740*	*Os03g0688300*	Calcium-dependent protein kinase, Positive regulator of drought stress tolerance and spikelet fertility
TraesCS4B02G031000	*AT3G60330*		Plasma membrane ATPase
TraesCS3B02G310300	*AT4G29120*	*Os01g0742300*	3-hydroxyacid dehydrogenase/ reductase domain proteins
TraesCS1A02G207000		*Os10g0546900*	Similar to Zinc finger, C3HC4 type family protein
TraesCS1A02G207300		*Os10g0547500*	Divalent ion symporter domain containing protein
TraesCS1A02G214300	*AT2G41705*	*Os10g0567000*	Camphor resistance CrcB protein family protein
TraesCS1A02G214600	*AT2G44650*	*Os10g0566700*	Chaperonin Cpn10 family protein
TraesCS1A02G215500	*AT4G17090*		Beta-amylase
TraesCS1A02G217300	*AT5G07470*	*Os10g0563600*	Similar to Peptide methionine sulfoxide reductase
TraesCS1A02G217400	*AT4G16970*	*Os10g0563500*	Protein kinase, core domain containing protein
TraesCS1A02G317300	*AT1G20650*	*Os05g0498900*	Serine/threonine protein kinase domain containing protein
TraesCS2D02G111500		*Os02g0118875*	NB-ARC domain containing protein
TraesCS2D02G111700		*Os07g0673900*	Hypoxia induced protein, early hypoxia signalling
TraesCS2D02G112000		*Os07g0673550*	Photosystem II protein PsbX family protein
TraesCS2D02G112100	*AT5G53637*	*Os04g0440400*	F-box domain, cyclin-like domain containing protein
TraesCS2D02G112600	*AT4G17910*	*Os03g0378200*	GWT1 family protein
TraesCS3B02G011400		*Os11g0173500*	Leucine-rich repeat
TraesCS3B02G011500		*Os11g0173500*	Pentatricopeptide repeat domain containing protein
TraesCS3B02G011600	*AT1G75500*		WAT1-related protein
TraesCS3B02G012100		*Os01g0111900*	Glutelin family protein
TraesCS3B02G012500	*AT3G03440*		Ubiquitin-protein ligase
TraesCS3B02G014100	*AT5G61290*		Putative Disulphide oxidoreductase
TraesCS3B02G017800		*Os11g0482400*	2OG-Fe (II) oxygenase domain containing protein
TraesCS3B02G310800	*AT1G51940*		LysM domain receptor-like kinase 3
TraesCS3B02G310900	*AT4G07670*	*Os01g0740600*	Transferrin receptor-like, dimerization domain
TraesCS3B02G311000	*AT4G07670*	*Os01g0740500*	Similar to glutamate carboxypeptidase 2
TraesCS3B02G506200	*AT4G15660*	*Os01g0936000*	Putative glutaredoxin-C3
TraesCS4A02G472700	*AT3G61970*	*Os06g0107800*	Similar to RAV-like protein
TraesCS4B02G028500	*AT3G22780*	*Os07g0176200*	
TraesCS4B02G031700	*AT5G39990*	*Os03g0692000*	Tesmin/TSO1-like, CXC domain containing protein Glycosyl transferase, family 14 protein

* Gene descriptors are extracted from EnsemblPlant database for Wheat, Arabidopsis, and Rice.

Rich genetic diversity and abundant allelic resources emerging from natural populations have been a key component of GWAS in addressing concerns of low resolution in the conventional linkage mapping approach [17]. The population structure analysis in our study highlighted the presence of abundant genetic diversity, subdividing the panel into seven subgroups: group I included post-green revolution cultivars from irrigated areas; group II included post-green revolution cultivars from rainfed areas; group III included landraces and their derivatives; group IV included derivatives of green-revolution cultivars; group V included green revolution cultivars adapted from CIMMYT; group VI included post-green revolution cultivars from CIMMYT; and group VII included elite cultivars with Inqalab-91 background. A higher proportion (i.e., 65 %) of the population showed admixture trend [21]. This data indicates the presence of ancestral and diverse alleles in our analysis that may have been ignored during the process of artificial selection and breeding improvement, because Ni uptake has not been targeted in previous genetic studies.

We identified 23 significant SNP markers associated with Ni concentration in leaf and seed. These 23 SNPs covered 15 loci on wheat genome, none of which were reported in the study of Bhatta*et al.* [20], the only reported study related to Ni in wheat grains. Among these, 9 loci were associated with $Seed_{Ni}$ concentration (Table 2). The random allelic distribution of these 9 loci among genotypes and their varied impact on $Seed_{Ni}$ (Fig. 3) further evidenced the fact that these alleles were ignored during selection. In these 15 loci, wheat genome had 248 high confidence protein coding genes according to the reference assembly IWGSC RefSeq v1.0. Relying on the information of wheat genome annotations and relative grass species, we identified descriptors for 156 genes. From these 156, exploring the published literature of characterized genes, we identified 32 genes involved in various aspects of heavy metal uptake in plants. These included plasma membrane ATPase, Ca-dependant protein kinases, and ABC (ATP-binding cassette) transporter like domain containing proteins, and others that were identified as active players for mineral uptake and transport in plants [28] as well as plant stress signalling [31]. Similarly, *TaABCC3* belongs to a subclass of ABC transporters and has been known for its involvement in stress response against heavy meatal accumulation [32].

Summarily, these genes and importantly the 15 loci, provide invaluable information for further studies to characterize Ni related genes using molecular biology approaches. Also, the low Ni uptake and resistant genotypes identified in our study can help cultivar development in stress affected areas.

Ethics statement

The research was conducted to satisfy Pakistan Agricultural Research Council Act and Quaid-i-Azam University Pakistan research safety standards.

CRediT authorship contribution statement

Luqman Bin Safdar: Data curation, Formal analysis, Investigation, Methodology, Validation, Writing - original draft, Writing - review & editing. **Fakhrah Almas:** Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. **Attiq ur Rehman:** Writing -

original draft. **Muhammad Jawad Umer:** Formal analysis, Writing - original draft. **Syed Mashab Ali Shah:** Data curation. **Siraj Uddin:** Validation, Writing - review & editing. **Shomaila Ashfaq:** Validation, Writing - review & editing. **Hamid Ur Rahman:** Validation, Writing - review & editing. **Umar Masood Quraishi:** Conceptualization, Methodology, Project administration, Resources, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest

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Appendix A. Supplementary data

Supplementary material related to this article can be found in the online version at doi: https://doi.org/10.1016/j.cpb.2020.100175.

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