Unveiling the physio-biochemical, photosynthetic and ionic Responses of wheat (*Triticum aestivum* L.) genotypes exposed to NaCl and chromium stress

Muhammad Ali[2*], Yu Chen[1,3], Salman Ahmad[4], Salman Aloufi[5], Fahad Al-Asmari[6], Tolga İzgü[7], Özhan Şimşek[8], Adel I. Alalawy[9], Siham M.AL-Balawi[10], Abdulrahman Alasmari[11], Meaad F. Alaida[12]

[1]*Institute of Botany, Jiangsu Province and Chinese Academy of Sciences (Nanjing Botanical Garden Mem. Sun Yat-Sen), Nanjing 210014, China；15150530195@163.com (Y. C.).*
[2]*Institute of Agro-Industry & Environment, The Islamia University of Bahawalpur, 63100, Pakistan*

[3]*Jiangsu Key Laboratory for the Research and Utilization of Plant Resources, Nanjing 210014, China*

[4]*Department of Agronomy, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Pakistan*

[5]*Department of Biotechnology, Faculty of Sciences, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia*

[6]*Department of Food and Nutrition Sciences, College of Agricultural and Food Sciences, King Faisal University, Al-Ahsa, Saudi Arabia*

[7]*Institute for BioEconomy (IBE), National Research Council (CNR), 50019 Sesto Fiorentino, Florence, Italy*

[8]*Erciyes University, Agriculture Faculty, Horticulture Department, Kayseri, 38030, Türkiye*

[9]*Department of Biochemistry, Faculty of Sciences, University of Tabuk, Tabuk 71491, Saudi Arabia*

[10]*Department of Biology, Faculty of Science, University of Tabuk P.O.Box:741,Tabuk,71491,Saudi Arabia*

[11]*Department of Biology, Faculty of Science, University of Tabuk, Tabuk, 71491, Saudi Arabia*

[12]*Biology Department, College of Science, Jouf University- Sakaka 41412-Saudi Arabia*

Corresponding Author's Email: 15150530195@163.com (Y.C.); muhammadali@iub.edu.pk (M.A)

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ABSTRACT

Heavy metal pollution and salinity is a serious ecological concerns posing a threat to agriculture sustainability and global food security. Chromium (Cr) accumulation in arable lands is of serious concern due to its long term persistent in the soil and strong detrimental impacts on crop yield. Soil salinization is also a primary abiotic stress in arid to semi-arid lands which restricts plant metabolism and sustainable growth. A hydroponic study was performed to investigate the effect of salinity (100 mM NaCl) and chromium (15 µM and 30 µM) in integrated and sole form on two wheat genotypes (Sahar and Lasani). In the current experiment, it was noticed that imposition of salinity stress notably reduces plant biomass, chlorophyll contents, Relative Water Contents (RWC), Membrane Stability Index (MSI), potassium / sodium (K^+/Na^+) ratio and gas exchange attributes in wheat seedlings. The effect of Cr on plant dry matter, physiology and photosynthetic activity varied with Cr concentration. Under low Cr level (15 µM), ceased plant growth and nutritional imbalance caused by salt stress was generally mitigated and this effect is more prominent in wheat genotype Sahar as compared to Lasani. The interactive stress of elevated Cr (30µM) and salt stress results in further reduction in plant biomass, water relations along with stomatal regulation as compared to two stresses (Cr and salinity) alone. The results of the current study may help in understanding the mechanisms involved in sustaining plant growth subjected to different abiotic stresses under the current climate change scenario.

Key words: Abiotic stress; Antioxidants; Leaf gas exchange; Oxidative stress; Wheat genotypes

1. INTRODUCTION

Wheat is a widely cultivated global staple crop as it tolerates a wide range of temperature and humidity and is a significant source of food and nutrition for one-third of the global population (Mottaleb *et al*. 2022; Jalil *et al.* 2024). Pakistan is the 4th largest producer of wheat in Asia and ranks 11th in the world but onset of abiotic environmental stress especially soil salinity and heavy metal toxicity limits its growth and productivity (Rehman *et al*. 2020). The arable land under wheat productivity in the world is significantly increased but during the last few decades, global warming and climate change in arid to semi-arid regions severely affected wheat crop yield (Manzoor *et al*. 2022). Degraded arable land that is characterized by high concentrations of certain soluble salts especially NaCl poses multiple negative impacts on agronomic crop yield and results in notable economic degradation especially in arid to semi-arid environment (Ali *et al*. 2021). The Food and

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Agriculture Organization (FAO) predicts that the application of saline water for crop irrigation, inappropriate irrigation land drainage and a noticeable increase in global temperature increase the amount of saline degraded land in different regions of the world (Negacz *et al*. 2022). Elevated salt concentration in the root medium reduces the plant water potential, which in turn inhibits proper cell division. It also damages plant antioxidant enzymes and plasma membrane function, causes stomatal closure, removes water from the plant and lowers the amount of CO_2 inside the olant photosynthetic sites (Parihar *et al*. 2015; Zulfiqar et al. 2022). High NaCl contents in the soil subjected to crop plants growth inhibition, imbalance nutrients uptake, specific ions toxicity and plants life sustaining process photosynthetic inhibition (Ali *et al*. 2017; Zulfiqar, 2021). Human induced soil salinity converts the arable land into salt prone zones and it is estimated that 50 % of cultivated land will be out of cultivation up to 2050 due to high soluble salt contents (Hussain 2019). Abiotic environmental stress such as drought, salinity and temperature variations alter wheat plant growth patterns and biochemical reactions that ultimately reduced grain yield (Mehmood *et al*. 2021; Alhaithloul *et al*., 2023; Hayat et al. 2024; Zulfiqar *et al.* 2024a). Wheat is moderately salt tolerant agronomic crop however; wheat plant physiology and biochemical process are disturbed when subjected to salt stress that leads to lower plant vigor and low grain yield. (Abobatta *et al*. 2020). In Pakistan, out of total 21 million hectares of arable land, 6.67 million are vulnerable to elevated saline stress. Due to its ability to withstand harsh environments and its multiple uses as a food source for humans, animal feed and a raw material for agro-based industry of the world, sustainable production of wheat is in the spotlight under current scenario of climate change (Cui *et al*. 2022).

Chromium (Cr) is found in all spheres of the environment including air, water and soil and its compounds are highly detrimental to plant growth (Stambulska *et al*. 2018). Chromium can enter the ecosystem as contaminated water, airborne particles and sludges that deteriorate the quality of water and soil ability to provide certain essential nutrients for plant growth not only near source but also on locations thousands of kilometer apart (Ali *et al*. 2013; Ma et al., 2024; Zulfiqar et al., 2024b). Another instance is the excessive use of Cr in leather and electroplating industry along with use of phosphorus (P) and other organic fertilizers which are known to have significant amount of Cr (Gupta *et al*.2013). Plants exposed to chromium stress show stunted growth (El Nemr *et al*. 2015), poor physiology (Shahid *et al*. 2017), less photosynthetic activity, inferior gas exchange attributes and plant water relations along with imbalance mineral nutrition (Lukina *et al*.

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2016). Although Cr can stimulate growth of certain plant species at lower concentration (Sathya *et al*. 2020), but its higher concentration in the growth medium may inhibit various metabolic activities in wheat and may even lead to a complete damage (Datta *et al*. 2011). Cr tends to bind sulfhydryl group of enzymes results in suppressed functioning of essential biological components. Heavy metals induced phytotoxicity is closely related to the generation of ROS in plants. It is observed that excess Cr in the growth medium leads to significant production of H_2O_2 and membrane lipid peroxidation in wheat plant (Adrees *et al*. 2015).

As salinity and Cr are toxic at all concentrations or above certain threshold level, their interaction and its influence on plant growth should be taken into consideration and investigated. Until now most researchers focus on response of plant to imposition of a single stress but in nature, plant often confront to more than one stresses, however very few studies in the literature are reported on the coincide behavior of salinity and metal element. The current experiment was conducted with an objective to investigate the alteration in growth, gas exchange and ionic response of two wheat genotypes under combine effect of NaCl and chromium. The working hypothesis is under current climatic change conditions, plants may have subjected to more than one abiotic stress which is a major reason for low growth and quality of wheat crop. We sought to clarify several stress indices under several abiotic stresses and this information will provide a novel approach to scientists working on salt effected and metal contaminated soils.

2. MATERIAL AND METHODS

2.1 Growth Conditions and Treatment Plan

The current project was carried out at The Islamia University of Bahawalpur (29.354º N, 71.691º E, 25.7 ºC and 28% humidity and 153 mm precipitation in the form of rainfall, Pakistan. Certified sterilized seeds of two wheat genotypes Sahar (V_1) and Lasani (V_2) were sown in moist sand culture. Wheat seedlings at two leaf stage were uprooted and transferred to Styrofoam sheet fixed on the upper surface of glass tubs having 50 liters of distilled water. Proposed salt (control and 100 mM NaCl) and Cr levels (15 µM and 30 µM) in sole and interactive form were mixed by calculating the required amount of NaCl and $K_2Cr_2O_7$. The wire house-controlled conditions experiment was conducted by following complete randomized design with split plot arrangement and each proposed treatment is repeated with four replications. Treatments include control (T_1), 15 µM L^{-1} Cr (T_2), 30 µM L^{-1} Cr (T_3), 100 mM NaCl, (T_4), 15 µM 1Cr + 100 mM NaCl (T_5), 30

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μM Cr + 100 mM NaCl (T_6). Oxygen was supplied to plants by artificial oxygen provision air pumps and half strength Hogland solution as proposed by Hoagland and Arnon, 1950 was provided to maize seedlings as a nutrient supplying media for growth. The pH range of the solution was maintained at 6 ± 0.5 till the harvesting of the wheat seedlings.

2.2 Plant growth and Physiological attributes

Wheat seedlings were harvested at seedling stage, plant shoot and root length were measured by using measurement scale and root area meter (WinRhizo, 2022A, Netherlands), while fresh and dry biomass was measured by using analytical weighing balance. Relative water contents (RWC) were calculated for wheat plant leaves according to the method adopted by Ahmed *et al*. 2022 by selecting 2 cm of fresh upper leaves (mid-rib free leaves). Fresh mass (FM) and dry mass (DM) of leaves disc were weighed and the fresh samples were placed overnight in stoppered vials containing ion-free distilled water for 24 hours turgid mass (TM). To calculate RWC, the following equation was applied:

$$\mathrm{RWC} = \frac{FW - DW}{TW - DW} \times 100$$

Fresh upper fourth leaves sample (0.2 g) were boiled in deionized distilled water (10 mL) in a water bath for a half-hour at 40 °C (C_1) and at 100 ºC for ten minutes (C_2). (Gautam *et al*. 2023). To calculate RWC, the following equation was applied:

$$\mathrm{MSI} = 1 - \left(\frac{EC_1}{EC_2}\right) \times 100$$

In both wheat genotypes, the chlorophyll contents in plant samples were determined by following the method described by Wellburn 1994 by using UV visible spectrophotometer (UV-1720, Shanghai, China) while leaf area was measured by using leaf area meter (WinFolia, 2022A, Netherlands).

2.3 Gas Exchange Attributes

Gas exchange attributes in seedlings of both wheat genotypes (photosynthetic rate and transpiration rate) were taken between 9.00 AM and 11.00 AM by taking young expanded leaf of

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each plant from each treatment in the leaf cuvette portion of infrared gas analyzer (IR202, Yokogawa, Japan) (Ali et al., 2025)

2.4 K^+ and Na^+ contents

Leaf tissues from wheat seedlings (0.5 g) was taken and samples for the determination of K^+ and Na^+ contents were prepared by following the method demonstrated by Chapman and Pratt 1961 through flame photometer (FP-910- Camspec, UK).

2.5 Chromium concentration in plant tissues

The chromium contents in plant tissues were determined by adopting differential centrifugation of subcellular fractions of roots and leaves. Root and leaves samples (1g) were homogenized and centrifuged at 3000 x g for 15 minutes at 4 ºC by adopting the method describe by Sun *et al*., 2023. The chromium contents in cell fractions of root and leaves of wheat genotypes were determined by using atomic absorption spectrophotometry (Zeng *et al*. 2011).

2.6 Statistical Analysis

All values reported in this study are analyzed by using statistical software statistics 8.1 (USA). The bars in the graph depict the values of four replicates and the error bars are the standard deviations. The bars not showing the same lower-case letters are significantly differ from one another at $P < 0.5$ (Steel and Torrie 1960).

3. RESULTS

3.1 Plant Biomass

The analyzed data under salinity and Cr stress regarding growth attributes of wheat genotypes (Figure 1 and Figure 2) reveals that salt stress exerted strong negative impacts on root and shoot length, fresh and dry biomass along with leaf area of both wheat genotypes used in the current project. Cr at low level (15 µM) did not significantly affect all examined morphological attributes; however, maximum reduction in terms of growth (more than 50 %) was observed under high chromium and salt level (30 µM Cr + 100mM NaCl). Combine effect of Cr and salt stress differ significantly among both wheat genotypes and it was observed that maximum fresh and dry biomass was shown by wheat genotypes Sahar as compared to wheat genotypes Lasani which depicts its tolerance against both abiotic stress.

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3.2 Physiological Attributes

In the present study, it was observed that in both wheat genotype seedlings, the application of a saline treatment (100 mM NaCl) and Cr substantially ($P < 0.05$) reduced membrane stability, RWC, and chlorophyll levels (Figure 3). To investigate variations among the wheat genotypes in terms of total chlorophyll contents, RWC and MSI, these attributes were calculated in leaf strips of NaCl and Cr treated wheat genotypes. Maximum values were observed at control where no Cr and salt stress were applied. The results show that high salt and Cr concentration restricted plant water contents, membrane integrity and chlorophyll in both under examined wheat genotypes and maximum reduction was displayed by wheat genotypes Lasani as compared to wheat genotype Sahar. Combined NaCl and Cr stress (15 µM Cr + 100 mM NaCl) results in slight increase in chlorophyll contents and plant water relations while at higher concentration (30 µM Cr + 100 mM NaCl), a significant reduction was observed as compared to salt and Cr stress alone.

3.3 Leaf Gas Exchange

Several plant gas exchange and photosynthetic parameters in both wheat genotypes under combine effect of salinity and Cr stress are shown in Figure 4. Maximum values for transpiration and photosynthetic rate was recorded at control while gradual increase of NaCl and Cr in the growth channel reduced the wheat seedlings capacity of photosynthesis and transpiration as minimum values were recorded under combined application of 30 µM Cr + 100 mM NaCl. Stomatal conductance and internal CO_2 concentration follow the same pattern and as compared to control, show inferior values (30% and 35%) under high salt and Cr stress. However Low Cr stress with salt stress (15 µM + 100 mM) exhibits little increase in gas exchange attributes and this effect is more observed in wheat genotypes Sahar as compared to wheat genotypes Lasani.

3.4 Sodium / Potassium **(K^+/Na^+) Ratio**

The concentration of K^+, Na^+ and K^+/Na^+ ratio in leaves of both wheat genotype examined in the current experiment under elevated saline and Cr toxicity were measured in view to detect the combine effect of these two-abiotic stress on ion homeostasis. It was noticed that increase in Na^+ contents were more reported by wheat genotypes Lasani as compared to Sahar. Sodium concentration decreased under sole application of Cr (15 and 30 µM L^{-1}) while a remarkable increase in Na^+ is noted when NaCl was applied @ 100 mM. When compared with the control treatment where no stress compared to control, exposure of plants to sole application of salt and Cr stress results in remarkable reduction in K^+ concentration while a minor increase in K^+ concentration was noted at low Cr and salt stress (15 µM Cr + 100 mM NaCl) which facilitate K^+ uptake. The increase in Na^+ and Cr influx under combine stress results in poor uptake of K^+ contents resulted in inferior K^+/Na^+ ratio in wheat seedlings (Figure 5). The results also show that the maximum K^+ contents under all treatments resulted in maintaining highest K^+/Na^+ ratio in wheat genotypes Sahar, showing better growth under high NaCl and non-saline conditions.

3.5 Chromium Concentration in Root and Shoot

In the current project, exposure of wheat seedlings to NaCl and Cr contamination and their combine effect on chromium uptake in plant root and shoot is illustrated in Figure 6. A significant increase in Cr concentration in wheat seedlings were noted with increasing Cr stress being significantly higher in roots than in shoots**.** The effect of salinity on Cr contents varied with plant genotype, organs and Cr level. A remarkable decrease in root and shoot Cr concentration (33% and 52%) in wheat genotype Sahar was noted when NaCl was applied to growth medium with low Cr level (15 µM Cr + 100 mM NaCl). In roots of both wheat genotypes, maximum Cr concentration was observed under sole application of Cr @ 30 µM and least value was recorded at control while in shoots, the highest Cr concentration was observed at combined stress of high Cr and salt stress. Wheat genotype Sahar depicts low Cr uptake under all treatments and show its tolerance against sole and combine stress of Cr and NaCl.

3.6 Relationship between Chromium and Sodium (Na^+) Uptake and Growth Attributes of Wheat Genotypes

The Spearman's correlation results indicated that the Cr concentration in the roots of Lasani and Sahar cultivar is significantly ($P \leq 0.01$) positively correlated with the Cr concentration in the shoots while a significant negative relationship was found with the growth and gas exchange parameters of both wheat cultivars. Similarly, Na^+ ion concentration is significantly negatively correlated with growth and gas exchange parameters of Lasani and Sahar cultivar (Figure 7).

4. DISCUSSION

Wheat is a most significant source of plant proteins and have high nutritional contents than any other cereal crop. Chromium (Cr) is among the toxic heavy metals extensively found in soil and

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water, causing environmental toxicity. Soil salinization under current global warming situation is the most brutal abiotic environmental stress restricting wheat crop production worldwide. Soil salinity poses a major constrain to global crop productivity as most of the agronomic plant species are glycophytes (Guarino *et al*. 2020). There are certain regions where soils contaminated with elevated levels of certain heavy metals simultaneously report high soluble salt concentrations and most of these soils are found in semi-arid areas where high temperature, extensive use of agrochemicals and some mining activities releases soluble salt and heavy metals (Zaman *et al*. 2018). In this study, application of Cr and salt stress in sole or in combined form found to be menacing for wheat plant growth by altering plant physiology, photosynthetic and specific ion toxicity. Reduction in root length, fresh and dry biomass might be due to higher accumulation of Na^+ and Cr causes toxicity in the rhizosphere, affecting permeability of cell membrane and causes accumulation of toxic ions at cellular level results in imbalance nutrient uptake, ceases the process of cell elongation and injuring hypocotyls (Zhang *et al*. 2020; Sheetal *et al*. 2016). Decreased plant height, shoot fresh and dry biomass is mainly due to consequent lower root growth, disturbed osmotic potential and less water and nutrient transport to aerial parts of the plant which ultimately results in reduced size and number of leaves under high salt accumulation (Stavridou *et al*. 2019; Moosa et al., 2024). High Cr stress disturbed plant photosynthetic activities and results in production of ROS which drastically reduced plant biomass (Wang *et al*. 2021). Similar reduction in plant biomass under Cr and NaCl stress was previously reported by Raja et al. 2023 in tomato and Javed et al. 2022 in maize.

Major physiological markers to sustain and improve plant productivity under abiotic stress environment are chlorophyll contents, plant cell membrane stability and plant water relations. In the current experiment, salt and Cr stress imposition drastically reduced the plant water contents and MSI in wheat and this effect was more eminent in wheat genotypes Lasani while least in wheat genotypes Sahar. Similar findings were previously reported by Mushtaq et al. 2021 in okra and Mustafa et al. 2024 in pepper. Elevated levels of Na^+ influx and ROS production resulting from higher NaCl and Cr contents in the growth medium have a substantial impact on plant balanced nutrient uptake. These both abiotic stress also enhances plasma membrane permeability which leads to low production of chlorophyll and alter stomatal opening and transpiration (Kumari *et al*.2018; Ramzan *et al*. 2023).

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Additionally, the production of ROS impose oxidative stress, severely damaging plant cell plasma membrane and lowers water retention capacity of plant cells which results in cell damage leading to cell death (Shah *et al*. 2017). Cr reduced chlorophyll contents which results in plant growth inhibition (Noman *et al*. 2020). Cr toxicity induces modifications and alteration in plant metabolism and suppresses production of pigments necessary in life retention of plants such as chlorophyll (Singh *et al*. 2017). Improvement in plant water relations and membrane stability at lower level of Cr under saline condition might be due to complex formation between Cr and Cl^- (Ertani *et al*. 2017) and these results were previously supported by Ali *et al*. 2012 in Barley.

When plants are subjected to salinity and heavy metal stress, different plant physiological bases shown insight reduction of plant gas exchange parameters (Sharifi and Bidabadi 2020). In current study, with increasing level of Na+ in the growth channel, reduced photosynthetic and transpiration rate along with stomatal conductance were recorded. Same results were previously reported by Liao et al. 2024 in maize. The maximum reduction in leaf gas exchange attributes was shown by wheat genotypes Lasani as compared to wheat genotypes Sahar. It might be a consequence of lower water contents and poor water availability in the root medium under saline environment which was responded by plant by taking adopted measures by packing stomata (Shahbaz and Ashraf 2013). Addition of Cr with NaCl at high concentration facilitate further reduction of plant gas exchange parameters as addition of chromium and its accumulation in plant upper ground parts results in poor plant metabolism and reduced leaf size and growth results in poor plant gas exchange. In our experiment, low concentration of Cr along with NaCl results in a slight improvement in plant gas exchange and these findings were earlier supported by Ali *et al*. 2011 in barley.

Production of plant life sustaining substances, proper plant metabolism and plant survival under abiotic environmental stress are highly dependent on plant K^+/Na^+ ratio. When plants are subjected to saline environment, the higher Na^+ in the rhizosphere hinders the uptake of K+ which ultimately results in lower K^+/Na^+ ratio which in return impact normal plant metabolism and physio-biochemical reactions (Kumar *et al*. 2021). Elevated concentration of Na^+ damage chlorophyll biosynthesis, cause oxidative damage to the plant roots and reduction in leaf turgor potential which are established as crucial parameters in salt induced growth inhibition in various agronomic and horticultural crops (Maqbool *et al*. 2020). The imbalance uptake of nutrients and

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specific ion toxicity results in poor plant dry matter built up and reduce plant tolerance against abiotic environmental stress. Cr accumulation in different organs of plant vary significantly and Cr was poorly translocated from roots to shoot in this study and similar findings were supported in mungbean (Jabeen *et al*. 2016) and in rice (Ma *et al*. 2016). Immobilized nature of Cr in vacuoles of root cells might be the reason might be the reason of higher accumulation of Cr in roots of the plant. Under combined effect of Cr and NaCl, reduction of Cr concentration in roots was noted. Less values of Cr in plant shoot was observed because the movement of Cr is confined from the root to the plant apex as a consequence of ion binding in the root at the place of cationic exchange and immobilization of Cr root cells (Sinha *et al*. 2018). Despite lacking a specific mechanism for Cr uptake, plant roots can absorb Cr along with other essential plant nutrients. Therefore, Cr may interfere with essential plant nutrients and also compete for the same carriers for its transport within plant (Guarino *et al*. 2020). These results are parallel with Samrana et al. 2020 who reported decrease in K^+ and other essential nutrients uptake in cotton under Cr stress. High salt concentration results in increased uptake and accumulation of Cr in plant as high Na^+ influx deteriorate membrane structure, increase electrolyte leakage and increase permeability of plant cell that facilitate more passage of Cr inside the plant cell (Singh *et al*. 2013).

CONCLUSION

Several arid to semi-arid areas of the world are simultaneously affected by chromium and high soluble salt stress. In the current study, the alteration in growth, gas exchange, biochemical and ionic response of two wheat genotypes under combine effect of NaCl and chromium were investigated. Although previous studies reported the interaction of salinity and heavy metals stress on agronomic and horticultural crops, however, their interactive effects on wheat are poorly understood. Soil salinity and heavy metals stress results in desertification of large agricultural land and remarkable economic losses in wheat and other crops. High salt and chromium stress accounts for obstructive changes in morpho-physiological features and also accounts for alteration in photosynthetic activity and mineral nutrition in wheat. Application of low Cr with salinity results in little improvement in plant biomass, physiology, gas exchange attributes along with improved K/Na ratio. Wheat genotype Sahar show improved growth at all treatments as compared to wheat genotypes Lasani. Our results provide useful information to scientists working on wheat in saline

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agriculture and Cr contaminated soils and offer new dimensions of research under multiple abiotic stresses.

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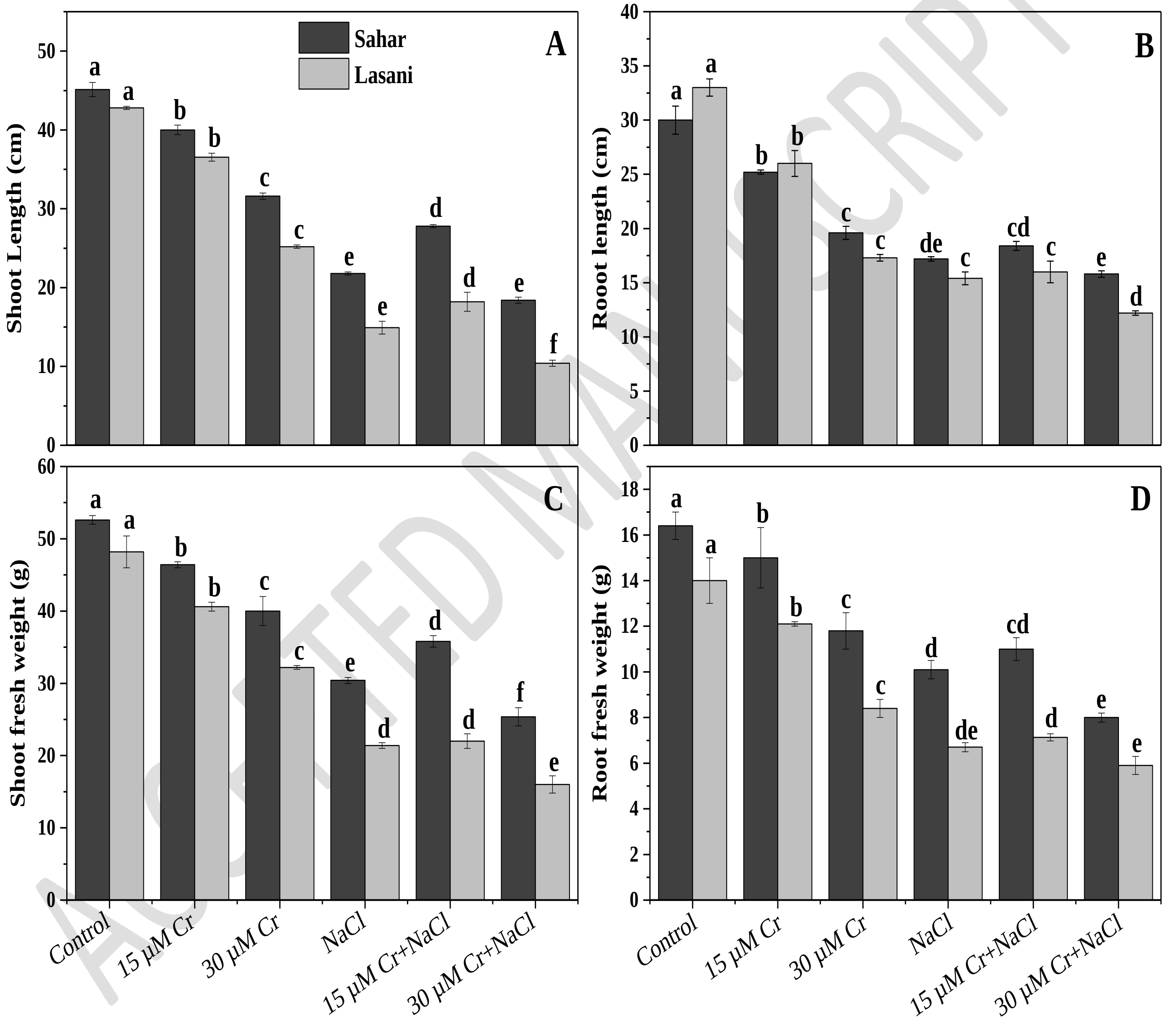

Figure. 1. Sole and combine effect of chromium and NaCl stress on plant fresh biomass of two wheat genotypes. The stated bar values show the average of four biological replications. The bars that do not have the same lowercase letter (LSD) differ from one another at *P* <0.5 level.

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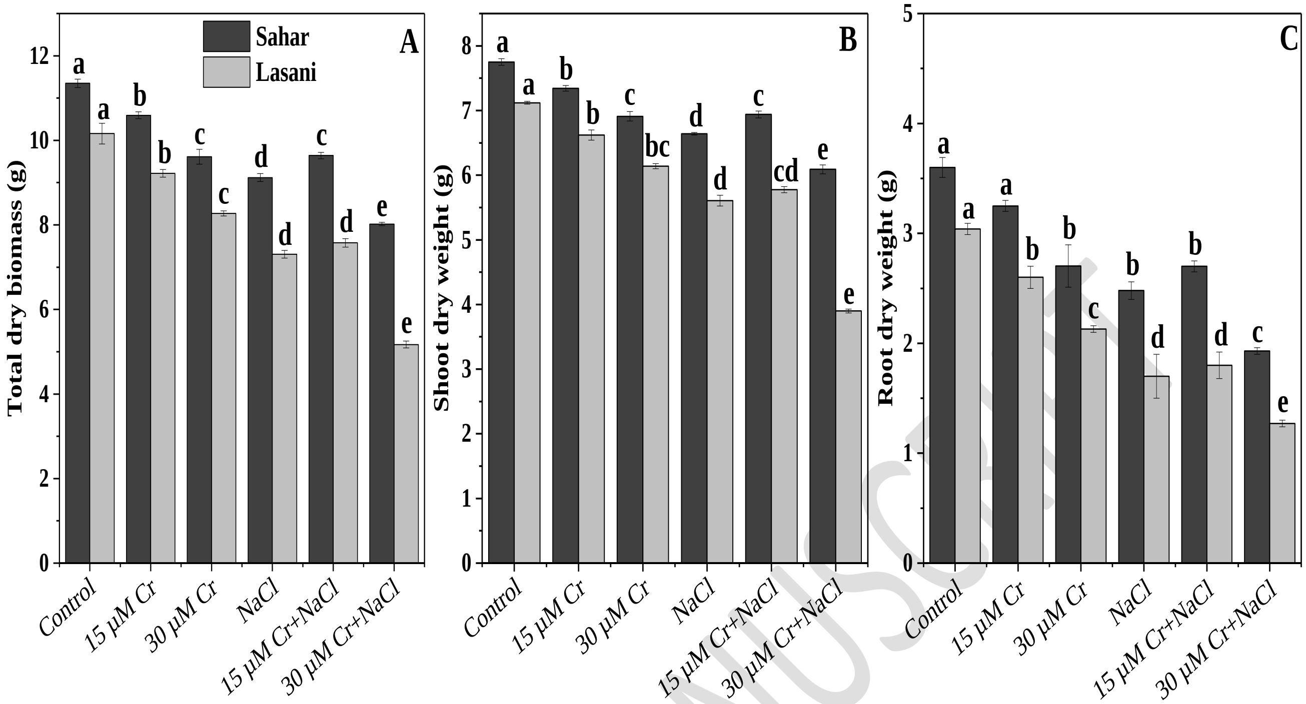

Figure. 2. Sole and combine effect of chromium and NaCl stress on plant dry biomass of two wheat genotypes. The stated bar values show the average of four biological replications. The bars that do not have the same lowercase letter (LSD) differ from one another at *P* <0.5 level.

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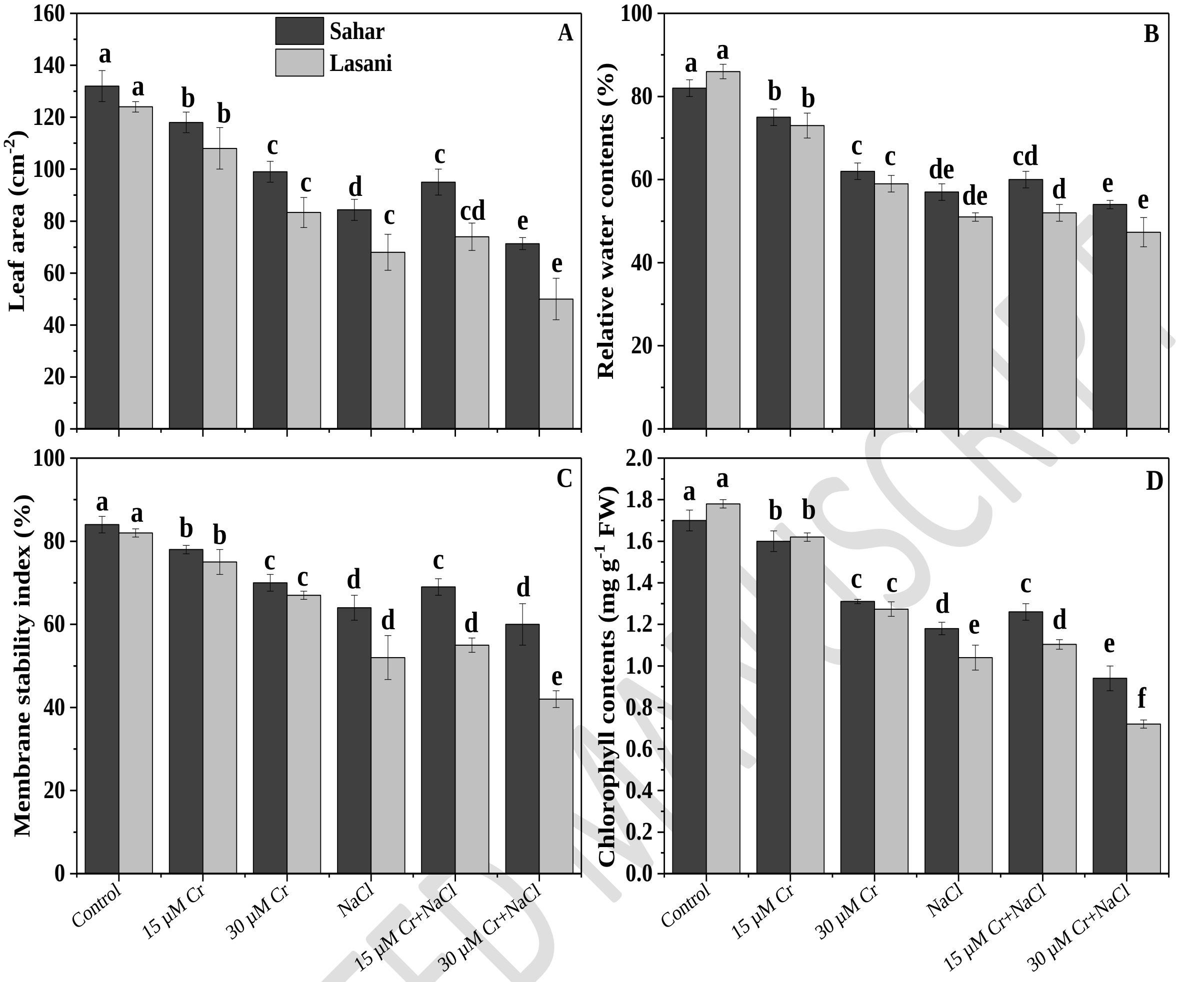

Figure. 3. Sole and combine effect of chromium and NaCl stress on physiological attributes of two wheat genotypes. The stated bar values show the average of four biological replications. The bars that do not have the same lowercase letter (LSD) differ from one another at *P* <0.5 level.

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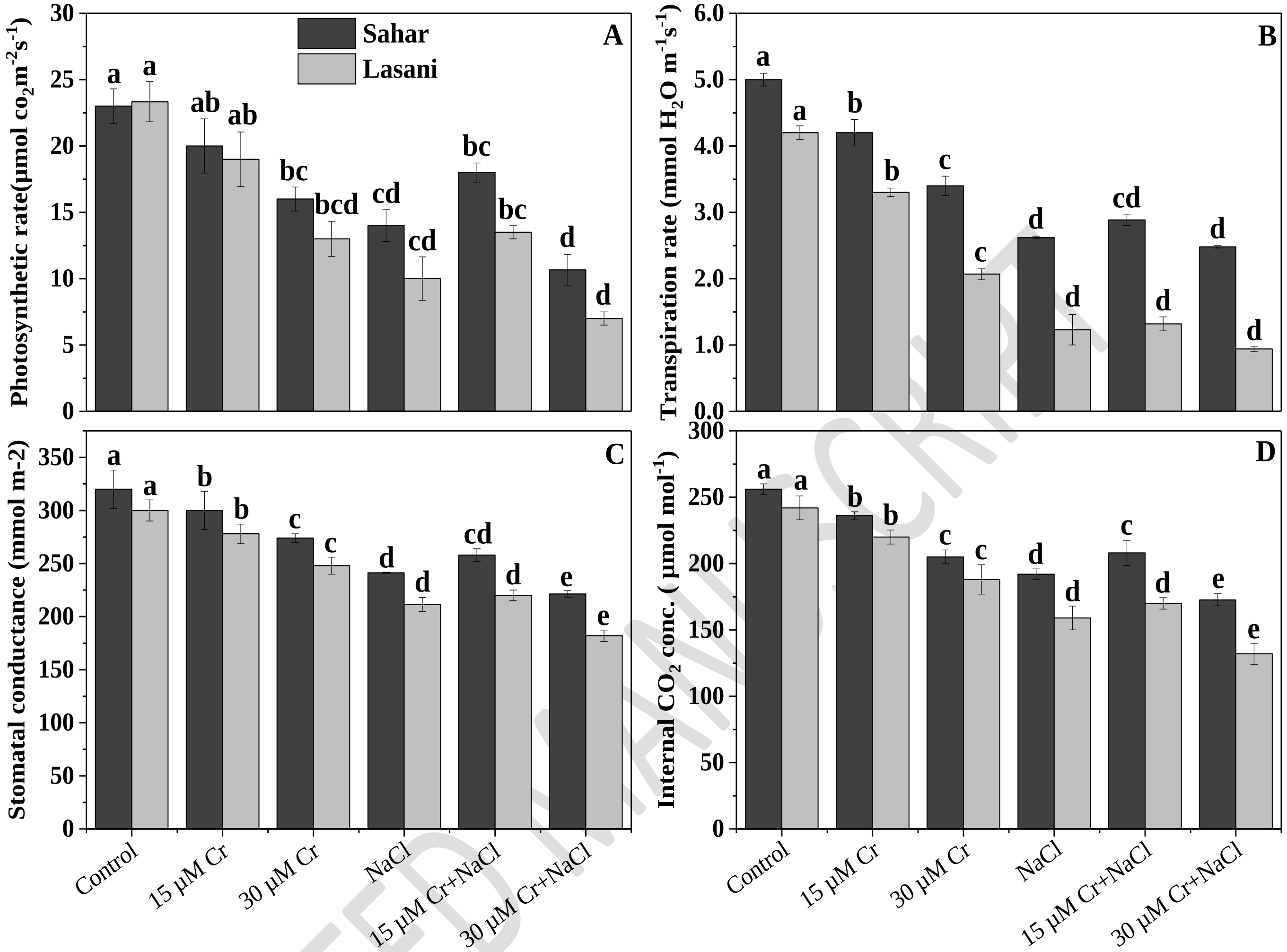

Figure. 4. Sole and combine effect of chromium and NaCl stress on p of two wheat genotypes. The stated bar values show the average of four biological replications. The bars that do not have the same lowercase letter (LSD) differ from one another at *P* <0.5 level.

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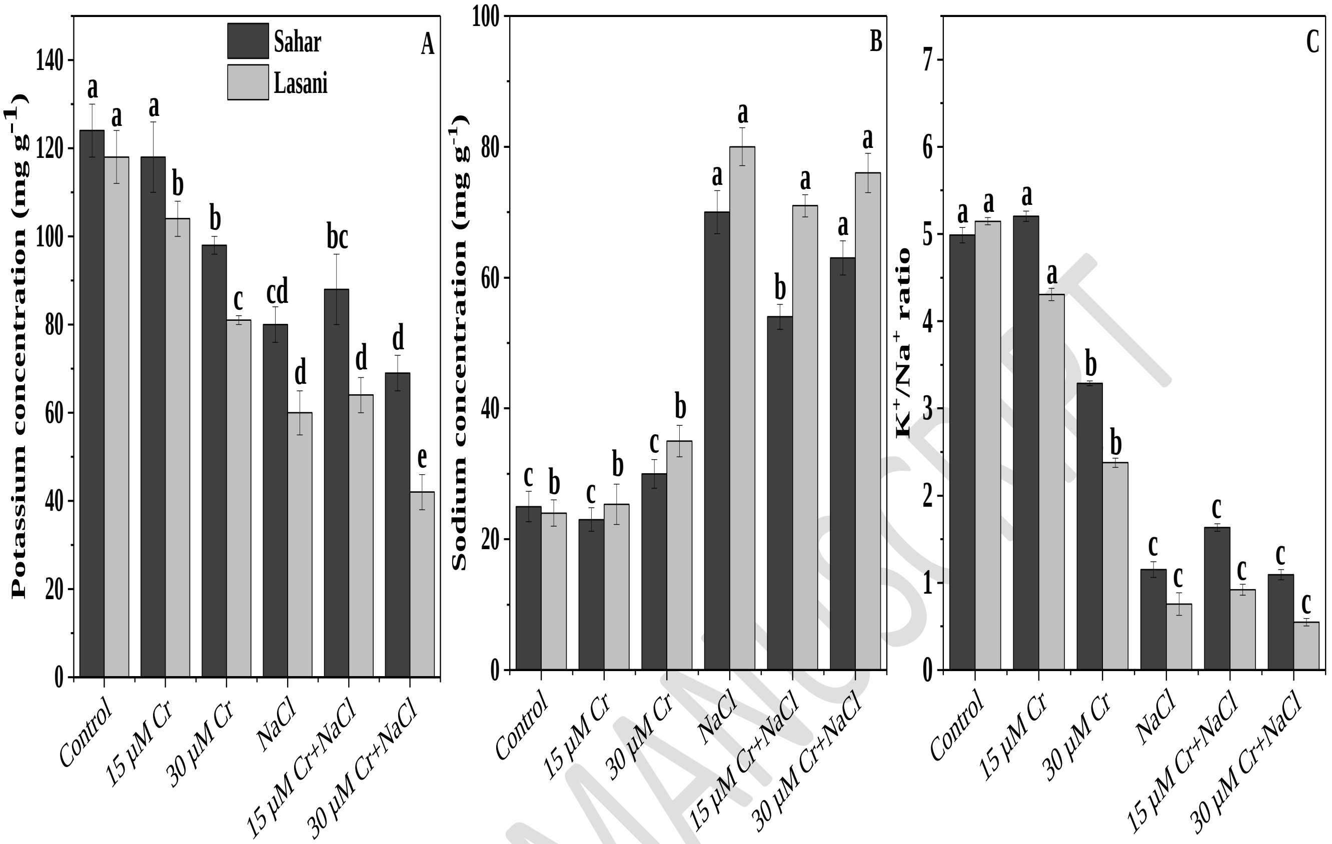

Figure. 5. Sole and combine effect of chromium and NaCl stress on K^+/Na^+ ratio of two wheat genotypes. The stated bar values show the average of four biological replications. The bars that do not have the same lowercase letter (LSD) differ from one another at *P* <0.5 level.

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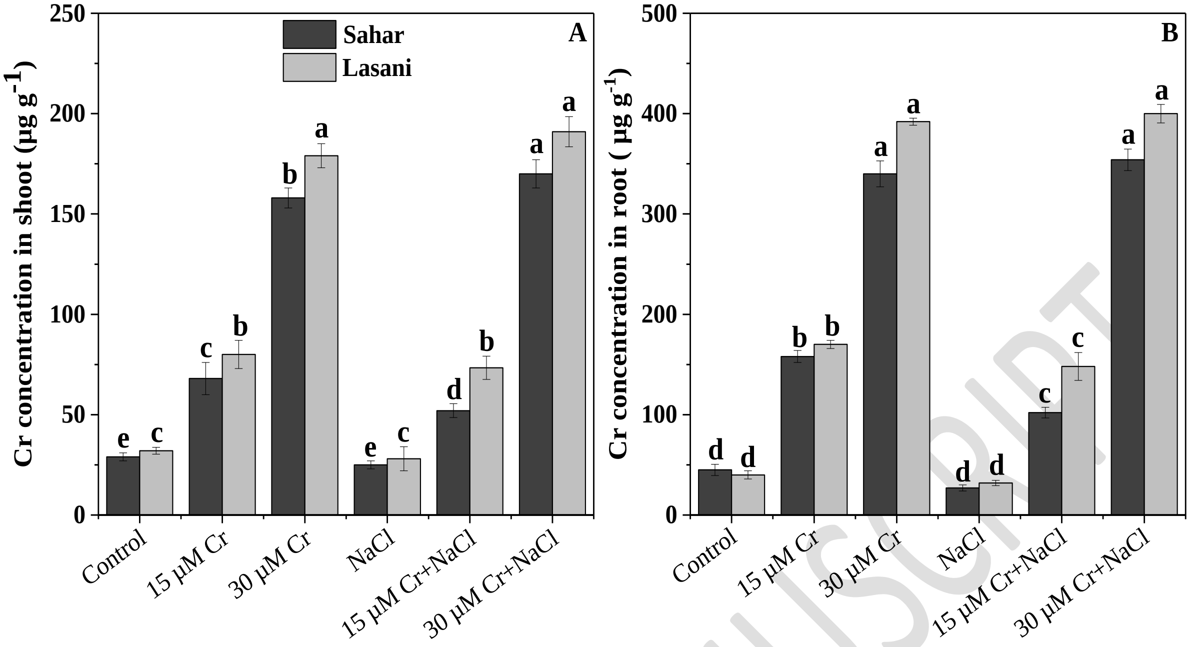

Figure. 6. Sole and combine effect of chromium and NaCl stress on chromium uptake in root and shoot of two wheat genotypes. The stated bar values show the average of four biological replications. The bars that do not have the same lowercase letter (LSD) differ from one another at *P* <0.5 level.

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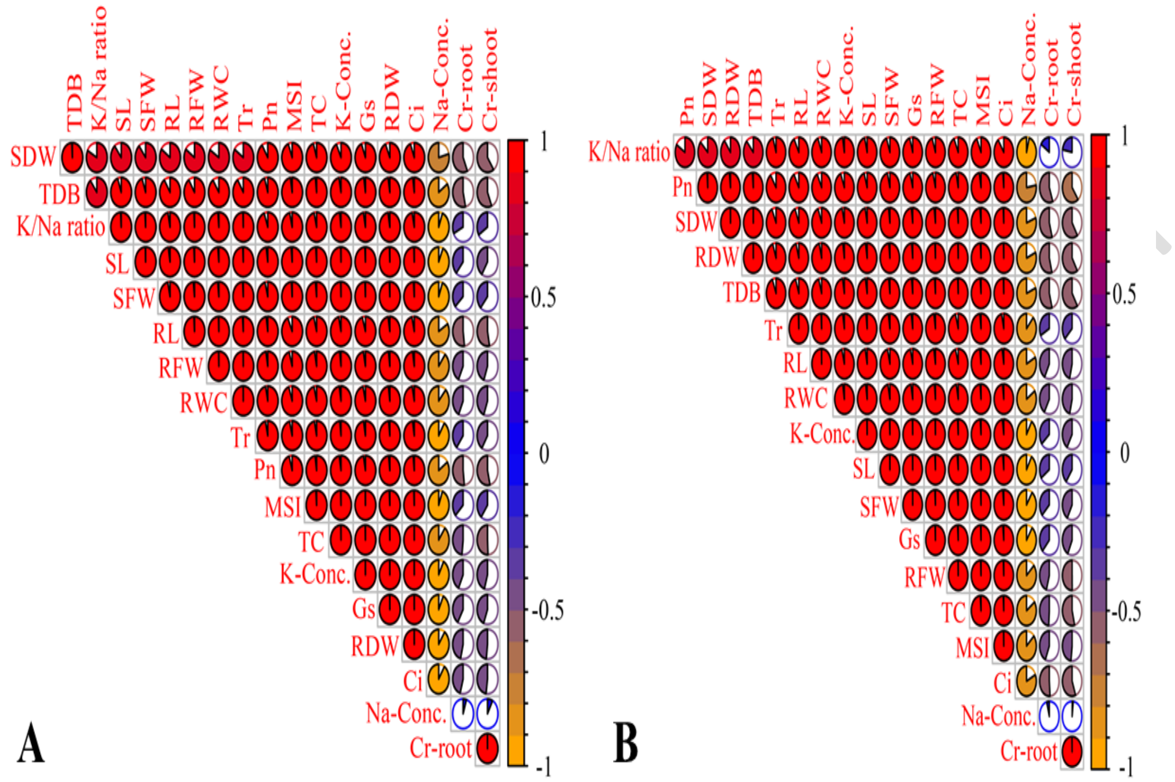

Figure. 7. Matrices of Spearman's correlation coefficients between shoot and root Cr concentrations and different measured variables of two wheat genotypes i.e. Lasani (A) and Sahar (B) at $P \leq 0.01$ (n = 18).

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