USE OF GENETIC VARIABILITY AND MINERAL NUTRITION TO MINIMIZE CADMIUM ACCUMULATION IN WHEAT

By
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2001-ag-1002
M.Sc. (Hons.) Soil Science

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY
IN
SOIL SCIENCE

INSTITUTE OF SOIL AND ENVIRONMENTAL SCIENCES
FACULTY OF AGRICULTURE
UNIVERSITY OF AGRICULTURE, FAISALABAD,
PAKISTAN
2014
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Faisalabad.

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Asif Naeem
2001-ag-1002
Dedicated to My Parents
ACKNOWLEDGEMENTS

Financial support by Higher Education Commission (HEC) of Pakistan to conduct this research under the project “Safe Food Production from Soils Contaminated with Cadmium” is gratefully acknowledged. I am also thankful to my employer, Pakistan Atomic Energy Commission (PAEC), for granting me permission to continue Ph.D. studies along with official assignments at office.

I express my sincere gratitude and thanks to my supervisor, Dr. Saif Ullah, Assistant Professor, Institute of Soil and Environmental Sciences (ISES), University of Agriculture (UAF), Faisalabad, for his continued guidance during the studies and constructive suggestions that have improved the quality of this dissertation. Appreciations and sincere thanks to Dr. Abdul Ghafoor, Professor (Rtd.) of Soil Science and Dr. Muhammad Farooq, Associate Professor, Department of Agronomy, UAF for serving on my committee and for their valuable comments and suggestions. Dissertation was also reviewed by Dr. Zed Rengel, Winthrop Professor, The UWA Institute of Agriculture during my stay at UWA for six months’ research training in his laboratory funded by HEC, Pakistan. I am highly thankful to him for hosting and guiding me to grow as a research scientist. Mr. Faqir Hussain, Ex-Head Soil Science Division, NIAB (Rtd.) deserves a lot of thanks for his motivating thoughts and reviewing the manuscript very critically.

Words can’t express feeling of thanks for my parents who wrote chapter of their life only for the sake of my happiness. It is my pleasure to especially thank Dr. Muhammad Zia-ur-Rehman, Assistant Professor, ISES, UAF who had always been helping me like an elder brother and Dr. Muhammad Sabir (Assistant Professor) for making my stay at the Institute unforgettable owing to his cheerful nature.

I pay sincere thanks to the academic and research staff at the Institute of Soil and Environmental Sciences who had been supportive at various stages of my Ph.D. studies. I offer many sincere thanks to my friends Tasneem Akhtar, Saqib Abubakar and Behzad Murtza for their assistance in executing experiments and analytical work.

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<td>APX</td>
<td>Ascorbate peroxidase</td>
<td>CEC</td>
<td>Cation Exchange Capacity</td>
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<td>CAT</td>
<td>Catalase</td>
<td>WWF</td>
<td>World Wide Fund</td>
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<td>GPX</td>
<td>Guaiacol peroxidase</td>
<td>NEQSP</td>
<td>National Environmental Quality</td>
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<td>SOD</td>
<td>Superoxide dismutase</td>
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<td>Standards of Pakistan</td>
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<td>MDA</td>
<td>Malondialdehyde</td>
<td></td>
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<tr>
<td>P</td>
<td>Phosphorus</td>
<td>Cr</td>
<td>Chromium</td>
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<tr>
<td>Cd</td>
<td>Cadmium</td>
<td>Se</td>
<td>Selenium</td>
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<td>Zn</td>
<td>Zinc</td>
<td>U</td>
<td>Uranium</td>
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<td>SCd</td>
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<td>RCd</td>
<td>Cadmium Concentration in Roots</td>
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<td>LSCd</td>
<td>Low shoot cadmium</td>
<td>HSCd</td>
<td>High shoot cadmium</td>
</tr>
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<td>FAO</td>
<td>Food and Agriculture Organization</td>
<td>ATSDR</td>
<td>Agency for Toxic Substances And Disease Registry</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
<td>PSCs</td>
<td>Pollution Safe Cultivars</td>
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<tr>
<td>ROSs</td>
<td>Reactive oxygen species</td>
<td>SEK</td>
<td>Swedish Krona</td>
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<td>RSi</td>
<td>Silicon concentration in roots</td>
<td>SSi</td>
<td>Silicon concentration in shoots</td>
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<td>CdTI</td>
<td>Cadmium translocation index</td>
<td>IARC</td>
<td>International Agency for Research on Cancer</td>
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<td>Si</td>
<td>Silicon</td>
<td>BCF</td>
<td>Bio-concentration factor</td>
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<tr>
<td>SEF</td>
<td>Shoot enrichment factor</td>
<td>EF</td>
<td>Enrichment factor</td>
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<tr>
<td>TF</td>
<td>Translocation factor</td>
<td>GEF</td>
<td>Grain enrichment factor</td>
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<td>SEF</td>
<td>Shoot enrichment factor</td>
<td>SAR</td>
<td>Sodium adsorption ratio</td>
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<td>RSTI</td>
<td>Root to shoot Cd translocation index</td>
<td>UAF</td>
<td>University of Agriculture, Faisalabad</td>
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<td>mM</td>
<td>Millimole</td>
<td>PHS</td>
<td>Porous hydrated silica</td>
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<tr>
<td>µM</td>
<td>Micromole</td>
<td>EC</td>
<td>Electrical conductivity</td>
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<td>Full Form</td>
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<tr>
<td>nm</td>
<td>Nanomole</td>
<td></td>
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<tr>
<td>DAT</td>
<td>Days after transplantation</td>
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<td>AOAC</td>
<td>Association of Official Analytical Chemists</td>
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<tr>
<td>LSD</td>
<td>Least significance difference</td>
<td></td>
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<tr>
<td>RSDM</td>
<td>Relative shoot dry matter</td>
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<tr>
<td>CV</td>
<td>Coefficient of variation</td>
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<tr>
<td>DM</td>
<td>Dry matter</td>
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<td>SoZn</td>
<td>Post-harvest soil Zn</td>
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<tr>
<td>UV</td>
<td>Ultra violet</td>
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<tr>
<td>TBA</td>
<td>Thiobarbituric acid</td>
<td></td>
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<tr>
<td>RDM</td>
<td>Root dry matter</td>
<td></td>
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<td>WUE</td>
<td>Water use efficiency</td>
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<td>ANOVA</td>
<td>Analysis of variance</td>
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<td>SoCd</td>
<td>Post-harvest soil Cd</td>
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<td>ESP</td>
<td>Exchangeable sodium percentage</td>
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<td>AAS</td>
<td>Atomic absorption spectrometer</td>
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<td>RRDM</td>
<td>Relative root dry matter</td>
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<tr>
<td>Ppm</td>
<td>Parts per million</td>
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<tr>
<td>SDM</td>
<td>Shoot dry matter</td>
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<tr>
<td>PVP</td>
<td>Polyvinylpyrrolidone</td>
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<tr>
<td>NBT</td>
<td>Nitroblue tetrazolium</td>
<td></td>
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<tr>
<td>TCA</td>
<td>Trichloroacetic acid</td>
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<td>PPFD</td>
<td>Photosynthetic photon flux density</td>
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ABSTRACT

Cadmium is toxic heavy metal soil pollutant and contamination of plant-based foods accounts for at least 70% of Cd intake by humans. Cultivating low-Cd plant species and optimum application of zinc (Zn) and silicon (Si) nutrients was hypothesized as a possible solution to avoid Cd intake. The research project is comprised of six separate experiments focusing on four major issues: i) identification of variation in Cd accumulation and tolerance among wheat cultivars under cultivation in Pakistan; ii) evaluating root zone acidification and antioxidants response of low and high Cd accumulating wheat cultivars to Cd stress; iii) underpinning the mechanism by which silicon could lower Cd in plants and optimizing its rate of application; and iv) determining the effect of combined Zn and Si application on Cd concentration in wheat grains. Considerable variation in shoot and root Cd concentration was observed among the wheat cultivars that was found to be regulated by differences in both absorption by roots and translocation to shoots. Decrease in root zone pH was not related to shoot Cd concentration of the cultivars and Cd concentration in low-shoot-Cd cultivars was related to sustained or higher activity of antioxidant enzymes which was not observed for high-shoot-Cd cultivars. Higher retention of Cd in roots of both low and high Cd accumulating cultivars while decrease in excessive transpiration only in HSCd cultivars with Si application proved to be the mechanisms suppressing Cd translocation to shoots. Higher increase in antioxidant activity with corresponding higher decrease in shoot Cd concentration in low-shoot-Cd cultivars suggested that improvement in antioxidant activity was associated with lowering Cd concentration in tissue. In soil, Si decreased Cd concentration in wheat cultivars by both decrease in plant available soil Cd and its translocation from roots to shoots. Moreover, application of Si at 150 mg kg⁻¹ proved to be the optimum level of Si that significantly lowered Cd concentration in wheat grains. The combined application of Zn and Si decreased Cd concentration in soil and consequently in grains and straw of wheat cultivars without affecting Zn concentration in grain and straw. Zinc decreased grain Cd concentration by lowering its translocation from shoot to grain and was depending on Zn uptake and translocation efficiency of wheat cultivars. Silicon induced improvement in grain yield and decline in Cd concentration was higher for salt-affected than normal soil and also for salt-sensitive compared to -tolerant wheat cultivars.
CHAPTER 1

INTRODUCTION

Pakistan is situated in arid to semi-arid regions and facing acute shortage of good quality irrigation water. Thus, farmers around big cities are compelled to use untreated city effluent to irrigate their soils. High nutrient contents and assured availability of untreated city effluent in periurban areas is another reason of its being popular source of irrigation. However, this city effluent receive discharge from different industries that contain toxic heavy metals including cadmium (Cd), human pathogens and have high sodium adsorption ratio. Continuous irrigation with such effluent have resulted in the accumulation of heavy metals along with soil salinity and/or sodicity problems (FAO, 1992). Other likely sources of Cd into soil are application of Cd contaminated phosphate fertilizers (Stephen and Calder, 2005) and atmospheric deposition (Alloway and Steinnes, 1999).

In plants, Cd inhibits uptake and translocation of nutrients such as nitrogen (Hernandez et al., 1996), zinc (Hart et al., 2002) and iron (Alcantara et al., 1994), interacts with water balance (Gong et al., 2005) and is involved in the production of reactive oxygen species (ROSs). In human body, critical organ for long-term Cd exposure is the kidney, where Cd principally affects renal tubular function. In extreme cases, chronic toxicity of Cd leads to pulmonary emphysema (shortness of breath) and bone fractures; Itai-Itai disease (Yeung and Hsu, 2005).

Cadmium is highly mobile in soil-plant system since it is easily absorbed and translocated to above ground tissues (Yang et al., 1998) and accumulation to phytotoxic levels may cause significant growth inhibition and yield loss. In plants, Cd could induce the production of ROS that could enhance lipid peroxidation resulting in loss of plasma membrane integrity leading to indiscriminate Cd absorption (Tian et al., 2012). Thus, plants equipped with defense mechanism against Cd toxicity, like enhanced antioxidant production, could be low in Cd accumulation in addition to better growth performance as reported by Wu and Zhang (2002) in barley. It was also shown that tocopherol (Vitamin E), an antioxidant in biomembranes, was crucial in Cd tolerance of plants (Collin et al., 2008). A number of studies have addressed the tolerance of cereals to Cd toxicity but its relationship with Cd accumulation
needs to be established. Cadmium could pose human or animal health risks at plant tissue concentrations those are not generally phytotoxic. This suggests that Cd accumulation in edible parts of plants should be checked so as to minimize its dietary intake to possible low level. It has been reported that at least 70% of average Cd intake by humans originates from plant foods (Wagner, 1993).

Avoiding anthropogenic Cd input to soil will ensure its low level in food but under some circumstances, P fertilizer application, soil loading with Cd has become a necessary evil. This situation urges the implication of some intervention or strategies to obtain low Cd food from these Cd contaminated soils. Use of soil amendments, phytoremediation, selection of low Cd accumulating species, nutrient optimization and other agronomic practices like crop rotation are the main strategies being undertaken to decrease soil-plant transfer of Cd. Application of various organic (compost, farmyard manure, biosoild and compost) and inorganic (lime, zeolites, P-compounds, Fe-oxides) amendments to immobilize (precipitate and/or fixation) Cd in soil presents a temporary solution to avoid Cd uptake by plants and inconsistent response with soil type and environmental conditions has been reported (Andersson and Siman, 1991; Li et al., 1996). Similarly, at present no plant with ability to tolerate and bioconcentrate high level of Cd (Chaney et al., 1997) has been identified so that it could be used for phytoextraction and if it would be available, proper disposal of the harvested parts will be another problem (Saifullah et al., 2009).

Variation in mineral uptake by plant has been recognized for many years (Saric, 1983) and there are now several examples exploiting these genetic differences to improve food safety (Li et al., 1995a). The Cd contamination has caused concern sufficient to exploit genetic variability in plants to produce low Cd food from Cd contaminated soil those are widely spread in occurrence. Difference in Cd concentration of the above ground edible parts of crops such as grains could be due to differential Cd absorption by roots, translocation from roots to shoots and a high translocation from shoots to grain (Arao and Ishikawa, 2006). Plant absorption is likely to be affected by desorption of Cd from soil into soil solution through release of various root exudates and also by cation exchange capacity of roots (root CEC). Differences in translocation from roots to shoots could arise from interaction of Cd with walls of vessels during translocation. Cultivars with genetically low level of Cd could give farmers greater
flexibility than restriction of crop production from soils with medium level of Cd contamination.

In addition to selection of genotypes, low Cd in cereal grains could also be achieved through optimizing plant nutrients in soil those may limit Cd uptake from soil and translocation within the plants. This strategy seems necessary if the cultivation of low Cd accumulating species/cultivars could not be adoptable because of their low yield potential and/or susceptibility to some diseases. Furthermore, low Cd accumulating behavior of plants may be improved through optimum application of essential nutrients which are antagonistic to Cd in absorption and translocation within plants. In this regard, Si and Zn have gained much attention in minimizing Cd in plants. Silicon is a beneficial plant nutrient and despite poor understanding of the mechanism involved, it is well known that Si decreases metal accumulation. Silicon deposition in root endodermis strengthens the casparian band that restricts the bypass inflow of metal from roots to shoots (Shi et al., 2005a). Co-precipitation of metal with Si as Cd-silicate in plants is another mechanism by which Cd transport is limited to the edible parts (Shi et al., 2005a). On the other hand, Zn is chemically similar to Cd and might act as competing cation for Cd absorption from soil by roots (Das et al., 1997) as well as have antagonistic interaction with Cd within the transport system of plants (Hart et al., 2002). It is also an integral part of various antioxidant enzymes of plants those strengthen plant body against Cd stress. It could also be supposed that under optimum Zn nutrition, Cd could not replace it in such enzymes to produce oxidative stress. Studies reveal that effects of Zn on Cd accumulation are not consistent and may differ with plant species and Zn and Cd concentration in soils. Moreover, studies on the interactive effect of Zn and Si needs to be elucidated for wheat cultivars grown in Pakistan.

Among soil factors, soil salinity could shift the equilibrium in favour of more soluble Cd species like CdCl₂ and CdCl⁺, thereby enhancing its availability to plants. Since salt-affected soils are wide spread in Pakistan and untreated city effluent also contain high amounts of soluble salts including Cl⁻ (Ghafoor et al., 2008), there would be pronounced availability of soil Cd to plants compared non-saline conditions (Weggler-Beaton et al., 2000). Thus, concentration and toxicity of Cd to plants is likely to be high on salt-affected compared to normal Cd contaminated soils.
Keeping in view the role of genetic variability and plant nutrition to minimize Cd accumulation by plants, present studies were planned with the following specific objectives:

1. To identify contrasting Cd accumulating wheat genotypes
2. To establish a relationship between Cd tolerance and accumulation in wheat genotypes differing in Cd accumulation.
3. To investigate the effect of Si application on Cd uptake, translocation and antioxidant activities in contrasting Cd accumulating wheat genotypes.
4. To explore the role of Si and Zn in combination on Cd accumulation and growth performance of wheat cultivars.
5. To investigate if presence of salts in soil does have any depressing effect on the antagonism between Cd and Zn for plant uptake?
CHAPTER 2

REVIEW OF LITERATURE

As a result of development of modern industry and agriculture, Cd has become a widespread pollutant of agricultural soils. It is one of the heavy metals toxic to both the plants and animals. This section reviews the sources, toxicity and various strategies to minimize Cd accumulation by edible parts of plants with special emphasis on differences in absorption by cultivar/species and mineral nutrition.

2.1. Contamination and Sources of Soil Cadmium

Generally the addition of Cd in soils is attributed to untreated sewage water irrigation (FAO, 1992), application of Cd containing phosphate fertilizers (Stephen and Calder, 2005) and atmospheric deposition (Alloway and Steinnes, 1999). But, the extent and distribution of these sources vary with the location. In Pakistan, use of raw sewage for irrigation, due to shortage of canal water, has become a common practice and has been reported to add heavy metals including Cd in soil. Qadir et al. (2000) found Cd concentrations in sewage of Faisalabad 3-fold (0.03 mg L$^{-1}$) higher than the permissible limit of 0.01 mg L$^{-1}$ for irrigation water (Ayers and Westcott, 1985). Mushtaq and Khan (2010) have reported even higher values of Cd (up to 0.13 mg L$^{-1}$) in effluent from various locations (Adiala, Pirwadhai, Texila and Wah factory) of Rawalpindi region. Similar results have been reported by Tariq et al. (2006) for Hayatabad Industrial Estate, Peshawar whereby a mean Cd concentration of 0.04 mg L$^{-1}$ with a range of 0.01 - .07 mg L$^{-1}$ was observed in effluent samples of eight industries. Effluent from none of the reported industries contained Cd below the permissible limit. But, these scientists attributed the Cd levels in effluents under the permissible level by comparing with permissible limit of 1.0 mg L$^{-1}$ proposed by National Environmental Quality Standards of Pakistan (NEQSP) (The Gazette of Pakistan, 2000). This permissible limit is 10-fold higher than the value described by Ayers and Westcott (1985), Rowe and Abdel-Magid (1995) and WWF (2007) and seems unrealistic as sewage of city drains below the permissible limit of 0.01 mg L$^{-1}$ found contaminating soils above permissible limit of 1.0 mg kg$^{-1}$ soil. This situation strongly
recommends that permissible level of Cd in city effluent must be reconsidered especially by NEQSP to ensure its loading in soil below permissible level. On the other hand, no accumulation of Cd in soils of 5 regions of Pakistan including Faisalabad, Multan, Lahore, Gujranwala and Kasur has been reported those were irrigated with untreated city waste effluent containing Cd from 0.001 to 0.002 mg L\(^{-1}\) (Murtaza \textit{et al}., 2012). These findings are contradictory to the 12-year earlier studies of Qadir \textit{et al}., (2000) in the suburbs of Faisalabad city that necessitates to identify whether there is a decrease in Cd load of city effluents or some other factors are responsible for this discordance.

Phosphate rock is used for the production of phosphate fertilizers owing to its high phosphate contents but the impurities it contains include various trace metals including Cd. These metals are transferred to P-fertilizers to varying amounts during production process. Contents of Cd in phosphate rock varies with origin and rock phosphates of sedimentary origin contain much higher concentrations of potentially hazardous elements (Cd, Cr, Se, and U) than that of igneous ones (Van Kauwenbergh, 2002). Cadmium contents in igneous phosphate rock of Finland ranges between 1-5 mg P kg\(^{-1}\) due to which fertilizers contribute only 4 % towards total Cd input into soils (FMAF, 2000). Javaid \textit{et al}., (2009) reported an average concentration of 7.2 mg Cd kg\(^{-1}\) of phosphate rock collected from Abottabad and Harripur districts of Pakistan which is below the limit of 10 µg Cd g\(^{-1}\) of phosphate rock implemented by European countries (Conceicaõ and Bonotto, 2006). Thus, instead of importing phosphate rock from Morocco/Egypt, local phosphate rock could be used for the production of P fertilizers to decrease the share of fertilizers in soil Cd load. Efforts are being undertaken to minimize Cd transfer to fertilizers during their manufacturing from phosphate rock containing high Cd contents. In New Zealand, for instance, based upon a voluntary agreement fertilizer manufacturing industries have decreased Cd concentration from 420 to 280 mg kg\(^{-1}\) of fertilizer P only in a few years (Taranaki Regional Council, 2005). Similarly, Swedish Board of Agriculture imposed a charge of SEK 30 per gram fertilizer Cd if the Cd content exceeds 5 mg kg\(^{-1}\) of fertilizer P and observed a decline in Cd contents of P fertilizers from 37 to 16 mg kg\(^{-1}\) fertilizer P within two years (Swedish EPA, 1997).

The fertilizer industries should be enforced to reduce the Cd content following options like; substitution of low-Cd phosphate rock with high-Cd one, production of phosphoric acid by thermal process instead of wet process, co-crystallization of Cd with CaSO\(_4\) anhydrite. Co-
crystallization seems most promising option but commercialization of this process is still under development. However, in developing countries, even system for monitoring Cd load of P fertilizer does not exist properly. It is thought that rational use of P fertilizers low in Cd contents could help reducing Cd accumulation in soil where fertilizers are the main contributors of soil Cd.

As far as the atmospheric deposition of Cd is concerned, maximum fall out is expected near the source of emission and it decreases away from the emission source. The atmospheric Cd comes from ferrous and non-ferrous metal production, combustion of fossil fuel and waste incineration (Alloway, 1999; ATSDR, 2012). Across the European Union, 3 g Cd ha\(^{-1}\) annum\(^{-1}\) has been reported to be deposited on agricultural lands (Kabata-Pendias and Mukherjee, 2007). The above said sources of atmospheric deposition do exist in Pakistan but surveys/studies on the atmospheric fall out of Cd on soils are lacking. The WHO (2000) has established a guideline value of 5 ng Cd m\(^{-3}\) of air for European Union. While in Pakistan, NEQSP has proposed a limit of 20 mg Cd m\(^{-3}\) of gaseous emission from any source of emission without any explanation to differ from the WHO standard. Probably, difference is based upon the quantity of atmospheric emission in the countries.

### 2.2. Cadmium Uptake and Transport in Plants

Although Cd is non-essential and toxic heavy metal, but yet it is easily taken up by plants. In plants, membrane potential of root epidermal cells might exceed -200 mV providing a strong driving force for the uptake of cations (Benavides et al., 2005). Toxic heavy metals compete with micronutrients and gain access into the plant cell via the same transporters operating for their uptake. Roth et al. (2006) and Papoyan et al. (2007) have reported the transport of Cd via the transmembrane carriers involved in uptake of Ca\(^{2+}\), Fe\(^{2+}\), Mg\(^{2+}\), Cu\(^{2+}\) and Zn\(^{2+}\). An iron transporter, OsNramp1, has been established as a Cd-influx transporter in the plasma-membrane of rice (Takahashi et al., 2011). Due to its high mobility and water solubility, Cd readily enters the roots through the cortical tissue and can reach the xylem via an apoplastic and/or symplastic pathway, complexed to organic acids or phytochelatins (Salt et al., 2000). Once loaded into the tracheary elements, Cd complexes spread throughout the entire plant following the water stream. Herren and Feller (1997) has attributed the grain accumulation of Cd in wheat to its mobility in phloem and xylem-phloem transfer in peduncle and lower
internodes. It has been hypothesized that Cd accumulation in developing fruits could occur via phloem-mediated transport, implicating a systemic diffusion of the heavy metals into the plant body (Benavides et al., 2005).

2.3. Cadmium Toxicity to Plants

Cadmium is toxic heavy metal and it can effect plant growth through a number of mechanisms. It can induce nutritional imbalance because of its physicochemical similarity to nutrient cations that lead to disorder in cell metabolism. Cadmium (1.03 Å) has been reported to decrease the uptake of Zn (0.83 Å) (Yang et al., 1996) and Ca (1.06 Å) (Obata and Umebayashi, 1997). In pea shoots, Cd inhibited NO₃⁻ reductase activity whereby absorption and transport of NO₃⁻ reduced from roots to shoots (Hernandez et al., 1996). Similarly, Cd induced inhibition of root Fe(III) reductase resulting in Fe(II) deficiency and severe decrease in photosynthesis as a consequence (Alcantara et al., 1994). The efflux of K⁺ from roots is a very good example of the sensitivity of K-ATPase and SH-groups of cell membrane proteins to Cd (Burzynski, 1987). Cadmium interacts with water balance by decreasing the size and number of xylem vessels (Gong et al., 2005). Being a non-redox metal, Cd has no direct role in the production of reactive oxygen species (ROSs) instead ROSs are the indirect consequence of Cd toxicity. It includes interaction with antioxidant system (Srivastava et al., 2004), disrupting the electron transport chain (Qadir et al., 2004) and disturbing the metabolism of essential elements (Dong et al., 2006). Peroxidation of lipid molecules by ROSs results in deterioration of biomembranes. So, in addition to restriction of electron transport and inhibition of chlorophyll synthesizing and Calvin cycle’s enzymes, damage to thylakoid membranes is another cause of decrease in photosynthesis.

2.4. Relationship between Cd Toxicity and Accumulation

In spite of severe toxic nature of Cd as described above, a number of plants can accumulate significant amounts of Cd without any visual toxicity and/or growth retardation. The phenomenon of Cd accumulation without any visual toxicity is not only related to Cd hyper accumulators like Arabidopsis halleri (Bert et al., 2003) and Thlaspi Caerulescens (Zha et al., 2004) but is also exhibited by many food crops. Garrett et al. (1998) reported that soil Cd up to 3.8 mg kg⁻¹ was not linked to phytotoxicity in wheat wherein wheat grain accumulated up
to 0.50 mg Cd kg\(^{-1}\). Yu et al. (2006) reported that more than 50 % of tested 43 rice cultivars produced similar or more grain yield at high level of soil Cd (75.69 to 77.55 mg kg\(^{-1}\)) and average grain biomass of Cd-pollution safe cultivars (Cd-PSCs defined as containing < 0.2 mg Cd kg\(^{-1}\) biomass according to National Food Hygiene Standards of China) was not significantly different from that of non-Cd-PSCs.

The absence of toxicity at a projected level of soil Cd confirms the occurrence of this phenomenon in every natural contaminated soil where chance of decrease in yield is more unlikely. According to Cheng et al. (2008), no relationship exists between Cd toxicity in terms of germination and seedling growth and concentration of Cd in shoots of some rice cultivars. Similarly, in maize when grown in nutrient solution supplemented with Cd up to 10 nM concentration, no toxicity symptom was observed on leaves irrespective of the tissue Cd concentration (Perriguey et al., 2008). Non-significant correlation between Cd tolerance and accumulation in wheat inbred lines (Ci et al., 2010) and between grain Cd concentration and tolerance in terms of germination and seedling growth in Japonica cultivars of rice (Cheng et al., 2008) suggested that these are independent traits. Stevens and McLaughlin (2006) reported that Cd could concentrate in harvestable parts of plant to the extent that is not toxic for them but could be harmful for humans. Ultimately, farmers would not get any prediction of grain Cd contents toxic to humans and animals based on yield. Cadmium level in food that could harm humans reaches well before any toxicity occurs, therefore, solving the former problem will ultimately result in the solution of later.

2.5. Cadmium Intake and Human Health

Human exposure to Cd mainly results from contamination of agricultural produce by this metal. Once Cd enters into the soil environment, it persists for longer periods and may leach down to ground waters or concentrated by crops growing in their body. In this way Cd may enter into food chain and could cause various health hazards in humans wherein critical organ for long-term Cd exposure is kidney. Dietary intake of Cd is at least 70 % of the total (Wagner, 1993) however; the share of a specific diet in Cd intake is determined by its consumption pattern which varies region to region. Of the total dietary Cd intake, for example, 30-36 % in Canada may be contributed by cereals (Dabeka, 1995), 43 % from the consumption of wheat flour in Sweden (Hellstrand and Landner, 1998) and 53 % from rice in Iran has been calculated
(Zazoli et al., 2008). Although no such calculation/survey exist in Pakistan, but being staple food, wheat could surely be the main contributor towards dietary intake of Cd.

Acute toxicity of Cd has not been reported but in extreme cases of chronic toxicity, Cd led to pulmonary emphysema (shortness of breath), renal tubular function and bone fractures; Itai-Itai disease (Yeung and Hsu, 2005). It may replace Ca in bones resulting in painful bone demineralization. International Agency for Research on Cancer has already classified Cd as a known human carcinogen of class I (IARC, 1993). To ensure Cd safe food, the World Health Organization (WHO) and CODEX Alimentarius Commission of the Food and Agriculture Organization of the United Nations (FAO) has proposed a limit of 0.1 mg Cd-kg$^{-1}$ of grain (WHO, 1989).

2.6. Minimizing Cd in Edible Parts of Plant

It is not only very difficult to avoid anthropogenic additions of Cd to soil but is also impossible to remove all the metals from root zone. Therefore, some suitable strategies must be undertaken to reduce the Cd burden of plant tissue. Stabilization in soil through organic and inorganic amendments, phyto-extraction, cultivation of low Cd accumulating cultivars and optimization of some essential/beneficial plant nutrients in soil are some promising options being used to minimize Cd uptake by food plants. But, most of these remain impracticable due to their high implementation costs and negative effects on soil quality. But, selection of low Cd accumulating cultivars and their further use in breeding programme and optimizing the supply of some plant nutrients antagonistic to Cd are most promising methods because it works on sustained basis with disturbing the soil-plant system.

2.7. Low Cd Accumulating Cultivars

Among the different strategies, exploitation of variation in mineral uptake by different cultivars/varieties within a species or among different species to produce low Cd food is a low cost and environmental friendly strategy. In this regard, Yu et al. (2006) have introduced the concept of Cd-PSCs i.e. cultivars accumulating Cd in edible parts low enough to be safely consumed by humans even when grown on contaminated soils. They tested rice cultivars on soils containing 1.75 to 1.85 mg Cd kg$^{-1}$ and reported 30 that out of 43 cultivars were Cd-PSCs as defined in section 2.3. Recently, Ueno et al. (2009) examined genotypic variations in shoot
Cd concentration in 146 rice accessions from rice core collection and found a large variation in the shoot Cd accumulation and Cd tolerance. Similarly, variation in Cd accumulation between different cultivars of spring bread wheat and durum wheat has been observed in a field study in southern Sweden (Stolt et al., 2002). It shows that there is considerable potential for selection of Cd-PSCs among cultivars. It is very easy approach to grow low Cd accumulating cultivars/varieties once they are screened out from the existing genetic pool. It will be of high significance for wheat, as it is higher Cd accumulator in grains than the other species. It was reported that wheat could accumulate Cd twice as much as the rye, oat and barley did (Puschenreiter and Horak, 2000). Further, accumulation from soil by roots and distribution within the plant is responsible for the variable Cd in grain.

2.7.1. Differential Absorption of Cd by Roots

Low Cd accumulating cultivars could decrease the active concentration of Cd in soil/rhizosphere to restrict its uptake by roots. Genetic factors those control the uptake Cd include anatomy/morphology and size of root system, production of root exudates, root association with mychorizal fungi and root colonizing bacteria and root CEC (Harris and Taylor, 2004; Arao and Ishikawa, 2006). Cadmium is taken up by roots from soil solution in concentration dependent manner i.e. higher solution Cd concentration will result in higher Cd uptake. Soil solution Cd is very sensitive to soil pH being higher at acidic and lower at basic pH. Roots could decrease or increase the pH of rhizosphere soil which in turn could enhance/depress the chemical processes including Cd desorption, dissolution and Cd-ligand complexation. Rhizosphere acidification due to organic acids exudation by roots will enhance the release of soil bound Cd from soil colloid and it was found that roots can decrease the pH within 1 mm of rhizosphere by a value of 1 unit (Murányi et al., 1994). In soil solution, Cd concentration measured at neutral pH was estimated to be doubled at a lower pH (Wagner, 1993). Moreover, Cieslinski et al. (1997) found that the release of organic acids from roots was cultivar dependent.

Higher Cd desorption from soil to soil solution was observed in case of wheat cultivars with higher release of organic acids (Nigham et al., 2000). Liue et al. (2007) have reported that differences in secretion of low molecular weight organic acids among plant cultivars may influence root uptake of Cd. However, root secretions may also precipitate Cd outside the roots.
Higher accumulation of organic complex of Cd in rice rhizosphere than that in non-rhizosphere soil may be interpreted as precipitation of Cd by root secretions outside the roots (Lin et al., 1998). It has been suggested by Murakami et al. (2007) that exudation of low molecular weight organic acids and mucilage could modify the bioavailability of Cd by effecting Cd speciation in soils.

Difference in uptake of cations by roots may also involve the cation exchange capacity (CEC) of the roots. It has been reported that Si increase the CEC of roots and consequently Cd bound to cell wall to higher extent in the roots (Gregar et al., 2004). Similarly, Wang et al. (2000) reported high affinity of Cd for roots containing Si bounds to its cell wall. The proportion of Cd\(^{2+}/Cd_{total}\) in the nutrient solution decreased with time (Chan and Hale, 2004) suggesting the preferential depletion of solution Cd\(^{2+}\) and/or addition of root exudates. Stolt et al. (2003) reported that the higher accumulation of Cd in the grain of durum wheat compared to bread wheat was associated with a higher total uptake by the plant.

A positive correlation between Cd accumulation by wheat cultivars and their number of root apices depicted that its uptake is also affected by morphology of roots (Berkelaar and Hale, 2000). The observations that apoplastic pathway of Cd is restricted to root tips and areas from where lateral roots originate (Moore et al., 2002) and that root tip is the most active region of the root for Cd\(^{2+}\) influx (Piñeros et al., 1998) further suggest that root anatomy is also critical in Cd uptake. Kubo et al. (2011) comprehensively studied the effect of root morphology of low and high grain Cd accumulating varieties of Japanese wheat. They observed double the number of root tips in high Cd grain varieties Kitakamikomugi and Nishikazekomugi compared to low Cd grain varieties Kitahonami and Nanbukomugi at 39 days of growth. Similarly, primary root was significantly longer and root frequency at soil depths of 0-25, 25-50 and in the whole soil volume was significantly higher in high Cd grain varieties at 73 and 97 days of growth. They concluded that root morphology is significantly related to Cd accumulation.

Generally to define Cd uptake potential of the roots bioconcentration factor also termed as enrichment factors (BCF/EF, ratio of metal concentration in plant tissue to its concentration in soil/growth medium) have been used. The cultivars with lower BCF could be cultivated on contaminated soils to avoid excessive metal transfer into food chain. Jamali et al. (2009) reported higher BCFs of various heavy metals including Cd for grains of wheat cultivars TJ-
83 and Mehran- 89 compared to Anmol and Abadgar grown on the same agricultural plots. Perhaps this is the only study revealing the subject matter in Pakistan.

Some scientists used different approaches for this purpose. In an interesting study on rice, rate of radial oxygen loss was found in strong negative correlation with Cd concentration of brown rice or its straw both in field and green house conditions (Wang et al., 2011). It indicated that genotypes with higher rate of radial oxygen loss had higher ability to limit Cd to brown rice/straw. Grafting shoots of Solanum (*S. integrifolium, S. melongena, and S. torvum*; Arao et al., 2008), and Noccaea (*Noccaea caerulescens*, Guimarães et al., 2009) with root stock of different Cd accumulating potential have revealed that shoot Cd concentrations are controlled by root properties.

### 2.7.2. Root to Shoot Translocation

Cadmium translocation from root to shoot and its distribution within plants could also be responsible for its lower concentration in grain and it is affected by the presence of intracellular binding sites, vacuolar sequestration, xylem loading and unloading, phloem transport (Harris and Taylor, 2004; Ishikawa et al., 2005a; Arao and Ishikawa, 2006). In two wheat cultivars with same total uptake, different grain Cd contents has been reported (Hart et al., 2006) which suggested differential translocation/redistribution within the plant. The same root uptake but rapid and greater root to shoot Cd translocation by indica cultivar Habataki’ (high in grain Cd) to that of japonica cultivar ‘Sasanishiki’ (low in grains Cd) revealed that root uptake is not responsible for the differential Cd accumulation in these rice cultivars (Uraguchi et al., 2009). These authors also observed a strong correlation between xylem sap Cd and its concentration in shoot and grain among 69 accessions of rice covering the genetic diversity of almost 32000 accessions and concluded that xylem transport is responsible for higher grain Cd. Liu et al. (2007) in a pot trial with 52 rice cultivars from different origins found great differences in Cd concentrations of straw, brown rice and grain chaff among the rice cultivars.

Higher Cd translocation requires higher concentration of Cd in root cells which is regulated by the expression of Cd transporters. The expression of transporters common to Cd and some micronutrient, like OsIRT1 and OsIRT2 iron (Fe) transporters (Ueno et al., 2010), may be affected by the concentration of that micronutrient in plants. Iron deficiency in rice plants resulted in over expression of these transporters that enhanced uptake and translocation.
of Cd (Ueno et al., 2010). Similarly, Fe-deficiency induced over expression of OsNRAMP1 in the roots of indica rice cultivars have been found to increase Cd uptake in root cells and the resultant higher Cd concentration in the cytoplasm of root cells. On the other hand, it is well known that vacuolar sequestration of Cd is the mechanism that limits its translocation. In durum wheat cultivars vacuolar retention of Cd in low-Cd cultivar is well documented (Chan and Hale, 2004; Harris and Taylor, 2004; Hart et al., 2006). It has been explained by Ueno et al. (2010) that mutation in amino acid of 80th position in OsHMA, a Cd transporter of Japanese rice cultivars Cho-kokoku and Anjana Dhan, resulted in loss of its ability to sequester Cd in root cell vacuole that enhanced its translocation from roots to shoots. The same has also been found true for Cho-ko-koku and Jarjan. However, absence of this phenomenon in cultivar Habataki, a higher Cd accumulator, suggested the involvement of some other mechanism. The ability of xylem-mediated Cd translocation into shoots has been shown as a major determinant for shoot Cd accumulation in many plants including rice (Hart et al. 2006; Mori et al. 2009; Uraguchi et al. 2009) Figure 2.1 explain how the vacuolar transport could render a plant to be higher accumulator of Cd.

![Fig. 2.1 Proposed model explaining the role of vacuolar sequestration of Cd (Adopted from Ueno et al., 2010)](image)

Under high soil Cd, accumulation of Cd in roots may exceed to extent that it is translocated to above ground edible portions of the plants (Sugiyama et al., 2007). Hart et al. (1998) found
1.5 to 4.5 times higher Cd translocation from roots to shoots in a bread wheat than in a durum wheat cultivar. Recombining shoots and rootstocks from high and low accumulating soybean lines led to a reduction in the Cd concentration of seed in high accumulating shoot grafted with rootstock with a capacity to accumulate high Cd, indicating that accumulation of Cd in seed was reduced by high accumulation in the root and was controlled by the rootstock cultivar (Sugiyama et al., 2007). Similarly, Greger and Löfstedt (2004) found in selected high and low grain-Cd accumulating cultivars of spring and winter bread wheat (Triticum aestivum L.) and of durum wheat (T. durum Desf.) that different Cd accumulation in grains was related to variations in the transfer from root to shoot and to the Cd concentration in shoot, flag leaf, and grain coats, but not to the uptake of Cd by roots. Chan and Hale (2004) have reported a high grain Cd accumulating durum wheat cultivar, Kyle, lower in root-Cd accumulation than a lower grain-Cd accumulating cultivar Arcola.

The roots to shoots translocation could be growth stage dependent. In isotopic studies for distribution of $^{106}$Cd among plant parts after an exposure of 24 h, the above authors have demonstrated that root-to-shoot transfer of Cd in Arcola was similar to that of Kyle at tillering then it ceased at flowering in Arcola but not in Kyle. No fraction of grain-Cd originated from $^{106}$Cd exposure which suggested that grain-Cd is a function of total shoot accumulation. Further, more basipetal translocation (translocation from shoots to roots) of Cd in low grain-Cd cultivar Arcola than higher grain-Cd cultivar Kyle at tillering suggested that roots to shoot transfer may not be transpiration linked as has also been reported by Salt and Rauser (1995). Contrarily, Florijn and van Beusichem (1993) have shown that Cd is linked to transpiration stream and its movement is affected by its interaction with the walls of the vessels and different extent of interaction in different cultivars may cause differential translocation from roots to shoots. According to Hart et al. (1998), the pattern of root and whole plant uptake and translocation from roots to shoots of Cd was similar to that of Zn.

Based on the competitive interaction between Cd and Zn, it could be interpreted that these differences in uptake and translocation of Cd may be reflection of different Zn uptake rather than intrinsic differences. But, unrelated uptake and translocation of Cd to Zn in near isogenic lines of durum wheat similar in $^{65}$Zn uptake and translocation nullified the above interpretation (Harris and Taylor, 2004). The rates of $^{109}$Cd translocation from roots to shoots following exposure of 48-60 h were 1.8 fold higher while $^{109}$Cd concentration in root-pressure
xylem exudates was 1.7 to 1.9 fold higher in high grain Cd accumulating line. During transport in xylem, Cd may transfer into phloem and this xylem-phloem transfer was suggested to be very important in grain Cd accumulation (Herren and Feller, 1997).

2.7.3. Cadmium Absorption and Redistribution within Plants
All of three physiological processes; higher uptake, root to shoot translocation and shoot to grain redistribution, or combination of any two may be present in the same plant. Liu et al. (2010) observed higher EFs as well as TFs in Suancaiwang and/or Beijingxiaoz 56 while lower in New Beijing 3 cultivars of Chinese cabbage and have attributed these genotypic differences to different uptake and internal distribution. Similarly, in a recent study pertaining to screening of 50 pakochi (Chinese cabbage) cultivars for Cd accumulation and partitioning, Chen et al. (2012) reported both enrichment factors (EFs) and translocation factors (TFs) of six cultivars were lower than 1.0 and further confirmed the suitability of growing Hangzhouyoudonger, Aijiaoheiye 333 and Zaoshenghuajing cultivars as Cd-PSCs on low Cd soils (≤1.2 mg/kg) without any risk of food contamination. Yan et al. (2010) evaluated 35 rice varieties for genotypic variation in Cd accumulation and distribution among plant parts. Growing these varieties with irrigation water containing 2 ppm Cd they observed up to 8-fold varietal differences in grain Cd concentration and shoot Cd accumulation. Cadmium concentration in grain was the highest in indica and the lowest in temperate japonica while Tongil-type and tropical japonica rice were intermediate to the above two genotypes in Cd concentration. But the physiological processes governing the higher grain Cd were different in each genotype. In indica rice, greater ability of Cd uptake, in Tropical japonica root-shoot translocation and in tongil-type shoot-grain redistribution resulted in the significantly higher grain than in temperate japonica. A significant correlation between the distribution ratios of Cd and Cd concentrations in brown rice which indicate that Cd concentration in rice grain is outcome of its transport from root to shoot and also from shoot to grain.

2.7.4. Breeding Crops for Low Grain-Cd
After the selection of low Cd accumulating cultivars, identification of their inheritance is required for developing a low Cd cultivar with all other desired traits. In durum wheat, a single dominant gene was found to be responsible for low grain Cd concentration. The near isogenic
lines of durum wheat with high/low grain Cd (genetically same except for Cd accumulation) with 2.5 times difference in Cd accumulation were developed and evaluated for other traits like yield and protein content of grains, days to maturity, lodging score and nutrient uptake (Clarke et al., 2002). Consequently, in 2004, they released first successful low-Cd wheat cultivar, Strongfield (Clarke et al., 2005), established it in fields and now they are incorporating the low Cd gene in each newly registered cultivar. Similarly in Germany, breeding programme was started and low Cd lines of sunflower were developed from 200 genotypes. Using these lines, hybrids with 50% reduction in kernel Cd were developed. The breeding programme for low Cd rice has also been started in Japan. But, we are yet in the very initial stage; the screening and selection process, and need much more effort to reach the destination, the low-Cd grain wheat. As the problem of Cd has been established, therefore, a breeding programme is utmost necessary with no delay to develop low Cd wheat cultivar with desired traits and it is easy in wheat owing to its self-crossing nature.

2.8. Mineral Nutrition and Cd Accumulation

After the development of low Cd cultivars, optimization of essential elements ranked 2nd among the strategies being attempted to ensure Cd safe food. Plant nutrients are the essential elements without which they can’t complete their life cycle. But, these essential elements do have some added benefits for plants to cope with biotic and abiotic stresses. Depressing solubility of Cd in soil and restricting its transport within the plant are two main mechanisms by which some nutrients could reduce the amount of Cd uptake by plants. The above mechanism could come into play while applying nutrients in quantities optimum for their growth. Application of the desired nutrients could help minimize Cd in food as long as we could not develop low Cd cultivars. Additionally, these could further improve the performance of low Cd cultivars. Mineral nutrients have been reported in this regard include Zn, Fe, Ca, Si etc. But, Zn and Si have gotten special attention owing to their higher requirement for plants than the other micronutrient and extraordinary resistance to abiotic stresses, respectively. Here we will discuss the role of Si and Zn in minimizing Cd accumulation in edible parts of plants.
2.8.1. Cadmium Accumulation in Relation to Silicon Nutrition

Silicon is a beneficial plant nutrient and might be accepted as essential one in near future owing to its vital role in plant stress tolerance. The absorbance of Si with the expense of energy, active absorbance, (Casey et al., 2003; Rains et al., 2006) reflects the Si hunger of plants. Based upon mitigation of Mn and Al toxicity by Si, it was thought if Si has any alleviative role under Cd toxicity? To answer this question, efforts have been made by various researchers which have been reviewed as under:

2.8.1.1. Effect of Si on Growth of Cd Stressed Plants

As a beneficial plant nutrient Si has been reported to improve plant growth under Cd contamination. Liang et al. (2005) have reported alleviative effect of Si on the growth of maize in a Cd contaminated soil. Enhanced production of maize biomass under Cd contaminated soil (10 mg Cd kg\(^{-1}\) soil) in response to Si applied as CaSiO\(_3\) was also reported by da Cunha and do Nascimento (2008) reported enhanced maize biomass production. Shoot and root growth may differ in their response to Si application and response may also be cultivar dependent. In nutrient solution with 200 \(\mu M\) Cd, Si application significantly recovered the growth of two peanut cultivars, Luhua 11 and Luzi 101, but growth recovery was higher in Cd sensitive cultivar Luhua 11 than the tolerant Luzi 101 (Shi et al., 2010). However, according to Song et al. (2009), the reverse was found true for Chinese cabbage. Lukačová Kuliková and Lux (2010) have observed a significant root growth promotion in response to Si addition (0.08 \(mM\)) in maize hybrid Reduta, while response was non-significant in other four hybrids (Novania, Valentína, Almansa and Szegedi). Cadmium affected rough and yellowish brown roots recovered to their original smoothness and white colour with the application of Si to nutrient solution.

In rice, Nwugo and Huerta (2008) reported that photosynthesis and chlorophyll fluorescence parameters were enhanced by Si but increasing Si from 0.2 to 0.6 \(mM\) did not further improve these growth parameters despite the significant increase in tissue Si contents. However, these researchers reported that in rice, 0.6 \(mM\) Si at 20 days of growth was required to resume Cd (2.5 \(\mu M\)) induced growth inhibition (Nwugo and Huerta, 2008). They further reported that decrease in stomatal conductance without affecting CO\(_2\) assimilation indicated that Si improved water use efficiency of rice plants. Similarly in maize, application of CaSiO\(_3\)
to Cd contaminated soil (10 mg Cd kg\(^{-1}\)) led to highly significant increase in root and shoot biomass of maize (da Cunha et al., 2008). But contradictory results were reported by Putwattana et al. (2010) who observed non-significant effect of Si fertilizer on growth of sweet basil (*Ocimum basilicum*) in a Cd contaminated soil (20 mg kg\(^{-1}\) soil).

2.8.1.2. Silicon Affects Morphology and Anatomy of Roots
As discussed in section 3.6 that root morphology and anatomy are very important variants regarding Cd accumulation. Silicon induced decrease Cd uptake may also be governed by its ability to modify root morphology and root anatomy. In hybrid maize grown with 100 uM Cd, lignification of endodermal cells increased from 11.7 to 15 % with the application of Si (5\(\mu\)M) (Lukačová et al. 2013). Development of endodermal Suberin lamella shifted towards root apex with the increase in Cd concentration and Si further moved it closer to root apex. Similarly, they observed an increase in development of tertiary endodermal cell wall but the effect was dependent on Si and Cd concentrations.

2.8.1.3. Silicon Nutrition and Enzymatic Antioxidant in Plants
The plants are equipped with the defense mechanism against different stresses and production of antioxidant, enzymatic or non-enzymatic, play a vital role in protecting against damage from reactive oxygen species those are generated through various physiological processes. The antioxidant capacity of plants is reported to increase under Cd stress (Lukačová et al., 2013). Silicon has been found to further increase the antioxidant ability of plants through enhancing the production of various enzymatic antioxidants (GPX, SOD and CAT) in maize (Lukačová et al., 2013), peanut (Shi et al., 2010) and Chinese cabbage (Song et al., 2009).

2.8.1.4. Silicon and Immobilization of Cd in Soils
It was found that Si could decrease the plant available fraction of Cd in soil through increasing soil pH but the effect was dependent upon nature and type of Si amendment. Incorporation of Na-silicate, furnace slag (a Si containing amendment) and other Si containing alkaline amendments resulted in higher soil pH compared to Si sources like biosolids (Chen et al., 2000). These researchers also reported furnace slag more effective in suppressing Cd uptake by rice and wheat than the calcium carbonate or steel slag. Liang et al. (2005) observed that Si
addition to Cd contaminated soils (20 and 40 mg kg\(^{-1}\)) both at high and low (400 and 50 mg kg\(^{-1}\), respectively) level decreased root and shoot Cd concentration and increased biomass of maize. Although only high level of Si increased soil pH, specifically bound Cd and Cd in Fe-Mn oxide fraction but decrease in Cd concentration of xylem sap was observed at both levels of Si. It indicated that Si decreases tissue Cd concentration both by lowering its availability in soil and decreasing translocation within the plants. Zhao and Saigusa (2004) observed increased pH of Andosol and an alluvial soil on incubation with porous hydrated calcium silicate (PHS) both under cultivated and flooded conditions. Adsorption of Cd in these soils increased with the increase in Si contents of PHS. Langmuir isotherm revealed high sorption maxima and binding capacity of PS treated soils compared to non-treated. As the soils used in these studies were acidic in reaction (pH) which shifted towards neutrality with the application of PHS, therefore, it could be thought that pH induced adsorption of Cd would only be possible in acid soils. But, the findings of da Cunha et al. (2008) nullified the above supposition. These authors reported alleviation of Cd toxicity to maize in acidic soil with the application of CaSiO\(_3\) and observed the effect due to increase of Cd to more stable soil fraction rather than increase in soil pH. It has been found that plant available Cd lowered to 18.8\% by Si fertilizer (Li et al., 2008) and silicate slag was found much effective in decreasing the exchangeable soil Cd (Cheng and Hseu, 2002).

2.8.1.5. Cadmium Absorption by Roots in Relation to Si Nutrition

Shi et al. (2005a) reported that Si significantly reduced the transport of apoplastic fluorescence tracer PTC (trisodium-8-hydroxy-1, 3, 6-pyrinesulphonate) from roots to shoots, suggesting that the heavy deposition of Si in the vicinity of endodermis might have blocked the bypass flow of Cd across the roots and restricted the apoplasmic transport of Cd to xylem. In Si treated plants compared to no Si treated plants in a solution culture experiment, Liang et al. (2007) reported that more Cd was bound to cell walls and less to symplasm, another mechanism by which Si reduces metal accumulation. But the above fact became ambiguous with the reports of Shi et al. (2005a) who have shown that most of the total root Cd was localized in symplasm, while remaining (13\%) was apoplastic in both +Si and -Si treatments. The other internal mechanisms by which Si could reduce metal in shoots include compartmentation of more Cd in vacuoles and reduction of membrane lipid peroxidation via stimulating antioxidants (Liang
et al., 2007). Similar to cultivar dependence of growth response, effect of Si on Cd accumulation both in roots and shoots have also been reported to vary with the cultivar. Lukačová and Lux (2010) have reported maximum Si (5 mM) induced depression in root and shoot Cd content (43 and 35 %, respectively) in Reduta while lowest in Almansa (12 %) and Valintena (6 %), hybrids of maize grown under 100 µM Cd. However, total uptake of Cd increased with Si application in hybrid Almansa. These findings are in line with the results of Vaculick et al. (2009) who have reported significantly higher root and shoot Cd and whole plant uptake in Jozefina maize hybrid with the application of Si. This phenomenon has not been explained by the authors except for stating the existence of hybrid or varietal specific interaction of plants. Nwugo and Huerta (2008) reported that under Cd contaminated nutrient solution, addition of Si significantly decreased root and shoot Cd concentration but effect was non-significant between 0.2 and 0.6 mM Si treatments in spite of significant difference in tissue Si concentration between the two treatments. Song et al. (2009) observed decreased Cd uptake and root to shoot translocation in Chinese cabbage with the addition of Si (1.5 mM) to Cd contaminated nutrient solution.

2.8.1.6. Silicon Nutrition and Cd Translocation to Shoots
Putwattana et al. (2010) have reported decrease in Cd concentration and whole plant accumulation and lowest transfer factor of Cd in Sweet basil (Ocilum basilicum) with application of Si fertilizer (10 and 20 % w/w) to Cd contaminated soil (20 mg kg\(^{-1}\) soil). Application of Si (1.8 mM) to nutrient solution containing 200 µM Cd significantly decreased shoot content of Cd of peanut resulting from lower root to shoot transfer of Cd.

In addition to increase in soil pH and decrease in phytoavailable Cd, Gu et al. (2011) found that steel slag and fly ash (amendments rich in silicates) were also effective in reducing stem to leaf translocation of Cd and they have interpreted it as co-precipitation of Cd with Si within the stem.

Wang et al. (2000) showed that cell wall-bound silica has a strong affinity for Cd. Thus, the elevated concentration of Si in Si-accumulating plants, such as rice, can significantly inhibit apoplastic Cd uptake by trapping Cd through covalent bonding as it diffuses through the cell wall and extracellular spaces. Furthermore, Neumann and zur Neiden (2001) showed Si-induced inhibition of symplastic heavy metal transport and suggested that intracellular Si is
able to form unstable silicates with heavy metals in the cytoplasm. They also stated that these silicates are then slowly dissociate and released metals are sequestered and bound in the vacuole by organic acids (Neumann and zur Neiden, 2001).

2.8.2. Zinc Antagonize Cd Absorption and Translocation

Of the essential nutrients affecting Cd accumulation in plants, Zn has been studied extensively. Due to its chemical similarity to Cd (Mengal and Kerkby, 2001), it could act as competitive ion for Cd absorption from soil by roots (Das et al., 1997) as well as interact with Cd within transport system of plants (Hart et al., 2002). Gomes et al. (2002) reported that Cd uptake is mediated through a Zn-transporter protein across the plasma membrane of yeast cells. It is also an integral part of various antioxidant enzymes of plants and under optimum supply; Cd could not replace it in such enzymes. It will prevent oxidative damage to integral parts of plants. Uptake of Cd have been shown to depend upon Zn contents of soil and under low Zn contents plants may accumulate higher levels of Cd even from marginal contaminated soil to exceed the regulatory levels. Generally, Zn application was reported to decrease Cd uptake and accumulation in plants (Oliver et al., 1997). Oliver et al. (1994) demonstrated that application of Zn @ 5 kg ha⁻¹ to wheat grown on marginally or severely Zn-deficient soils decreased concentration of Cd in grains up to 50%. Supporting the above findings, Hart et al. (2002) attributed the competitive interaction between Cd and Zn for uptake and transport to the existence of a common transport system on the plasma membranes.

Zinc was also shown to interfere with phloem-mediated Cd transport in durum wheat, possibly by competing with Cd for binding sites of a common transporter protein on the plasma membranes of sieve tube cells (Cakmak et al., 2000). Wu and Zhang (2002) found that Zn application decreased Cd uptake in barley (Hordium vulgare L) plants; improving growth and reducing membrane damage. Another interactive study between Cd and Zn in barley (cv. Karatay-94) grown under field conditions showed that Cd concentrations in flag leaf and grain decreased with increasing Zn application (Akay and Koleli, 2007). Jiao et al. (2004) have shown significant effect of Zn (20 mg kg⁻¹) on Cd accumulation, grain Cd concentration and translocation to seed in both durum wheat and flax seed. It indicates that Zn exhibited antagonism for both Cd uptake and translocation to grain.
The effects of Zn nutrition on Cd uptake and accumulation in plants have not shown consistent results instead it varied with plant, level of Zn and Cd and form of Zn fertilizers. Zinc has shown to increase the antioxidant response of wheat genotypes but increase was dependent upon Zn level and genotype (Sanaeiostovar et al., 2012). Zhong-qiu et al. (2005) confirmed from $^{109}$Cd tracer study that inhibitory effect of Zn on roots and shoots Cd concentration of spring wheat (Triticum aestivum, L. cv. Brookton) grown at uniform Cd concentration of 20 gmol/L was marginal and non-consistent up to Zn level of 200 gmol/L but decrease was consistent and significant at high rates (>200-2000 gmol/L). Zinc nitrate [(Zn(NO$_3$)$_2$] have been shown to be more effective in reducing shoot Cd concentration and improving biomass of two maize genotypes Jidan 209 and Changdan 374 then ZnCl$_2$ and ZnSO$_4$ which were at par with each other (Zhang and Song, 2006). But in roots, Cd concentration was the lowest with ZnCl$_2$ and highest with ZnSO$_4$. When grown at high level of soil Cd (10 and 25 mg Cd kg$^{-1}$), bread and durum wheat cultivars showed necrosis but symptom were more intense in durum wheat under Zn deficiency (Koleli et al., 2004). Application of Zn (10 mg kg$^{-1}$ soil) alleviated Cd toxicity symptoms but without accompanied decline in its concentration in shoot. Synergism has also been reported between Cd and Zn. Nan et al. (2002) reported enhanced Cd uptake with the application of Zn. Zhu et al. (2003) summarized that inhibitory effect of Zn on Cd accumulation in wheat was observed at Zn fertilization level that was itself toxic to plants and this decrease did not improve plant growth.

A positive correlation between Cd and Zn contents both in roots and shoots of 20 rice cultivars, observed by Liu et al. (2003), suggest cooperative absorption of these minerals in rice. But, Moraes et al. (2010) found a significant negative correlations between the concentration of Cd and micronutrients (including Zn) and positive among the concentration of micronutrients in upland rice grains. Moreover, the expected growth dilution of Cd like that of micronutrients was not observed in high yielding cultivars. Hence, it is presumed that plant regulate the accumulation of toxic and essential elements into the grains by some different mechanisms.

2.8.3. Interactive effect of Si and Zn on Cd accumulation

Based upon the chemical similarities between Cd and Zn, there have been reports on the use of Si in alleviating the toxicity of both Cd and Zn on contaminated soils. It could be thought that
application of Si to inhibit Cd uptake might also decrease Zn uptake by plants. But information in this regard is lacking and limited only to inhibition of Zn toxicity to plant in Zn contaminated soils. Gu et al. (2012) found that Si supply (0, 0.5 and 1.8 mM) significantly improved shoot biomass and decreased Zn concentration in roots, shoots and xylem sap of rice under 200 μM Zn contamination. They further showed that concentration of biologically active Zn²⁺ decreased because of Zn-Si co-localization in the cell walls of sclerenchyma of roots and other metabolically less active tissues resulting in 10 % increase of cell wall fraction of Zn. Song et al. (2011) have reported that effect of Si nutrition (1.5 mM) on Zn stressed (0.15 and 0.2 mM) plants was very similar to that on Cd stressed. They observed increase in root morphological attributes (total surface area, total length and no. of root tips), antioxidant enzymes [SOD, CAT, ascorbabe peroxidase (APX)] whereas decrease in MDA, H₂O₂ and shoot Cd contents. On the other hand in a recent study (Masarović et al. 2012) Si has been found ineffective in inhibiting toxic effect of Zn on growth and its concentration in shoot although Zn concentrations of growth medium were equal or below the lower level used by Song et al. (2011). These observations indicate that effect of Si on Zn nutrition remains un-elucidated and interaction may be more complex in soil system.

2.8.4. Soil salinity and Cd absorption by plants

Among soil factors, soil salinity could shift the soil-solution chemical equilibrium in favour of more soluble Cd species like CdClO₂ and CdCl⁺ thereby enhancing its availability to plants (Weggler-Beaton et al., 2000). The lower adsorbing ability of these species to soil than free Cd²⁺ ions increases Cd mobility at the soil-root interface. Moreover, these complexes can enhance transport of Cd across plasma membrane which results in increased soil-plant transfer of Cd under salinity. It was shown that combined stress of NaCl and Cd caused higher plasma membrane permeability and enhanced production of oxygen radicals and H₂O₂ in comparison to Cd and NaCl treatments alone in wheat (Muhling and Lauchli, 2003). Thus, the problem of Cd accumulation in edible parts of plants could become even more severe when plants are exposed to salinity stress (Shafi et al., 2009, 2010) which is the case in countries like Pakistan where approximately 26 % of the total irrigated land is salt-affected (Pakistan Bureau of Statistics, 2010) and untreated waste effluent of poor quality (high EC, SAR and/ or ESP) is being use to irrigate the crops (Ghafoor et al., 2008).
Over all, it could be concluded that Cd contamination is common to most of the agricultural soils due to application of phosphate fertilizers and untreated sewage irrigation. Consequently, plants growing there could contain significant amount of Cd that may bio-accumulate in humans consuming contaminated plants as food. Growing low Cd accumulating varieties/ species could be a better option to produce low Cd food. In low Cd accumulating cultivars, genes responsible for low Cd character must be identified and incorporated in cultivars with desired traits of yield and quality. Furthermore, optimization of Zn and Si nutrition of plants could enhance the benefit. Response to these nutrients by varieties differing in Cd accumulation also needs to be studied.
CHAPTER 3

Screening of Wheat Genotypes for Shoot Cadmium Accumulation

3.1. Abstract

The exploitation of genetic differences among various cultivars of wheat for producing low Cd food requires better understanding of the Cd absorption potential and translocation capabilities of the cultivars. A hydroponics study was carried out to identify low Cd accumulating wheat cultivars. Fifteen cultivars (Chakwal-97, Shahkar-95, Chenab-2000, Mairaj-2008, Faisalabad-2008, LU-26S, Iqbal-2000, Sehar-2006, Lasani-2008, Inqlab-91, SH-2002, Auqab-2000, AS-2002, Farid-2006, Kohistan-97) approved for general cultivation in Pakistan were used. Seeds were got germinated and 10-day old seedlings were transplanted to Johnson’s nutrient solution. Two weeks after transplanting, seedlings were exposed to four levels of Cd (0, 15, 30 and 45 µM) and plants were harvested two weeks after Cd exposure. In most of the cultivars, root and shoot dry matter was negatively affected even at the lowest level of Cd stress whereas some of them did not show any toxic effect. Shoot and root Cd concentration showed significant differences among cultivars. Lasani-2008 and Iqbal-2000 had the lowest while Sehar-2006 and Inqlab-91 were contained the highest concentration of Cd in their shoots. Both absorption by roots and translocation to shoots seems to play role in regulating differential Cd concentration in shoots of wheat cultivars. There was non-significant relationship was observed between relative dry matter and Cd concentration in roots or shoots suggesting that cultivars with low tissue Cd but higher tolerance could be selected for utilizing as parent material for the development of low Cd cultivars.

3.2. Introduction

Among the most toxic heavy metals, Cd is of special concern because of its high mobility in soil-plant system and toxicity to living organisms at very low concentration (Ok et al., 2011a). Owing to high mobility of Cd in soil plant system, it is easily absorbed and accumulated by plant shoots (Ok et al., 2011b) and phytotoxic levels may cause significant growth inhibition and yield loss. In addition to phytotoxicity, it has been reported that at least 70% of average
Cd intake by humans originates from contaminated plant based foods (Clemens et al., 2013). Therefore, Cd accumulation in edible parts of plants should be checked to possible low level so that these could be consumed by human beings without harmful effects.

The utilization of genetic variation in Cd uptake among plants especially wheat and rice provides an opportunity to produce low-Cd food. New cultivars/varieties developed from traditional varieties or lines not known for Cd accumulation potential may randomly be higher or lower in Cd than traditional cultivars. In Canada, a breeding programme was established in early 1990s and they released first successful low-Cd durum wheat cultivar “Strongfield” in 2004 (Clarke et al., 2005).

In Pakistan, wheat is a major staple food and can be a major source of Cd intake by humans. However, despite the well documented Cd contamination of untreated city effluent and soil in suburbs of big cities, no such breeding programme exists instead we don’t have even started screening and selection process with the coordination among research and development organizations. This situation warrants that the existing genetic material of wheat should be characterized for Cd absorption by roots and translocation from roots to shoots to help plant breeders. Keeping in view the above facts, a hydroponic experiment was conducted with the following objectives:

1. To identify contrasting Cd accumulating wheat cultivars
2. To investigate a relationship between Cd tolerance and its accumulation in wheat cultivars.

3.3. Materials and Methods

3.3.1. Nursery raising and transplantation

Seeds of fifteen bread wheat (Tritium aestivum L.) cultivars, approved for general cultivation in Pakistan, were collected from the Department of Plant Breading and Genetics, UAF. Seeds were then surface sterilized in 3 % v/v H₂O₂ solution for 10 minutes followed by rinsing in distilled water. These disinfected seeds were sown in polyethylene-lined iron trays containing acid-washed sand. Moisture content in germination trays was maintained at 10% w/w until seedlings were 10 days old. Those were then transplanted into foam-plugged holes (two seedlings per hole) in polystyrene sheets floating on plastic tubs (25 L capacity) containing
Johnson’s nutrient solution (Johnson et al., 1957) placed in Wire House, Institute of Soil and Environmental Sciences, UAF. The composition of the nutrient solution is given in Table 3.1. pH of the nutrient solution was measured daily with a SensoDirect pH 200 pH meter (Lovibond, USA) and was maintained at 6.50±0.01 using 1% w/v NaOH. The nutrient solution was continuously aerated using an aeration pump; the nutrient solution was replaced weekly.

3.3.2. Application of treatments and experimental design

While replacing nutrient solution 15 days after transplantation (DAT), three levels of Cd (15, 30 and 45 $\mu$M) were added in nutrient solution as CdCl$_2$.H$_2$O. A control without Cd for all the cultivars was also maintained to compare the effect of Cd stress on dry matter production. The experiment was laid out in completely randomized factorial design with five replicates.

3.3.3. Harvesting and chemical analyses

At day 30 of growth in nutrient solution (15 days after Cd exposure), plants were harvested. Seedlings were separated into roots and shoots and washed with distilled water to remove any adhering material. Washed samples were blotted dry and put into a convection oven adjusted to 65±5 °C. After 72 h of oven drying, shoot and root dry matter yield was recorded. The dried samples were then ground in a stainless steel grinding mill and digested in di-acid as described by AOAC (1990). Cadmium in digests was determined by an Atomic Absorption Spectrophotometer (Solar S-100, Thermo Electron, USA).

3.3.5. Calculations and statistical analyses

Relative root and shoot dry matter was calculated by dividing the mass under Cd by the one under control and was also referred as Cd tolerance Index of roots/shoots accordingly.

\[
\text{Relative Dry Matter (Cd tolerance index)} = \frac{DM \text{ under Cd treatment}}{DM \text{ under control}}
\]

Root to shoot Cd translocation abilities of the wheat cultivars were assessed by Cd translocation index which was calculated as the ratio of Cd concentration in roots to that in shoots (Ghosh and Singh, 2005).

\[
\text{Root to shoot Cd translocation Index} = \frac{\text{Cd concentration shoots}}{\text{Cd concentration in roots}}
\]
Cultivars were ranked in decreasing order of Cd concentration in their roots and shoots and Cd translocation index so as the cultivar receiving the highest rank was containing the highest Cd concentration or having the highest Cd translocation index. The data regarding root and shoot dry matter and Cd concentration in these plants tissues were statistically analyzed following ANOVA technique and least significance difference (LSD) test was applied to differentiate the treatment differences (Steel et al., 1996) using Statistix Version 8.1 software.

3.4. Results

3.4.1. Relative root and shoot dry matter
As observed visually, roots of Cd stressed plants were shorter with rough surface and light brown in pigmentation compared to that growing under no-Cd stress. The relative root dry matter (RRDM, ratio of the root dry matter under Cd to that without Cd) was significantly different (p = 0 at α 0.05) among the wheat cultivars with both the main and interaction effects being significant (Table 3.1). Coefficient of variation (CV) for relative root dry matter was 21.5, 16.2 and 20.5 % at 15, 30 and 45 µM Cd stress, respectively. The value of relative root dry matter was above one in wheat cultivars Chakwal-97 at 15 µM, in Chenab-2000 and Iqbal-2000 at 15 and 30 µM and in Iqbal-2000 at all the levels of Cd stress indicating enhanced root dry matter production in these cultivars at specified Cd stress. In cultivars including Shahkar-95, Faisalabad-2008, SH-2002 and Farid-2006 at 15 µM, Chenab-2000, Mairaj-2008 and Sehar-2006 at 15 and 30 µM and Lasani-2008 at all the level of Cd, relative root dry matter was statistically equal to one which depicts that root dry matter production in these cultivars remained unaffected at specified levels of Cd stress. Irrespective of whether Cd negatively affected root dry matter production or not, in some cultivars RRDM did not decrease with increase in the levels of applied Cd (Lasani-2008). In others, it either decreased up to 30 µM and no further decrease was recorded (Inqlab-91) or it decreased continuously up to 45 µM of Cd stress as in Chenab-2000. Likewise relative root dry matter, large variation (CV > 9.8%) in relative shoot dry matter (RSDM) was recorded which was highly significant among the wheat cultivars and levels of the applied Cd stress (p = 0 at α 0.05) but their interaction was non-significant (Table 3.1). The values of relative shoot dry matter were 0.909, 0.826 and 0.763 at 15, 30 and 45 µM Cd stress, respectively.
Table 3.1. Relative root and shoot dry matter of wheat cultivars exposed to different levels of Cd stress.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Relative Root Dry Matter</th>
<th>Relative Shoot Dry Matter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 μM Cd</td>
<td>30 μM Cd</td>
</tr>
<tr>
<td>Chakwal-97</td>
<td>1.245 ab</td>
<td>0.893 i-l</td>
</tr>
<tr>
<td>Shahkar-95</td>
<td>0.948 g-j</td>
<td>0.793 m-r</td>
</tr>
<tr>
<td>Chenab-2000</td>
<td>1.322 a</td>
<td>1.064 def</td>
</tr>
<tr>
<td>Mairaj-2008</td>
<td>0.989 efg</td>
<td>0.990 efg</td>
</tr>
<tr>
<td>Faisalabad-2008</td>
<td>0.913 g-l</td>
<td>0.707 q-t</td>
</tr>
<tr>
<td>LU-26S</td>
<td>0.838 l-p</td>
<td>0.833 l-p</td>
</tr>
<tr>
<td>Iqbal-2000</td>
<td>1.253 ab</td>
<td>1.184bc</td>
</tr>
<tr>
<td>Sehar-2006</td>
<td>0.971 f-i</td>
<td>0.932 g-k</td>
</tr>
<tr>
<td>Lasani-2008</td>
<td>1.053 def</td>
<td>1.00 efg</td>
</tr>
<tr>
<td>Inqlab-91</td>
<td>0.891 i-l</td>
<td>0.672 t</td>
</tr>
<tr>
<td>SH-2002</td>
<td>0.983 e-h</td>
<td>0.894 i-l</td>
</tr>
<tr>
<td>Auqab-2000</td>
<td>0.760 o-s</td>
<td>0.791 n-r</td>
</tr>
<tr>
<td>AS-2002</td>
<td>0.659 t</td>
<td>0.656 t</td>
</tr>
<tr>
<td>Farid-2006</td>
<td>0.961 g-j</td>
<td>0.881 j-m</td>
</tr>
<tr>
<td>Kohistan-97</td>
<td>0.754 p-s</td>
<td>0.705 rst</td>
</tr>
<tr>
<td>Mean</td>
<td>0.954</td>
<td>0.881</td>
</tr>
<tr>
<td>CV %</td>
<td>21.5</td>
<td>16.2</td>
</tr>
<tr>
<td>LSD</td>
<td>Cultivar</td>
<td>Cd</td>
</tr>
<tr>
<td></td>
<td>0.023</td>
<td>0.051</td>
</tr>
</tbody>
</table>

Means sharing different letter(s) are significantly different from each other at probability level of 0.05.
Maximum relative shoot dry matter was 0.954 for Shahkar-95 which was 1.46-fold of the minimum (0.653) recorded for Faisalabad-2008. The RSDM values of 0.954, 0.932 and 0.926 for cultivars Shahkar-95, Iqbal-2000 and Lasani-2008, respectively showed that considerable tolerance to Cd stress exists in these cultivars. Moreover, regression between relative root and shoot dry matter revealed that tolerance to Cd stress in roots and shoots was not related to each other (R² values approaching to zero).

3.4.2. Cadmium concentration in roots and shoots

Considerable variation (CV ≥ 3.5 %) among wheat cultivars was recorded for Cd concentration in roots. The differences in root Cd concentration were significant among cultivars, application rates and their interaction was significant as well (p = 0 at α 0.05) (Table 3.2). Cadmium concentration in roots varied from 94 to 123, 114 to 140 and 150 to 167 mg kg⁻¹ with average values of 112, 130 and 157 mg kg⁻¹ at 15, 30 and 45 µM Cd in nutrient solution, respectively. With increasing level of Cd stress in nutrient solution, Cd concentration in roots increased but variation among the cultivars narrowed. Across the range of Cd levels tested, Lasani-2008, Inqlab-91 and SH-2002 cultivars contained the highest concentration of Cd in their roots but it was the lowest in cultivars Mairaj-2008 and AS-2002. Among shoots of wheat cultivars, the range of Cd concentration was wider (CV ≥ 7.1 %) than that of roots. Moreover, these differences were found significant for both cultivars and Cd levels whereas their interactive effect was non-significant (Table 3.2). Average Cd concentration in shoots was 60, 71 and 97 mg kg⁻¹ at 15, 30 and 45 µM Cd in nutrient solution, respectively. On average and also across the three implemented levels of Cd stress, Lasani-2008 contained the lowest (64 mg kg⁻¹ DM) whereas Inqlab-91 and Sehar-2006 contained the highest concentration of Cd in their shoots (85 and 83 mg kg⁻¹ DM, respectively). Overall, Cd concentration in roots was approximately 2-fold of that in shoots. The R² values of linear regression between Cd concentration in roots and/ or shoot and its stress level in nutrient solution ranged between 0.92 – 1.0 for roots and 0.93 – 1.0 for shoots which indicates that Cd was absorbed in a concentration dependent manner i.e. Cd absorption increased with increase in its concentration in rooting medium (nutrient solution).
Table 3.2. Cadmium concentration in roots and shoots of wheat cultivars exposed to different levels of Cd stress

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Cd concentration in roots (mg kg(^{-1}) DM)</th>
<th>Cd concentration in shoots (mg kg(^{-1}) DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 (\mu M) Cd</td>
<td>30 (\mu M) Cd</td>
</tr>
<tr>
<td>Chakwal-97</td>
<td>106 op</td>
<td>117 j-n</td>
</tr>
<tr>
<td>Shahkar-95</td>
<td>112 m-p</td>
<td>124 ij</td>
</tr>
<tr>
<td>Chenab-2000</td>
<td>113 mno</td>
<td>128 hi</td>
</tr>
<tr>
<td>Mairaj-2008</td>
<td>94 r</td>
<td>114 mno</td>
</tr>
<tr>
<td>Faisalabad-2008</td>
<td>113 mno</td>
<td>128 hi</td>
</tr>
<tr>
<td>LU-26S</td>
<td>111 nop</td>
<td>134 gh</td>
</tr>
<tr>
<td>Iqbal-2000</td>
<td>115 lmn</td>
<td>134 gh</td>
</tr>
<tr>
<td>Sehar-2006</td>
<td>117 j-n</td>
<td>133 gh</td>
</tr>
<tr>
<td>Lasani-2008</td>
<td>122 i-l</td>
<td>136 g</td>
</tr>
<tr>
<td>Inqlab-91</td>
<td>116 j-n</td>
<td>140 g</td>
</tr>
<tr>
<td>SH-2002</td>
<td>123 ijk</td>
<td>138 g</td>
</tr>
<tr>
<td>Auqab-2000</td>
<td>114 mno</td>
<td>135 gh</td>
</tr>
<tr>
<td>AS-2002</td>
<td>98 qr</td>
<td>134 gh</td>
</tr>
<tr>
<td>Farid-2006</td>
<td>116 k-n</td>
<td>135 gh</td>
</tr>
<tr>
<td>Kohistan-97</td>
<td>105 pq</td>
<td>119 j-m</td>
</tr>
<tr>
<td>Mean</td>
<td>0.954</td>
<td>0.881</td>
</tr>
<tr>
<td>CV %</td>
<td>7.2</td>
<td>6.1</td>
</tr>
<tr>
<td>LSD</td>
<td>Cultivar</td>
<td>Cd</td>
</tr>
<tr>
<td></td>
<td>4.45</td>
<td>1.99</td>
</tr>
</tbody>
</table>

Means sharing different letters are significantly different from each other at probability level of 0.05.
3.4.3. Translocation of Cd in wheat varieties

For root to shoot Cd translocation index, both the main and interactive effects were highly significant (Table 3.3). The TI of the wheat cultivars with 15, 30 and 45 µM Cd treatments ranged from 0.374 to 0.656, 0.446 to 0.633 and 0.521 to 0.701, respectively. Cultivars with lowest concentration of Cd in shoots, Lsanai-2008 and/or Iqbal-2000, showed the lowest TI across the range of tested Cd treatments (Table 3.4). Similarly, the highest concentration of Cd in shoots of Inqlab-91 and Sehar-2006 was linked to higher root to shoot TI in these cultivars (Table 3.4). Like Cd concentration in roots, differences in TI among cultivars also narrowed with increasing stress level of Cd in nutrient solution.

Table 3.3. Root to shoot Cd translocation index of wheat cultivars at different levels of Cd stress

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>15 µM Cd</th>
<th>30 µM Cd</th>
<th>45 µM Cd</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chakwal-97</td>
<td>0.537 n-t</td>
<td>0.564 i-r</td>
<td>0.593 e-m</td>
<td>0.565</td>
</tr>
<tr>
<td>Shahkar-95</td>
<td>0.510 stu</td>
<td>0.555 l-s</td>
<td>0.558 l-s</td>
<td>0.541</td>
</tr>
<tr>
<td>Chenab-2000</td>
<td>0.531 o-t</td>
<td>0.557 l-s</td>
<td>0.621 d-h</td>
<td>0.570</td>
</tr>
<tr>
<td>Mairaj-2008</td>
<td>0.656 a-d</td>
<td>0.633 c-f</td>
<td>0.614 d-j</td>
<td>0.634</td>
</tr>
<tr>
<td>Faisalabad-2008</td>
<td>0.496 tuv</td>
<td>0.541 m-t</td>
<td>0.574 gp</td>
<td>0.537</td>
</tr>
<tr>
<td>LU-26S</td>
<td>0.560 k-s</td>
<td>0.568 i-q</td>
<td>0.616 d-i</td>
<td>0.581</td>
</tr>
<tr>
<td>Iqbal-2000</td>
<td>0.449 v</td>
<td>0.465 uv</td>
<td>0.597 e-l</td>
<td>0.504</td>
</tr>
<tr>
<td>Sehar-2006</td>
<td>0.570 h-q</td>
<td>0.580 g-o</td>
<td>0.689 ab</td>
<td>0.613</td>
</tr>
<tr>
<td>Lasani-2008</td>
<td>0.374 w</td>
<td>0.446 v</td>
<td>0.521 q-t</td>
<td>0.447</td>
</tr>
<tr>
<td>Inqlab-91</td>
<td>0.579 g-o</td>
<td>0.562 j-r</td>
<td>0.691 ab</td>
<td>0.611</td>
</tr>
<tr>
<td>SH-2002</td>
<td>0.514 r-u</td>
<td>0.539 n-t</td>
<td>0.640 b-e</td>
<td>0.564</td>
</tr>
<tr>
<td>Auqab-2000</td>
<td>0.524 p-t</td>
<td>0.529 o-t</td>
<td>0.658 a-d</td>
<td>0.570</td>
</tr>
<tr>
<td>AS-2002</td>
<td>0.655 a-d</td>
<td>0.565 i-r</td>
<td>0.701 a</td>
<td>0.640</td>
</tr>
<tr>
<td>Farid-2006</td>
<td>0.529 o-t</td>
<td>0.531 o-t</td>
<td>0.588 f-n</td>
<td>0.549</td>
</tr>
<tr>
<td>Kohistan-97</td>
<td>0.612 d-k</td>
<td>0.626 d-g</td>
<td>0.679 abc</td>
<td>0.639</td>
</tr>
<tr>
<td>Mean</td>
<td>0.540</td>
<td>0.551</td>
<td>0.623</td>
<td></td>
</tr>
</tbody>
</table>

LSD Cultivar Cd Cultivar x Cd
0.030 0.014 0.052

Means sharing different letters are significantly different from each other at probability level of 0.05.
Table 3.4. Ranking of cultivars with respect to Cd concentration in roots, shoots and its root to shoot translocation index

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Root Cd</th>
<th>Shoot Cd</th>
<th>Cd T. Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chakwal-97</td>
<td>97</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Shahkar-95</td>
<td>6</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Chenab-2000</td>
<td>5</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Mairaj-2008</td>
<td>1</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Faisalabad-2008</td>
<td>9</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>LU-26S</td>
<td>8</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Iqbal-2000</td>
<td>8</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Sehar-2006</td>
<td>9</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>Lasani-2008</td>
<td>13</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Inqlab-91</td>
<td>11</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>SH-2002</td>
<td>12</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Auqab-2000</td>
<td>7</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>AS-2002</td>
<td>4</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>Farid-2006</td>
<td>10</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Kohistan-97</td>
<td>3</td>
<td>11</td>
<td>7</td>
</tr>
</tbody>
</table>

Means sharing same ranks do not differ significantly at $\alpha = 0.05$.

3.4.6. Relationship between Cd concentration and tolerance in wheat cultivars

The regression analysis between Cd concentration and relative dry matters of roots and shoots of wheat cultivars revealed that no relationship exists between these parameters indicating that Cd concentration and tolerance are independent traits in these wheat cultivars (Fig. 3.2). It was also observed that even with a considerably high Cd concentration in tissue, shoot and root dry matter remained unaffected in several wheat cultivars.

![Figure 3.2](image_url). Relationship between Cd concentration and relative dry matter (tolerance index) of wheat cultivars (a and b stands for roots and shoots, respectively)
3.5 Discussion

3.5.1. Root and shoots dry matter

Cadmium is toxic heavy metal and easily absorbs by plant roots. Selection of low Cd accumulating plants species could help avoid its dietary intake by human beings. Toxicity of Cd is well documented (Sarwar et al., 2010) and symptoms of Cd toxicity, as observed in this study, are in agreement with those of Lukačová Kulikova and Lux (2010). These authors reported that roots of Cd treated maize hybrids were rough and brown pigmented while leaf blades were necrotic/chlorotic with decreased leaf area. Although root and shoot dry matter of some of the tested cultivars stimulated or remained unaffected but most of them showed significantly lower dry matter under Cd stress (Table 3.2 and 3.3). The enhanced dry matter production under Cd stress could be attributed to higher Cd tolerance of those cultivars. Perriguey et al. (2008) did not observe Cd toxicity to maize hybrids grown in nutrient solution containing 10 µM Cd instead toxicity was observable at 100 µM Cd. Interestingly, Lukacˇova´ Kulikova and Lux (2010) have recorded higher root dry mass of maize plants even at 100 µM Cd stress than that grown without Cd stress. However, increased dry matter in Cd stressed plants remains still un-elucidated.

3.5.2. Cadmium concentration in roots and shoots

In the present studies, roots contained almost two fold higher Cd compared to shoots at all the Cd application rates. The regression analysis revealed that Cd was absorbed by wheat cultivars in concentration dependent manner i.e. a highly significant linear relationship was observed between tissue and external Cd concentration. Results pertaining to retention of the major portion of Cd in roots are confirmatory to several earlier studies on wheat (Stolt et al., 2003; Gregar and Löfstedt, 2004) and barley (Vassilev et al., 2004). Vassilev et al. (2004) also reported linear relationship between Cd concentration in barley (Hordeum vulgare L. cv. Ribeka) and its stress level when plants were exposed to 42 ppm Cd level in sand culture.

There was considerable variation among wheat cultivars for Cd concentration in roots and shoots. The absorption by roots and/or internal distribution could be responsible for this variation. Variation in Cd accumulation among different cultivars of spring bread wheat and durum wheat was reported by Stolt et al. (2003) in a field study. According to these authors, higher accumulation of Cd in shoots of durum wheat compared to bread wheat was associated
with higher plant absorption. However, Gregar and Löfstedt (2004) have shown difference in both Cd absorption and distribution between the durum and spring wheat and these differences were lower for genotypes compared to cultivars within each genotype. In the present studies, Lasani-2008 and Iqbal-2000 with the lowest shoot Cd and the highest root Cd could be considered as shoot Cd excluders. There was no relationship between Cd concentration and its tolerance both in roots and shoots of wheat cultivars (Fig. 3.2). Similarly, correlation between Cd tolerance and accumulation in inbred lines of wheat (Ci et al., 2010) and Japonica rice (Cheng et al., 2008) was found non-significant which suggest that these traits are independently regulated. Also Stevens and McLaughlin (2006) reported that Cd could concentrate in harvestable parts of plants to the extent that is not toxic for them but could be harmful for humans.

3.5.3. Root to shoot translocation of Cd
Differential Cd translocation from roots to shoots in wheat cultivars was observed in present studies. It was reported by earlier scientists (Harris and Taylor, 2004; Ishikawa et al., 2005a; Arao and Ishikawa, 2006) which was attributed to the presence of intracellular binding sites and biochemical processes like vacuolar sequestration, xylem loading and unloading and phloem transport. Hart et al. (2006) reported different Cd contents in grains of two wheat cultivars with the same total uptake which suggested differential translocation/redistribution within the plant. However, with the same root absorption but rapid and greater root to shoot Cd translocation in a high grain indica rice cultivar (Habataki) compared to that of low grain japonica cultivar (Sasanishiki) revealed that root uptake was not responsible for the differential Cd accumulation in these rice cultivars (Uraguchi, 2009). In durum wheat cultivars, vacuolar retention of Cd in low-Cd cultivar is well documented (Harris and Taylor, 2004; Hart et al., 2005, 2006). In high and low grain-Cd accumulating cultivars of spring, winter (Tritium aestivum L.) and durum wheat (T. durum Desf.) cultivars, Greger and Löfstedt (2004) concluded that different amounts of Cd accumulation in grains was related to variations in the transfer from roots to shoots and to the Cd concentration in shoots, flag leaves, and grain coats, but not to the uptake of Cd by roots. Chan and Hale (2004) reported a high grain Cd accumulating durum wheat cultivar, Kyle, lower in root-Cd accumulation than a lower grain-Cd accumulating cultivar Arcola. In near isogenic lines of durum wheat, rates of $^{109}$Cd
translocation from roots to shoots following exposure of 48-60 h were 1.8 fold higher while $^{109}$Cd concentration in root-pressure xylem exudates was 1.7 to 1.9-fold higher in high grain Cd accumulating line (Harris and Taylor, 2004).

Similar findings were obtained by Liu et al. (2010) who reported a large variation in translocation index of cabbage cultivars. But the range of translocation index of cabbage cultivars was much wider (0.24 – 2.62) than that of wheat cultivars where TI from none of the cultivars exceeded 1.0 and this discrepancy may be attributed to the differences in abilities of cabbage and wheat plants to transfer Cd or to differences in growth conditions of studies, i.e. nutrient solution vs. soil culture. The narrowing of the difference for Cd accumulation in roots and its transfer to shoots among wheat cultivars beyond 15 $\mu$M Cd could be attributed to Cd toxicity induced loss of membrane integrity which resulted in unregulated uptake of Cd. Further, the unregulated inflow of Cd was also cultivar dependent. The Cd concentrations used in this study do not correspond to soil solution Cd of even highly contaminated soils. Therefore, it is necessary that the response of these cultivars to Cd stress should also be studied under natural field conditions. Overall, variability in Cd concentration among wheat cultivars provide opportunity to obtain low Cd wheat grain food.

3.6. Conclusions
Both absorption by roots and translocation to shoots seems to play role in regulating differential Cd concentration in shoots of wheat cultivars. There was no relationship between relative dry matter and Cd concentration in roots and shoots suggesting that cultivars with low tissue Cd but higher tolerance could be selected for utilizing as parent material for the development of low Cd cultivars with all other desired traits of yield, disease resistance and nutritional quality.
ROOT ZONE ACIDIFICATION AND ANTIOXIDANT RESPONSE OF WHEAT CULTIVARS DIFFERING IN SHOOT Cd UPTAKE

4.1. Abstract

Plant species differing in shoot Cd accumulation could differ in root zone acidification potential and production of antioxidant enzymes. Based on screening study, selected low and high shoot Cd cultivars were evaluated for their ability to modify the root zone pH and effect on the production of antioxidant enzymes under Cd stress. Plants were grown in Johnson’s nutrient solution and change in pH of nutrient solution was measured daily following exposure to 15 µM Cd stress. Fifteen days after Cd exposure, plants were harvested and antioxidant enzymes and other physiological parameters were measured and dry matter was recorded. The growth response to Cd stress and Cd concentration both in roots and shoots of wheat cultivars confirmed the results of initial screening study, i.e. the Cd concentration was low in shoots of cultivars Iqbal-2000 and Lasani-2008 while high in Inqlab-91 and Sehar-2006. Wheat cultivars tended to induce a higher mean daily decrease in pH of Cd contaminated nutrient solution (0.199 units) than with no Cd (0.177 units). The decrease in pH of uncontaminated nutrient solution was lower (0.141 units) for Lasani-2008 compared to rest of the cultivars. Under Cd stress, activities of antioxidant enzymes catalase (CAT) and ascorbate peroxidase (APX) decreased, superoxide dismutase (SOD) remained unaffected whereas that of guaiacol peroxidase (GPX) increased significantly. The decrease in activity of CAT and APX and improvement in GPX was higher in low-shoot-Cd cultivars. Lipid peroxidation (malondialdehyde content) significantly increased in all the cultivars under Cd stress and it was higher in high-shoot-Cd cultivars. It is concluded that decrease in pH was not related to shoot Cd concentration of cultivars. However, low Cd concentration in low-shoot-Cd cultivars was related to sustained or higher activity of antioxidant enzymes which was not recorded for high-shoot-Cd cultivars.
4.2. Introduction

Cadmium absorption is likely to be affected by root-induced desorption of Cd from soil into soil solution through modification of rhizosphere pH depending upon the extent of release of root exudates and ratio of cation to anion absorption. Under Cd stress, non-accumulating species could be thought regulating higher pH in rhizosphere by either no exudation of organic acid anions or shift in cation/anion uptake ratio in favour of anions.

In plants, Cd imposes many negative effects on growth and development manifested by the visual toxicity symptoms, changes in biochemical and physiological processes and growth inhibition (Maksymiec et al., 2007). The Cd toxicity in plants increases the production of reactive oxygen species (ROSs) such as superoxide radicals (O$_2^-$), singlet oxygen (1O$_2$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (Sanità di Toppi, 2007; Sharma and Dietz, 2009). It involves the replacement of essential redox elements with Cd in different electron transport chains operating within plant cells, causing interruption in electron flow (Sigfridsson et al., 2004). Thus, inadequate supply of electrons at the acceptor end could result in enhanced production of ROSs. The ROSs are also produced during normal metabolism of plants, but their concentration remains relatively low due to antioxidant defense system. However, enhanced production of ROSs due to Cd$^{2+}$ stress could disturb the balance between their generation and removal, resulting in oxidative damage, such as lipid peroxidation (Lin et al., 2007; Razinger et al., 2008) as evidenced by the presence of byproduct malondialdehyde (MDA) in the cytosol. Thus, a plant with a relatively high activity or a capacity to enhance activity of antioxidant enzymes is likely to be less prone to tolerant oxidative damage under Cd stress.

Although there is increasing evidence that species differ in their ability to modify root zone pH and to produce antioxidant enzymes, there is limited information on how these processes are related to Cd absorption and tolerance in genotypes that either do or do not accumulate Cd in shoots. Therefore, the present work was carried out to investigate the root zone pH modification and the antioxidant enzyme producing potential of Cd accumulating and non-accumulating wheat cultivars under Cd stress.
4.3. Materials and Methods

4.3.1. Growing plants

A hydroponic study was conducted in glasshouse, Institute of Soil and Environmental Sciences, UAF. Based on the findings of preliminary screening, two non-accumulating (low-shoot-Cd cultivars, Iqbal-2000 and Lasani-2008) and two accumulating (high-shoot-Cd cultivars Inqlab-91 and Sehar-2006) wheat cultivars were grown in this study. These high- and low-shoot-Cd cultivars hereafter will be referred as HSCd and LSCd cultivars, respectively. Seeds of selected cultivars were surface sterilized in 3 % v/v H$_2$O$_2$ solution for 10 minutes followed by rinsing in distilled water. These disinfected seeds were sown in polyethylene-lined iron trays containing acid-washed sand. Moisture content in germination trays were maintained at 10% w/w until seedlings were 10 days old.

Seedlings were then transplanted into foam-plugged holes (two seedlings per hole) in polystyrene sheets floating on plastic pots (2 L capacity) containing Johnson’s nutrient solution. The composition of the nutrient solution is given in Table 3.1. The pH of the nutrient solution was measured daily with a SensoDirect pH 200 pH meter (Lovibond, USA) and was maintained to 6.50±0.01 using 1% w/v NaOH. The nutrient solution was continuously aerated using an aeration pump; the nutrient solution was replaced weekly.

Cadmium stress at 15 µM Cd L$^{-1}$ as CdCl$_2$·H$_2$O was imposed while replacing nutrient solution 15 days after transplantation (DAT). A control without Cd for all the cultivars was maintained to compare the effect of Cd on growth and other physiological parameters. There were two sets of three replications (keeping conditions similar for each set). One set was for enzyme assay and physiological/biochemical analyses, while the other was for growth parameters and chemical analysis.

4.3.2. Measurement of pH and gas exchange

Following exposure to Cd, dynamics of pH in nutrient solution was recorded daily till plant harvest (15 days). After pH measurement, the solution pH was readjusted to 6.50±0.01 using 1% w/v NaOH.

Gas exchange parameters and chlorophyll contents were recorded for 3 consecutive days after Cd exposure between 9:00 am and 11:00 am using an LCi-SD portable Infrared Photosynthetic Meter (ADC Bioscientific Ltd., England) and a Chlorophyll Meter SPAD-502.
(Minolta, Japan), respectively. For photosynthetic measurements, the mid part of the youngest fully expanded leaf of each main tiller was enclosed in the leaf chamber and measurements were made under constant leaf temperature (30 ± 1 °C), CO₂ concentration (400 ppm) and photosynthetic photon flux density (PPFD, 1000 μM m⁻² s⁻¹). Chlorophyll contents were estimated at the middle of each fully expanded leaf from main tillers. Four measurements were recorded and averaged.

4.3.3. Harvesting and chemical/ biochemical analyses

At day 30 of growth period in nutrient solution (15 days after Cd exposure), the plants were harvested. One set (three reps) was used for the measurement of dry matter of shoots and roots, whereas the second set of three replicates was used for the assay of antioxidant enzymes.

The seedlings from the 1st set were separated into roots and shoots, washed with 1% v/v acetic acid followed by rinsing in distilled water. The washed samples were blotted dry and put into a convection oven adjusted to 65±5 °C. After 72 h of oven drying, shoot and root dry matter was recorded. The dried samples were ground in a stainless steel grinding mill and digested following the procedure described by AOAC (1990). One gram of dried plant sample was placed in a conical flask, 5 mL concentrated HNO₃ and 5 mL of HClO₄ were added and kept overnight. Next day, additional 5 mL concentrated HNO₃ was added and the samples were digested on a hot plate. The digested material was cooled, diluted to 50 mL volume with distilled water, filtered through Whatman filter paper No. 42 and stored in air-tight bottles. Cadmium in digests was determined by an Atomic Absorption Spectrophotometer (AAS) (Model Thermo Electron S-Series). The biochemical analyses were performed on the second set of three replicates. Leaf samples were collected for enzyme assay, snap frozen in liquid N and stored at –20 °C.

4.3.3.1 Crude extract preparation

All steps involved in the extraction of enzymes were carried out at 4 °C. The composite sample of fresh leaves (0.3 g) was homogenized in 2 mL of ice cold 50 mM potassium phosphate buffer in 0.2 mM EDTA (pH 7.8) and 2 % (w/v) polyvinylpyrrolidone (PVP). The homogenate was collected in eppendorfs and centrifuged at 12,000 × g for 20 min at 4 °C. The supernatant comprising crude enzyme extract was decanted into new eppendorfs and stored at –20 °C. The
spectrophotometric measurements regarding total soluble proteins and antioxidant enzymes were recorded with a Shimadzu (Japan) UV-2550 spectrophotometer.

### 4.3.3.2. Total proteins

Total proteins were determined following the method of Bradford (1976). Bovine serum albumin solution was used as the standard.

### 4.3.3.3. Catalase (CAT) activity

Catalase activity was measured as a decrease in absorbance of enzyme extract following the reduction of H$_2$O$_2$ by catalase (Cakmak and Marschner, 1992). The reaction mixture contained 1.7 mL 25 mM phosphate buffer in 0.1 mM EDTA (pH 7.0) and 100 μL of enzyme extract. The reaction was initiated by adding 200 μL of 100 mM H$_2$O$_2$, and an initial decrease in absorbance was measured up to 30 s at 240 nm. The activity was calculated using extinction coefficient of 39.6 mM$^{-1}$ cm$^{-1}$ for H$_2$O$_2$ and expressed as units (U) mg$^{-1}$ protein. One unit of enzyme was defined as 1 mM of substrate reacted per min per mg protein.

### 4.3.3.4. Superoxide dismutase (SOD) activity

Superoxide dismutase activity was estimated using nitroblue tetrazolium (NBT) test (Gong et al., 2005). The NBT reagent was prepared by mixing 196 mL of K-phosphate buffer (50 mM in 0.1 mM EDTA, pH 7.8), 0.387 g methionine and 0.103 g NBT. Fifty (50) μL of enzyme extract and 3 mL of NBT reagent were mixed in a glass test tube and placed in the dark. The reaction was started by adding 4 mL of 320 μM riboflavin under dim light (15 W, 60 μM m$^{-2}$ s$^{-1}$). Blanks and controls were run in the same manner but without illumination and enzyme extract. The reaction was stopped by replacing the test tubes in the dark; absorbance was measured at 560 nm. The quantity of enzyme that caused 50 % inhibition in rate of NBT reduction under the conditions of assay was defined as one unit of enzyme.

### 4.3.3.5. Ascorbate peroxidase (APD) activity

Ascorbate peroxidase was measured following H$_2$O$_2$-dependent oxidation of ascorbate as a decrease in absorbance at 290 nm for 30 s (Nakano an Asada, 1981). The reaction mixture contained 1.7 mL of 25 mM phosphate buffer (pH 7.0), 100 μL enzyme extract, 100 μL of 20
mM H$_2$O$_2$ and 100 µL of 0.5 mM ascorbic acid solution. Ascorbate peroxidase activity was calculated from the extinction coefficient of 2.8 mM$^{-1}$ cm$^{-1}$ for the ascorbate and was expressed as units (U) mg$^{-1}$ protein. One unit of enzyme was defined as 1 mM of substrate reacted per min per mg protein.

4.3.3.6. Guaiacol peroxidase (GPX) activity
The GPX was measured as an increase in absorbance due to oxidation of guaiacol (2-methoxyphenol) (Egley et al., 1983). The reaction mixture contained 1.7 µL potassium phosphate buffer (25 mM in 0.1 mM EDTA, pH 7.0), 100 µL 1 % w/v guaiacol, 100 µL 1 mM H$_2$O$_2$ and 100 µL enzyme extract. The reaction was started by adding guaiacol to the reaction mixture, and an increase in absorbance was recorded at 470 nm. Guaiacol peroxidase activity was calculated using extinction coefficient of 26.6 mM$^{-1}$ cm$^{-1}$ for guaiacol and was expressed as units (U) mg$^{-1}$ protein. One unit of enzyme was defined as 1 mM of substrate reacted per min per mg protein.

4.3.3.7. Lipid peroxidation
Lipid peroxidation was assessed by measuring malondialdehyde (MDA), a byproduct of lipid peroxidation, according to Shalata and Tal (1998). The reaction mixture, 1 mL of enzyme extract and 3 mL of 2 % v/v thiobarbituric acid (TBA) in 20 % v/v trichloroacetic acid (TCA), was heated at 95 ºC in a water bath for 30 min and cooled in ice quickly. The cooled mixture was centrifuged at 10,000 xg for 10 min, and absorbance of the supernatant was recorded at 532 nm. Absorbance at 600 nm was subtracted from that at 532 nm to make correction for nonspecific turbidity. The MDA content (nM g$^{-1}$ Fresh Matter) were calculated using extinction coefficient of 155 mM$^{-1}$ cm$^{-1}$.

4.3.4. Statistical analyses
The data pertaining to root-induced pH changes, plant growth response, biochemical attributes and Cd accumulation in plants were statistically analyzed using ANOVA. The LSD test was applied to differentiate the treatment differences (Steel et al., 1996) using Statistix Version 8.1 software package.
4.4. Results

4.4.1. Effect of Cd on root and shoot dry matter

There was significant difference in relative root dry matter of cultivars, Cd treatments and their interaction (Table 4.1). Except Iqbal-2000, root dry matter was significantly decreased under 15 µM Cd stress. Negative effect of Cd stress on root dry matter of HSCd cultivar Inqlab-91 (25.5 %) and Sehar-2006 (15.9 %) was significantly higher than LSCd cultivar Lasani-2008 (8.0 %). Although shoot dry matter tended to decrease with increase in Cd stress, but negative effect remained non-significant for all the cultivars. Overall, the highest shoot dry matter was recorded for Inqlab-91 (2.96 g pot⁻¹) whereas it was minimum for Iqbal-2000 and Sehar-2006 (2.17 and 2.00 g pot⁻¹, respectively).

Table 4.1 Effect of Cd on root and shoot dry matter (g pot⁻¹) of wheat cultivars

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>No Cd</th>
<th>15 µM Cd</th>
<th>Mean</th>
<th>No Cd</th>
<th>15 µM Cd</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Roots</td>
<td>Shoots</td>
<td></td>
<td></td>
<td>Shoots</td>
<td></td>
</tr>
<tr>
<td>Iqbal-2000</td>
<td>0.646 d</td>
<td>0.614 d</td>
<td>0.63</td>
<td>2.06</td>
<td>1.95</td>
<td>2.00 c</td>
</tr>
<tr>
<td>Lasani-2008</td>
<td>0.714 c</td>
<td>0.657 d</td>
<td>0.69</td>
<td>2.50</td>
<td>2.45</td>
<td>2.48 b</td>
</tr>
<tr>
<td>Inqlab-91</td>
<td>0.980 a</td>
<td>0.730 c</td>
<td>0.86</td>
<td>3.01</td>
<td>2.91</td>
<td>2.96 a</td>
</tr>
<tr>
<td>Sehar-2006</td>
<td>0.847 b</td>
<td>0.712 c</td>
<td>0.78</td>
<td>2.32</td>
<td>2.02</td>
<td>2.17 c</td>
</tr>
<tr>
<td>Mean</td>
<td>0.80</td>
<td>0.68</td>
<td>—</td>
<td>2.47</td>
<td>2.33</td>
<td>—</td>
</tr>
</tbody>
</table>

LSD

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Cd</th>
<th>Cult. × Cd</th>
<th>Cultivar</th>
<th>Cd</th>
<th>Cult. × Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.036</td>
<td>0.026</td>
<td>0.237</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values in column followed by different letters are statistically significant at α = 0.05.

4.4.2. Changes in pH of nutrient solution

The pH of Johnson nutrient solution was adjusted to 6.5 and change resulting from the activity of roots was recorded daily onward to exposure of plants to Cd stress and continued for 15 days until harvesting (Table 4.1). All the cultivars tended to induce a decrease in pH of the nutrient solution and decrease was significantly higher for no-Cd treated (0.199 units) than Cd treatment (0.177 units) nutrient solution. The cultivar Lasani-2008 induced pH significantly lower (0.141 units) compared to the rest of cultivars those were at par with each other.
Table 4.2 Decrease in pH of nutrient solution (units day\(^{-1}\)) following growth of wheat cultivars for 15 days

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Decrease in pH (units/day)</th>
<th>No-Cd</th>
<th>15 (\mu M) Cd</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iqbal-2000</td>
<td></td>
<td>0.227</td>
<td>0.186</td>
<td>0.207 a</td>
</tr>
<tr>
<td>Lasani-2008</td>
<td></td>
<td>0.145</td>
<td>0.136</td>
<td>0.141 b</td>
</tr>
<tr>
<td>Inqlab-91</td>
<td></td>
<td>0.220</td>
<td>0.200</td>
<td>0.210 a</td>
</tr>
<tr>
<td>Sehar-2006</td>
<td></td>
<td>0.205</td>
<td>0.184</td>
<td>0.195 a</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>0.199 a</td>
<td>0.177 b</td>
<td>——</td>
</tr>
<tr>
<td>LSD</td>
<td>Cultivar</td>
<td>Cd</td>
<td>Cult. (\times) Cd</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.029</td>
<td>0.015</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Values followed by different letter(s) are statistically significant among each at \(\alpha = 0.05\).

4.4.3. Cadmium concentration in roots and shoots

Cadmium concentration in roots and shoots differed significantly among cultivars (Fig. 4.1). The LSCd cultivars Lasani-2008 and Iqbal-2000, contained the minimum Cd concentration in shoots (35 and 36 mg kg\(^{-1}\) DM, respectively) that differed non-significantly between them. Maximum SCd (58 mg kg\(^{-1}\) DM) was found in Sehar-2006 followed by Inqlab-91 (44 mg kg\(^{-1}\) DM). In roots, maximum concentration of Cd (270 mg kg\(^{-1}\) DM) was recorded in Lasani-2008 while it was minimum (181 mg kg\(^{-1}\) DM) in Inqlab-91. The LSCd cultivars had higher Cd concentration in roots compared to HSCd cultivars. Further, RCd differed non-significantly between LSCd cultivars whereas the reverse was true for HSCd cultivars differing significantly with each other for RCd. Overall, cultivars in decreasing order of root Cd concentration ranked as Iqbal-2000 > Lasani-2008 > Sehar-2006 > Inqlab-91.

4.4.4. Chlorophyll contents and gas exchange

Chlorophyll content and photosynthetic rate was significantly different among the wheat cultivars and exposure to Cd stress caused a decrease in these parameters (Table 4.3). On an average, chlorophyll content in leaves decreased from 36.6 without Cd to 33.1 under 15 \(\mu M\) Cd stress which corresponds to 9.5 \% decrease. Decrease in chlorophyll content was 6.62, 7.30, 7.93 and 15.37 \% for Iqbal-2000, Lasani-2006, Inqlab-91 and Sehar-2006, respectively.
Fig. 4.1. Cadmium concentration in roots and shoots of wheat cultivars grown under 15 \( \mu M \) Cd stress. Cadmium concentration both in roots and shoots without Cd treatment were not detectable. Bars sharing different letter are significantly different from each other at probability level of 0.05 (LSD: Root Cd = 7.99; Shoot Cd 5.94)]. Error bars indicates standard error (SE).

Photosynthetic rate of cultivars (Table 4.3) was also significantly lower (20.2 \( \mu M \) CO\(_2\) m\(^{-2}\) s\(^{-1}\)) under Cd stress compared to those grown without Cd stress (22.8 \( \mu M \) CO\(_2\) m\(^{-2}\) s\(^{-1}\)). Similar to chlorophyll contents, maximum decline in photosynthetic rate (20.87 %) was recorded for Sehar-2006 whereas it was minimum in Lasani-2008 (3.91 %).

Leaf stomatal conductance (\( cm \) H\(_2\)O m\(^{-2}\) s\(^{-1}\)) was statistically similar among wheat cultivars but Cd stress decreased it. Interaction between wheat cultivars and Cd stress was non-significant (Table 4.4). Decrease in stomatal conductance with exposure to Cd stress was negligible for Iqbal-2000 (1.0 %), Lasani-2008 (1.32 %) and Inqlab-91 (3.00%) whereas Sehar-2006 showed considerable decrease 9.09 % in stomatal conductance. Effect of Cd stress on leaf transpiration rate (\( mm \) H\(_2\)O m\(^{-2}\) s\(^{-1}\)) was non-significant whereas it differed significantly among cultivars (Table 4.4). Maximum transpiration rate was recorded for Lasani-2008 (6.93 \( mm \) of H\(_2\)O m\(^{-2}\) S\(^{-1}\)) while it was minimum (5.04 \( mm \) of H\(_2\)O m\(^{-2}\) s\(^{-1}\)) for Iqbal-2000.
Table 4.3. Effect of Cd stress on leaf chlorophyll content and gas exchange attributes of wheat cultivars

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>No-Cd</th>
<th>15 µM Cd</th>
<th>Mean</th>
<th>No-Cd</th>
<th>15 µM Cd</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chlorophyll content</td>
<td>Photosynthetic rate (µM CO₂ m⁻² s⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iqbal-2000</td>
<td>34.8</td>
<td>32.5</td>
<td>33.7 b</td>
<td>19.6</td>
<td>18.5</td>
<td>19.1 b</td>
</tr>
<tr>
<td>Lasani-2008</td>
<td>35.8</td>
<td>33.2</td>
<td>34.5 b</td>
<td>24.5</td>
<td>23.5</td>
<td>24.0 a</td>
</tr>
<tr>
<td>Inqlab-91</td>
<td>35.6</td>
<td>32.8</td>
<td>34.2 b</td>
<td>22.2</td>
<td>19.0</td>
<td>20.6 b</td>
</tr>
<tr>
<td>Sehar-2006</td>
<td>40.0</td>
<td>33.9</td>
<td>36.9 a</td>
<td>25.0</td>
<td>19.8</td>
<td>22.4 a</td>
</tr>
<tr>
<td>Mean</td>
<td>36.6 A</td>
<td>33.1 B</td>
<td>—</td>
<td>22.8 A</td>
<td>20.2 B</td>
<td>—</td>
</tr>
</tbody>
</table>

LSD

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Stomatal Conductance (cM H₂O m⁻² s⁻¹)</th>
<th>Transpiration rate (mM H₂O m⁻² s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iqbal-2000</td>
<td>6.11</td>
<td>5.02</td>
</tr>
<tr>
<td>Lasani-2008</td>
<td>6.31</td>
<td>7.01</td>
</tr>
<tr>
<td>Inqlab-91</td>
<td>6.44</td>
<td>6.39</td>
</tr>
<tr>
<td>Sehar-2006</td>
<td>6.49</td>
<td>6.72</td>
</tr>
<tr>
<td>Mean</td>
<td>6.34 a</td>
<td>6.28</td>
</tr>
</tbody>
</table>

Values sharing different letter are significantly different from each other at probability of 0.05.

4.4.5. Activities of antioxidant enzymes

Activities of antioxidant enzymes viz. Catalase (CAT), Ascorbate peroxidase (APX), Guaiacol peroxidase (GPX) and Superoxide dismutase (SOD) in leaves of wheat cultivars are given in Table 4.4. Catalase activity was significantly different between Cd treatments but differences for cultivars and interactive effect of Cd and cultivar differed non-significantly (Table 4.4). The LSCd cultivars Iqbal-2000 and Lasani-2008 showed sustained activity of CAT under Cd stress compared to no-Cd treated plants. Contrarily, HSCd Inqlab-91 and Sehar-2008 could not sustain CAT activity under Cd stress and showed significant decrease from 16.17 to 13.17 (20 %) and 15.34 to 13.68 (11 %) U min⁻¹ mg⁻¹ protein, respectively.

Unlike CAT, APX differed significantly both among wheat cultivars and Cd treatments but their interaction was non-significant (Table 4.4). Cultivar Sehar-2006 showed significantly lower APX activities (47.6 U mg⁻¹ protein) than the rest of cultivars that were statistically at par among each other in APX activity (60.0 – 63.4 U mg⁻¹ protein). In addition to lower APX activity among the wheat cultivars, Sehar-2008 also showed higher decline in activity of this enzyme (33 %) whereas minimum decrease was observed for Lasani-2008 (17 %).
magnitude of decline in the activity of APX (21 %) under Cd stress was observed for Iqbal-2000 and Inqlab-91.

Activity of GPX was statistically similar among wheat cultivars; however, unlike CAT and APX, it increased in all the cultivars when plants were exposed to Cd stress (Table 4.5). Increase in GPX activity was higher in LSCd cultivar Iqbal-2000 (40 %) and Lasani-2008 (27 %) than HSCd Inqlab-91 and Sehar-2006 showing 12 and 9 % decrease, respectively.

Although it tended to decrease under Cd stress (≤ 7 %), but activity of SOD remained statistically similar among stressed and no-Cd stressed plants but differed significantly among cultivars (Table 4.4). It was the maximum (203 U min\(^{-1}\) mg\(^{-1}\) protein) in Lasani-2008 whereas the minimum (161 U mg\(^{-1}\) protein) in Sehar-2006. In Iqbal-2000 and Sehar-2006, SOD activity (190 and 180 U mg\(^{-1}\) protein, respectively) was significantly lower than Lasani-2008, higher than Sehar-2006 and similar between them.

Table 4.4 Activity of antioxidant enzymes (Units mg\(^{-1}\) protein) in leaves of wheat cultivars grown under Cd stress

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>No-Cd</th>
<th>15 μM Cd</th>
<th>Mean</th>
<th>No-Cd</th>
<th>15 μM Cd</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAT</td>
<td>APX</td>
<td></td>
<td>CAT</td>
<td>APX</td>
<td></td>
</tr>
<tr>
<td>Iqbal-2000</td>
<td>16.3</td>
<td>16.1</td>
<td>16.2</td>
<td>70.7</td>
<td>56.1</td>
<td>63.4 a</td>
</tr>
<tr>
<td>Lasani-2008</td>
<td>15.8</td>
<td>15.7</td>
<td>15.7</td>
<td>68.9</td>
<td>57.5</td>
<td>63.2 a</td>
</tr>
<tr>
<td>Inqlab-91</td>
<td>16.2</td>
<td>13.2</td>
<td>14.7</td>
<td>67.1</td>
<td>52.9</td>
<td>60.0 a</td>
</tr>
<tr>
<td>Sehar-2006</td>
<td>15.3</td>
<td>13.7</td>
<td>14.5</td>
<td>56.9</td>
<td>38.4</td>
<td>47.6 b</td>
</tr>
<tr>
<td>Mean</td>
<td>15.9 a</td>
<td>14.7 b</td>
<td></td>
<td>65.9 a</td>
<td>51.2 b</td>
<td></td>
</tr>
</tbody>
</table>

LSD

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Cd</th>
<th>Cult (\times) Cd</th>
<th>Mean</th>
<th>Cultivar</th>
<th>Cd</th>
<th>Cult (\times) Cd</th>
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<tbody>
<tr>
<td>NS</td>
<td>1.14</td>
<td>NS</td>
<td>4.72</td>
<td>NS</td>
<td>3.34</td>
<td>NS</td>
</tr>
</tbody>
</table>

Cultivar GPX SOD

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>No-Cd</th>
<th>15 μM Cd</th>
<th>Mean</th>
<th>No-Cd</th>
<th>15 μM Cd</th>
<th>Mean</th>
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<tr>
<td></td>
<td>CAT</td>
<td>APX</td>
<td></td>
<td>CAT</td>
<td>APX</td>
<td></td>
</tr>
<tr>
<td>Iqbal-2000</td>
<td>82</td>
<td>114</td>
<td>98</td>
<td>191</td>
<td>189</td>
<td>190 b</td>
</tr>
<tr>
<td>Lasani-2008</td>
<td>83</td>
<td>106</td>
<td>94</td>
<td>206</td>
<td>199</td>
<td>203 a</td>
</tr>
<tr>
<td>Inqlab-91</td>
<td>99</td>
<td>111</td>
<td>105</td>
<td>182</td>
<td>178</td>
<td>180 b</td>
</tr>
<tr>
<td>Sehar-2006</td>
<td>86</td>
<td>94</td>
<td>90</td>
<td>166</td>
<td>155</td>
<td>161 c</td>
</tr>
<tr>
<td>Mean</td>
<td>88 b</td>
<td>106 a</td>
<td></td>
<td>186</td>
<td>180</td>
<td></td>
</tr>
</tbody>
</table>

LSD

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Cd</th>
<th>Cult (\times) Cd</th>
<th>Mean</th>
<th>Cultivar</th>
<th>Cd</th>
<th>Cult (\times) Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>7.85</td>
<td>NS</td>
<td>10.1</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Means sharing different letter are significant among each other at probability of 0.05.

4.4.6. Lipid peroxidation

Lipid peroxidation in leaves of wheat cultivars was measured in terms of malondialdehyde (MDA) content which is a byproduct from peroxidation of polyunsaturated lipids. Differences
among wheat cultivars and effect of Cd stress both were significant whereas their interaction was non-significant for MAD content (Table 4.5). Low-shoot-Cd cultivars, Iqbal-2000 and Lasani-2008, contained significantly lower MDA content in their leaves (51.7 and 45.8 nM g\(^{-1}\) fresh biomass, respectively) compared to that of HSCd cultivars Inqlab-91 and Sehar-2006 containing 64.1 and 62.7 nM MDA g\(^{-1}\) fresh biomass of leaves (Fig. 4.3). While growing in Cd containing nutrient solution, more decrease in MDA content for Inqlab-91 (26 %) and Sehar-2006 (47 %) compared to LSCd cultivars Iqbal-2000 and Sehar-2006 (22 % for both) was observed. The linear regression revealed negative relationship between activity of antioxidant enzymes against MDA content (R\(^2\) ≤ 0.64) with the exception of GPX (R\(^2\) = 0.023) (Fig. 4.2b) which indicates that the cultivars with higher antioxidant activities are more tolerant to Cd toxicity stress.

Table 4.5. Malondialdehyde content in leaves of cultivars as affected by Cd stress.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>No-Cd</th>
<th>15 µM Cd</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iqbal-2000</td>
<td>46.6</td>
<td>56.9</td>
<td>51.7 b</td>
</tr>
<tr>
<td>Lasani-2008</td>
<td>41.2</td>
<td>50.4</td>
<td>45.8 b</td>
</tr>
<tr>
<td>Inqlab-91</td>
<td>56.7</td>
<td>71.6</td>
<td>64.1 a</td>
</tr>
<tr>
<td>Sehar-2006</td>
<td>50.7</td>
<td>74.7</td>
<td>62.7 a</td>
</tr>
<tr>
<td>Mean</td>
<td>15.9 a</td>
<td>14.7 b</td>
<td></td>
</tr>
</tbody>
</table>

LSD

<table>
<thead>
<tr>
<th></th>
<th>Cultivar</th>
<th>Cd</th>
<th>Cult × Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.93</td>
<td>Cultivar</td>
<td></td>
<td>NS</td>
</tr>
</tbody>
</table>

Means sharing different letter are significant among each other at probability of 0.05.

4.5. Discussion

The aim of the study was to evaluate the ability of low and high shoot Cd containing cultivars to modify the pH of nutrient solution and production of antioxidant enzymes both under Cd contaminated and uncontaminated nutrient solution.

4.5.1. Wheat growth and Cd accumulation

The four wheat cultivars used in this study were selected out of 15 cultivars based on low (Iqbal-2000 and Lasani-2008) and high (Inqlab-91 and Sehar-2006) shoot Cd concentration during the initial screening study. Although on higher side, the trend of wheat cultivars for Cd concentration both in shoot and roots confirmed the results of screening study.
Fig. 4.2. Relationship between activities of antioxidant enzymes and MDA contents in leaves of wheat cultivars.

The shoot Cd concentration ranged between 35 to 36 and 44 to 58 mg kg\(^{-1}\) dry matter for LSCd and HSCd cultivars, respectively. Cadmium translocation index (ratio of shoot Cd to root Cd) revealed that higher/lower shoot Cd was the outcome of corresponding higher and/or lower Cd translocation to shoot. Similarly, in high and low grain-Cd cultivars of spring, winter \((Triticum aestivum\) L.) and durum wheat \((T. durum\) Desf.) cultivars, Greger and Löfstedt (2004) found that different Cd concentration in grains was related to variations in the transfer from root to shoot.

These results are also in line with the findings of Hart et al. (1998) who found 1.5 to 4.5 times higher Cd translocation from roots to shoots in a bread wheat than in a durum wheat cultivar. Near isogenic lines of durum wheat with higher grain Cd have shown 1.8 fold higher rate of \(^{109}\)Cd translocation from roots to shoots and 1.7 to 1.9 fold higher \(^{109}\)Cd concentration in root-pressure xylem exudates compared to that of low grain Cd lines (Harris and Taylor, 2004).

With considerable higher Cd concentration, shoot dry matter of wheat cultivars was similar to that of low Cd containing cultivars which confirmed that the traits of Cd accumulation and tolerance were not related to each other and are independently controlled.
within plants. Cadmium accumulation without inhibitory toxicity symptoms and/or effect on growth and physiology of plants has been shown by many plants. For example, wheat (Garrett et al., 1998) and barley (Vassilev et al., 2004) have been reported to show no Cd toxicity while growing in highly Cd contaminated conditions. Likewise, Cheng et al. (2008) reported that Cd toxicity in terms of reduction in seedling germination and growth was related to its concentration in shoots of some rice cultivars. On the other hand, root dry matter, photosynthetic rate, stomatal conductance and transpiration rate were severely affected by Cd stress in HSCd cultivars only. Non-significant relationship of tissue Cd concentration with Cd tolerance in terms of shoot growth of wheat (Ci et al., 2010) and seed germination and seedling growth of Japonica rice cultivars (Cheng et al., 2008) also suggest that these traits are independently regulated.

### 4.5.2. Activities of antioxidant enzyme

The presence of Cd in plant cells produces ROSs which are scavenged by enzymatic or non-enzymatic antioxidants. Thus, we hypothesized that antioxidant activity would enhance/sustain in low Cd wheat cultivars consequently such cultivars could be less prone to oxidative damage caused by Cd stress. Compared to uncontaminated environment, increased activities of antioxidant enzymes were expected under Cd stress so that the excessively produced ROSs may be scavenged to avoid oxidative stress. However, the response to Cd stress was depending on wheat cultivars and it was also enzyme specific. Activities of CAT and APX tended to decrease (LSCd cultivars), or significantly decreased (HSCd cultivars) while that of SOD remained unaffected when plants were exposed to Cd stress compared to non-stressed. Moreover, increase in activity of GPX was observed which was higher for HSCd cultivars compared to LSCd cultivars.

Contrasting results are available for the effect of Cd on the activity of the antioxidant enzymes. Zhao et al. (2011) reported decrease in SOD while increase in CAT and POD activities in wheat and corn seedlings under Cd stress up to 1 mg L⁻¹. Hassan et al. (2005) reported a higher activity of SOD in low compared to high grain Cd rice cultivar that decreased in both cultivars under Cd stress. They concluded that expected increase in SOD activity would have happened shortly after exposure to Cd and decreased thereafter with the passage of time. In other studies on wheat, (Dandan et al., 2011; Amirjani, 2012) rice (Shah et al., 2001) and
peas (Sandalio et al., 2001), decrease in CAT and SOD activities have been reported. These authors have suggested that Cd toxicity produced too many reactive oxygen radicals to be scavenged by these enzymes resulting in reduced activities of these enzymes in plants cells. Moreover, Dandan et al. (2011) have also attributed the decrease in activity of antioxidants to the increase of Cd in heat death protein (enzyme) fraction of plant cells that interfered with the enzyme system. Decreased antioxidant activity could have also been associated with degradation of enzyme system caused by peroxisomal proteases or to photo-inactivation of enzymes (Sandalio et al., 2001).

The MDA content in leaves increased significantly in all the wheat cultivars with exposure to Cd stress. Overall, the increase was less pronounced in LSCd compared to HSCd cultivars. The significant negative correlation between activities of antioxidant enzymes and MDA contents under Cd contaminated nutrient solution indicated that the cultivars with higher antioxidant activity were less prone to lipid peroxidation of membranes and vice versa. In low Cd rice cultivars Hassan et al. (2005) also reported less elevated level of MDA in low than high grain Cd rice cultivar were in negative correlation with the activity of SOD and CAT. Similarly, Zhao (2011) have reported that increased MDA content with increasing Cd stress level in hydroponic was in negative correlation with SOD and showed positive correlation with CAT and APX. Shi et al. (2010) also found the involvement of highly efficient antioxidant system in Cd-tolerance of peanut cultivar Luzi 101 compared to Cd-sensitive Luhua 11.

4.5.3 Change in root zone pH

Plant uptake is likely to be affected by dissolution of soil bound Cd through release of various root exudates that could decrease or increase the pH of rhizosphere. In the present study, cumulative change in pH of nutrient solution over 15 days of growth was found significantly different among the cultivars. Under Cd stress, the ability of roots to negatively modify the pH of nutrient solution substantially decreased compared to control leading to lower degree of average decrease (Table 4.2). Lesser decrease in pH under Cd stress might be due to reduced root activity as a result of Cd toxicity since roots are more sensitive to Cd toxicity than shoots. The wheat cultivar Lasani-2008 induced significantly lower decrease in pH (0.141 units) compared to the rest of cultivars that were statistically at par with each other in lowering root zone pH. The LSCd cultivar Iqbal-2000 was similar to HSCd cultivars for decreasing the pH
of Cd contaminated nutrient solution which indicated that the low SCd was not linked to the maintenance of alkaline rhizosphere in nutrient solution. Decrease in pH might enhance Cd uptake by promoting Cd dissolution in soil but in current study the Cd was already in soluble form. Contrarily, Gill et al. (1999) reported a significant difference in pH decreasing potential of 12 wheat cultivars over 18 days of growth which had strong negative correlation with P uptake. Murányi et al., 1994 found a 1 unit decrease in pH within 1 mm of rhizosphere and Cieslinski et al. (1997) found that release of organic acids from roots of wheat was cultivar dependent.

4.6. Conclusion
The growth response to Cd stress and Cd concentration both in roots and shoots of wheat cultivars are in line to the results of screening study i.e. the Cd concentration was low in cultivars Iqbal-2000 and Lasani-2008 and high in Inqlab-91 and Sehar-2006. The change in decrease in pH of rooting medium had not shown any relation to shoot Cd concentration of cultivars probably due to having no effect on solubility of Cd in nutrient solution as all the Cd was already in soluble form. The LSCd cultivars as well as one of the high Cd cultivars showed substantial tolerance to Cd for growth and gas exchange. Sustained or significantly higher increase in the activity of antioxidant enzymes which were positively and negatively correlated with Cd tolerance and MDA contents, respectively suggesting that Cd concentration and tolerance in wheat cultivars was related to the activity of antioxidant enzymes.
CHAPTER 5

CADMIUM ACCUMULATION AND ANTIOXIDANT RESPONSE OF WHEAT GENOTYPES TO SILICON APPLICATION IN HYDROPONICS

5.1. Abstract

The effect of Si application on growth, antioxidant activity and Cd concentration in two each of high and low shoot Cd (HSCd and LSCd) cultivars was evaluated in hydroponics experiment. Based on initial screening study, the LSCd cultivars, Iqbal-2000 and Lasani-2008 and HSCd cultivars, Inqlab-91 and Sehar-2006, were grown in Jonson’s nutrient solution without and with 15 $\mu$M Cd. Cadmium stress (15 $\mu$M Cd as CdCl$_2$.$H_2$O) was imposed 15 days after seedling transplantation in hydroponics with and without three levels of Si (2, 4 and 6 $mM$) as monosilicic acid (SiO$_2$.nH$_2$O). In LSCd cultivars, Si enhanced dry matter either non-significantly or increase was lower compared to HSCd cultivars. Silicon treated HSCd wheat cultivars showed higher improvement in chlorophyll contents and photosynthesis compared to LSCd cultivars. On the other hand, Si application did not affect stomatal conductance and transpiration rate in LSCd cultivars whereas decreased them in HSCd cultivars. Conversely, Cd stress had higher negative effect on chlorophyll contents and photosynthesis in sensitive HSCd cultivars compared to tolerant LSCd cultivars. Thus, Si increased chlorophyll contents and photosynthesis that could be regarded as the alleviation of Cd toxicity in Cd stressed plants. Improvement or reduction in gas exchange attributes of Si treated plants was limited to 2 or 4$mM$ Si application. The Si treatment enhanced the activities of CAT, APX, GPX and SOD antioxidant enzymes but depressed MDA content in Cd stressed wheat cultivars. Generally, the increase (Antioxidants) or decrease (MDA) was higher in LSCd cultivars compared to HSCd cultivars. Increase in antioxidant activity with Si treatment alleviated lipid peroxidation only in HSCd sensitive cultivars as indicated by decreased MDA contents in leaves. Application of Si significantly caused low RCd concentration only in LSCd cultivars while that of SCd in both the HSCd and LSCd cultivars. The decrease in SCd concentration seems due to both the decreased absorption and less translocation from roots to shoots. Similar to root and shoot dry matter, 4$mM$ remained the optimum level (except 2$mM$ for RCd in Lasani-2008)
reducing Cd concentration in wheat cultivars beyond which it caused no further decrease in Cd concentration. Overall, optimum level of Si enhancing growth, gas exchange attributes and antioxidant activity was either 4 mM or 2 mM in fewer parameters indicating that the optimum level of Si for wheat cultivars lay between these treatments.

5.2. Introduction

Accumulation of Cd to phytotoxic levels may cause significant growth inhibition and yield loss. In plants, Cd induces the production of ROS that could enhance lipid peroxidation. Lipid peroxidation can result in loss of plasma membrane integrity leading to indiscriminate Cd uptake (Tian et al., 2011). Thus, plants equipped with defense mechanism against Cd toxicity, like enhanced antioxidant production, could be thought to have low tissue Cd in addition to better growth performance as has been reported for barley (Wu and Zhang, 2002).

Plants have evolved different mechanisms to avoid Cd uptake and accumulation in grains including exclusion at root level, compartmentalization of Cd and production of stress proteins (Clemens et al, 2013). The different potential of plants to exclude Cd absorption and alleviate its toxicity provide an opportunity to use low Cd species to obtain Cd free food. Cadmium absorption, translocation and toxicity in plants have also been shown to be affected by interaction with mineral nutrients such as Zn (Zhu et al., 2003; Saifullah et al., 2013) and Si (Liang et al., 2007). Therefore, in addition to selection of low Cd genotypes, mineral nutrients can also be used to decrease Cd entry into food chain through minimizing Cd accumulation in cereal grains (Sarwar et al., 2010). This strategy seems foreseeable in case the cultivation of low Cd accumulating species/cultivars seems impracticable because of their low yield potential and/or susceptibility to some disease.

Silicon has been categorized as ‘quasi-essential’ because of its important role in improving growth and quality of a number of plant species especially under adverse growth conditions (Epstein and Bloom, 2005). In wheat and many other members of the Poaceae family, Si is actively absorbed by roots (Rains et al., 2006; Currie and Perry, 2007). Silicon deposition in root endodermis can strengthen the casparian band to restrict the bypass flow of Cd from roots to shoots. It can also co-precipitate with Cd as Cd-silicate in vacuole which could again limit the Cd transport to edible parts in rice (Shi et al., 2005a).
In a number of studies especially on rice and maize, Si has been shown not only to restrict Cd absorption and translocation but also to alleviate its toxicity through enhancing activity of antioxidant enzymes (da Cunha et al., 2008, Liang et al., 2005, Nwugo and Huerta, 2008; Lukacova et al., 2013). But, studies on wheat are lacking especially in Pakistan, despite the fact that wheat is the most important cereal crop in Pakistan (Economic Survey of Pakistan, 2012-13) and ranked number two for production worldwide in 2009 (FAO, 2011). In a recent study, (Rizwan et al., 2012), Si application decreased the grain Cd concentration in durum wheat. Since the cultivar of durum wheat used was sensitive to Cd stress, it remained un-elucidated that the decrease was due to reduction in absorption or it was associated with growth dilution caused by Si induced increase in dry matter. Moreover, effect of Si on growth, Cd accumulation and activities of antioxidant could also differ with Cd accumulation and/or tolerance levels of plants.

Keeping in view the above facts, a hydroponics experiment was conducted using four cultivars of bread wheat (Triticum aestivum L.) with low and high Cd accumulation abilities in shoots. Main objectives of the study were to investigate the impact of Si on Cd detoxification with regard to activities of antioxidant enzyme, plant growth and Cd absorption and translocation in two high and two low shoot Cd wheat cultivars.

5.3. Materials and Methods

5.3.1. Nursery raising and transplantation

Based upon the findings of initial screening study (study 1), two high shoot Cd cultivars (Inqlab-91, Sehar-2006) and two low shoot-Cd wheat cultivars (Iqbal-2000 and Lasani-2008, respectively) were used in this study. These high and low shoot-Cd cultivars will be hereafter referred as HSCd and LSCd cultivars, respectively. Seeds of selected cultivars were surface sterilized with 3% H$_2$O$_2$ solution for 10 minutes and washed with distilled water. These disinfected seeds were sown in polyethylene lined iron trays containing acid washed sand. Moisture contents were maintained at 10% of sand weight in germination trays until seedlings were 10 days old. Seedlings were transplanted into foam plugged holes (two seedlings per hole) in polystyrene sheets floating on plastic pots (2 L capacity) containing Johnson’s nutrient solution. The composition of the nutrient solution was same as used in study 1 (Table 3.1). The pH of the nutrient solution was measured daily with Senso Direct pH 200 pH meter (Lovibond,
USA) and was maintained at 6.50±0.01 using 1% NaOH or HCl. The nutrient solution was continuously aerated using an aeration pump and after each seven days of growth period, the nutrient solution was replaced.

5.3.2. Silicon and cadmium application
While replacing nutrient solution after 2nd week of growth completion, plants were exposed to 15 µM Cd as CdCl₂·H₂O and three levels of Si (2, 4 and 6 mM) as mono silisic acid (SiO₂·nH₂O). The experiment, two sets of three replications each, was laid out in completely randomized design (CRD). The seedlings were harvested after 30 days of growth period. One set was harvested for enzyme assay and physiological/biochemical analysis and the other for growth response and chemical analysis.

5.3.3. Measurement of Gaseous exchange attributes
Measurement of gas exchange attributes was carried out as described in section 4.3.2.

5.3.4. Plant harvesting and chemical analysis
At day 30 of growth period in nutrient solution (15 days after Cd exposure), plants were harvested. One set (three reps) was used for the measurement of dry matter of shoots and roots, whereas the second set of three replicates was used for the assay of antioxidant enzymes. Seedlings from the 1st set were separated into roots and shoots, washed with 1% v/v acetic acid followed by rinsing in distilled water. The washed samples were blotted dry and put into a convection oven adjusted to 65±5 °C. After 72 h of oven drying, shoot and root dry matter was recorded. The dried samples were ground, digested following the procedure described by AOAC (1990) as described in section 4.3.3. Cadmium in digests was determined with Atomic Absorption Spectrophotometer (AAS) (Model Thermo Electron S-Series). However, for Si determination shoot and root samples (0.2 g) were digested in Teflon beakers with 2 mL of 50 % NaOH and 5 mL of 35 % H₂O₂ in five increments of 1 mL each. The digests were diluted to 50 mL with distilled water and stored in plastic bottles for Si determination. Aliquot of the digests (1 mL) was taken in 25 mL polypropylene flask and 5 mL of 1.0 N H₂SO₄ and 5 mL of 0.3 M ammonium heptamolybdate adjusted to pH 7.0 with 5 N NaOH was added. After 2 minutes of reaction time, 2.5 mL of 20 % tartaric acid and 1 mL reducing solution was added.
to the mixture. The mixture was diluted to volume and absorbance was taken at 650 nm using UV spectrophotometer (Shamazu, Japan). The reducing solution was prepared as follows: Sodium bisulphate (12.5 g) was dissolved in 100 mL DI water and 1.0 g of sodium sulphite along with 0.2 g 1-amino-2-naphthol-4-sulphonic acid was dissolved in 12.5 mL DI water. Both the solutions were mixed, shaken well and diluted to 125 mL final volume.

5.3.5. Enzymes assay and measurement of other biochemical and physiological parameters
Enzyme assay and biochemical/physiological analysis were carried out (2nd set of three reps) as described in section 4.3.3.

5.3.6. Statistical analysis
The data regarding plant growth, Cd accumulation and biochemical/physiological attributes of plants in response to Si application were statistically analyzed following ANOVA technique and LSD test was applied to differentiate the treatment differences (Steel et al., 1996) using Statistix Version 8.1 software package.

5.4. Results
5.4.1. Root and shoot dry matter
Cadmium stressed wheat cultivars produced significantly different root dry matter (RDM) (Table 5.1). However, application of Si had significant stimulating effect on RDM of all the Cd stressed wheat cultivars. Higher Si induced increase in RDM was recoded for HSCd cultivars compared to LSCd Cd cultivars. The increase in RDM with increasing level of 2 to 6 mM Si application was: 6.1, 8.1 and 7.1% for Iqbal-2000; 6.2, 5.9 and 4.1 % for Lasani-2008; 15.9, 24.3 and 22.5 % for Inqlab-91 and 10.2, 19.2 and 20.4 % for Sehar-2006. Beyond 4 mM Si application, no further increase in RDM was observed in wheat cultivars except Lasani-2008, in which improvement in RDM became stagnant over 2 mM Si application. Similar to RDM, Cd stressed wheat cultivars produced significantly different shoot dry matter (SDM) (Table 5.1). The maximum SDM was observed for Inqlab-91 (1.84 g pot⁻¹) while the minimum for Iqbal-2000 (1.11 g pot⁻¹). Effect of Si application on SDM of LSCd cultivars Iqbal-2000 and Lasani-2008 and one of the LSCd cultivar Inqlab-91 was non-significant. However, SDM of Sehar-2006 significantly increased with the addition of Si. The highest increase of 20.3 %
was observed at 4 mM Si application beyond which no further improvement in SDM was observed.

Table 5.1 Effect of Si nutrition on root and shoot dry matter (g pot⁻¹) of Cd stressed wheat cultivars

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Iqbal-2000</th>
<th>Lasani-2008</th>
<th>Inqlab-91</th>
<th>Sehar-2006</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>RDM (g pot⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.295 de</td>
<td>0.319 bc</td>
<td>0.218 h</td>
<td>0.287 ef</td>
<td>0.280</td>
</tr>
<tr>
<td>Si 2 mM</td>
<td>0.313 (6.1) cd</td>
<td>0.339 (6.2) a</td>
<td>0.253 (15.9) g</td>
<td>0.317 (10.2) c</td>
<td>0.305</td>
</tr>
<tr>
<td>Si 4 mM</td>
<td>0.319 (8.1) bc</td>
<td>0.338 (5.9) ab</td>
<td>0.271 (24.3) fg</td>
<td>0.343 (19.2) a</td>
<td>0.318</td>
</tr>
<tr>
<td>Si 6 mM</td>
<td>0.316 (7.1) c</td>
<td>0.332 (4.1) abc</td>
<td>0.267 (22.5) g</td>
<td>0.346 (20.4) a</td>
<td>0.315</td>
</tr>
<tr>
<td>Mean</td>
<td>0.311</td>
<td>0.332</td>
<td>0.252</td>
<td>0.323</td>
<td>—</td>
</tr>
</tbody>
</table>

| SDM (g pot⁻¹) |            |             |            |            |      |
| Control       | 1.10       | 1.68        | 1.78       | 1.13       | 1.42 b |
| Si 2 mM       | 1.13 (4.6) | 1.76 (4.2)  | 1.87 (4.4) | 1.27 (12.1) | 1.51 a |
| Si 4 mM       | 1.13 (4.9) | 1.76 (3.9)  | 1.89 (5.3) | 1.36 (20.3) | 1.53 a |
| Si 6 mM       | 1.10 (1.7) | 1.71 (1.3)  | 1.80 (0.3) | 1.32 (16.5) | 1.47 ab |
| Mean          | 1.11 e      | 1.73 b      | 1.84 ab    | 1.26 de     | —     |

Values (means with % increase or decrease over control in parenthesis) followed by different letter(s) are statistically significant at α = 0.05.

LSD
Root dry matter: Cultivar, 0.010; Treatment, 0.013; Cultivar × Treatment, 0.020
Shoot dry matter: Cultivar, 0.08; Treatment, 0.08; Cultivar × Treatment, NS

5.4.2. Effect of Si nutrition on chlorophyll contents and gas exchange of wheat cultivars

Gas exchange attributes were significantly different among cultivars, treatments and interactive effect of treatment and cultivars (Fig. 5.1). Cadmium stressed wheat cultivars had significantly different chlorophyll contents in decreasing order of Inqlab-91 > Lasani-2008 > Iqbal-2000 > Iqbal-2006 (Fig. 5.1 a). Effect of Si on chlorophyll contents was cultivar dependent and also varied with Si application rate. Among LSCd cultivars, Si had no effect on chlorophyll contents of Iqbal-2000 while it increased (6.19 %) for Lasani-2008 only at the highest level of Si addition (6 mM). As for as HSCd cultivars are concerned, chlorophyll contents increased up to 4 mM Si addition and increase of 22.9 and 15.7 % was recorded at this Si level for Sehar-2006 and Inqlab-91, respectively. Silicon had more pronounced effect on chlorophyll contents in HSCd cultivars compared to LSCd cultivars in which no (Iqbal-2000) or comparatively lesser increase (Lasani-2008) was observed.
The photosynthetic rate ($\mu M$ CO$_2$ m$^{-2}$ s$^{-1}$) of Cd stressed LSCd cultivars remained unaffected with Si while that of HSCd increased (Fig. 5.1 b). Moreover, Si application over 4mM showed no further improvement in photosynthesis. The increase in photosynthetic rate of Inqlab-2000 and Sehar-2006 with 4mM Si was 35.0 and 36.1 %, respectively. Contrary to chlorophyll contents and photosynthetic rate, leaf stomatal conductance ($cM$ H$_2$O m$^{-2}$ s$^{-1}$) decreased in Si treated Cd stressed HSCd cultivars while that of LSCd cultivars remained unaffected (Fig. 5.1c). The decrease in stomatal conductance ranged between 11 - 20 and 12 - 22 % for of Inqlab-91 and Sehar-2006, respectively. Application of Si beyond 4 mM did not further decrease the stomatal conductance in Cd stressed plants of HSCd cultivars. As a consequence of decrease in stomatal conductance, transpiration rate ($mM$ H$_2$O m$^{-2}$ s$^{-1}$) also decreased in Si treated Cd stressed HSCd wheat cultivars while that of LSCd remained unaffected (Fig. 5.1 d). The decrease in transpiration rate ranged between 11 - 22 and 14 - 31 % for Inqlab-91 and Sehar-2006, respectively. Over all, Si improved the leaf chlorophyll contents and photosynthesis but decreased stomatal conductance and transpiration in HSCd cultivars while that of LSCd cultivars remained unaffected.

5.4.3. Activities of antioxidant enzymes in Cd stressed wheat cultivars

Under Cd stress, the HSCd cultivar Sehar-2006 showed significantly lower activities of CAT and APX antioxidant enzymes compared to rest of the three cultivars (Fig. 5.2 a and b). Silicon treated wheat cultivars showed increase in CAT activity however, no increase was recorded beyond 4 mM Si application (Fig. 5.2 a). The LSCd cultivars, Iqbal-2000 and Lasani-2008, showed higher increase of 65.2 and 43.1 %, respectively compared to that of HSCd cultivars Inqlab-91 (39.8%) and Sehar-2006 (31.3%). The effect of Si application on the activity of APX was similar to that of CAT (Fig. 5.2 b).

However, increase in APX activity was not related to whether a cultivar is low or high in shoot-Cd concentration. At 4mM Si application, the optimum level enhancing APX activity, the maximum increase in APX activity was recorded for Iqbal-2000 (49.8 %) while it was minimum (35.2 %) for Inqlab-91. Under Cd stress, the activity of GPX was low in Iqbal-2000 compared to rest of three cultivars which were statistically similar to each other (Fig. 5.2 c).
Fig. 5.1. Effect of Si nutrition on chlorophyll content (a), photosynthetic rate (b), stomatal conductance (c) and transpiration rate (d) of Cd stressed wheat cultivars.

The bars followed by different letter(s) are statistically significant at $\alpha = 0.05$ [LSD: Chlorophyll = 2.11; Photosynthetic rate = 2.19; Stomatal conductance = 27.85; Transpiration rate = 0.72]. Error bars indicates standard error (SE).
Fig. 5.2. Effect of Si nutrition on activities of Catalase (CAT; a), Ascorbate peroxidase (APX; b), Guacol peroxidase (GPX; c) and superoxide dismutase (SOD; d) antioxidant enzymes of Cd stressed wheat leaves. The bars followed by different letter(s) are statistically significant at $\alpha = 0.05$ [LSD: CAT = 2.47; APX = 9.22; GPX = 10.09; SOD = 10.12]. Error bars indicates standard error (SE).
It was also enhanced by Si application but contrary to CAT and APX, GPX activity did not increased beyond 2 mM Si addition. Likewise CAT, higher improvement in GPX activity was shown by LSCd cultivars Iqbal-2000 and Lasani-2008 (21.4 and 22.6 %, respectively) relative to HSCd cultivars Inqlab-91 and Sehar-2006 showing increase of 14.3 and 10.5 %, respectively. The activity of SOD was significantly low in Cd stressed HSCd cultivars compared to LSCd cultivars (Fig. 5.2 d). Similarly, Si enhanced SOD activity and enhancement was more pronounced in LSCd compared to HSCd cultivars. Like CAT and GPX, 4 mM Si was the optimum level for enhancing activity of SOD in these cultivars. The increase in activity of SOD at 4mM Si application was 21.3, 15.9, 10.8 and 11.2 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively. Overall, among the antioxidant enzymes the maximum increase (11.9 - 67.4 %) was recorded for CAT activity while the minimum (7.1-23.6 %) for SOD.

However, increase in APX activity was not related to whether a cultivar is low or high in shoot-Cd concentration. At 4mM Si application, the optimum level enhancing APX activity, the maximum increase in APX activity was recorded for Iqbal-2000 (49.8 %) while it was minimum (35.2 %) for Inqlab-91. Under Cd stress, the activity of GPX was low in Iqbal-2000 compared to rest of three cultivars which were statistically similar to each other (Fig. 5.2 c). It was also enhanced by Si application but contrary to CAT and APX, GPX activity did not increased beyond 2 mM Si addition.

Likewise CAT, higher improvement in GPX activity was shown by LSCd cultivars Iqbal-2000 and Lasani-2008 (21.4 and 22.6 %, respectively) relative to HSCd cultivars Inqlab-91 and Sehar-2006 showing increase of 14.3 and 10.5 %, respectively. The activity of SOD was significantly low in Cd stressed HSCd cultivars compared to LSCd cultivars (Fig. 5.2 d). Similarly, Si enhanced SOD activity and enhancement was more pronounced in LSCd compared to HSCd cultivars. Like CAT and GPX, 4 mM Si was the optimum level for enhancing activity of SOD in these cultivars. The increase in activity of SOD at 4mM Si application was 21.3, 15.9, 10.8 and 11.2 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively. Overall, among the antioxidant enzymes the maximum increase (11.9 - 67.4 %) was recorded for CAT activity while the minimum (7.1-23.6 %) for SOD.
5.4.4. Silicon nutrition and malondialdehyde content in leaves of wheat cultivars

Under Cd stress, LSCd cultivars showed significantly low leaf malondialdehyde (MDA) content compared to HSCd cultivars (Fig 5.3). Moreover, Si treated LSCd cultivars responded no change in MDA contents where as in HSCd cultivars, MDA contents significantly decreased with Si application. Increasing Si beyond 4 mM did not further decrease MDA contents in these cultivars. Among HSCd cultivars, decrease in MDA concentration was higher in Sehar-2006 (24.5 %) compared to Inqlab-91 (11.4 %) at 4 mM addition.

![Fig. 5.3. The MDA contents in leaves of Cd stressed wheat cultivars treated with different rates of Si. The bars followed by different letters are significantly different at α 0.05 (LSD cultivar × Treatment = 8.88). Error bars indicates standard error (SE).]

5.4.5. Cadmium concentration in roots and shoots of wheat cultivars

In non-Si treated treatment, root Cd concentration (RCd) differed significantly in low shoot Cd cultivars while there was no significant difference for RCd in high shoot Cd cultivars (Table 5.3). The maximum RCd (123 mg kg⁻¹) was recorded in Lasani-2008 while minimum (92 mg kg⁻¹) in Iqbal-2000. Application of Si decreased Cd concentration in LSCd cultivars while it remained unaffected in high shoot Cd cultivars. However, response level of Si differed in both of LSCd and HSCd cultivars for decrease in RCd concentration. The decrease in RCd at 4 mM Si application was 11.5 and 10.4 % in Iqbal-2000 and Lasani-2008, respectively. Beyond these levels of Si, effect on RCd was non-significant for wheat cultivars.
Regarding shoot-Cd concentration (SCd), cultivars remained consistent with results of initial studies, i.e. Iqbal-2000 and Lasani-2008 showed low while Inqalab-91 and Sehar-2006 high Cd concentration in shoots. The interaction between Si and cultivars for SCd remained non-significant and contrary to RCd, Si application decreased SCd both in low and high shoot-Cd cultivars. In both groups of cultivars, no decrease in SCd was observed beyond 4 mM Si application. Maximum decrease in SCd was recorded in Iqbal-2000 while minimum in Sehar-2006 at either level of Si application. The decrease in SCd ranged between 14.2 to 26.3 % and 23.6 to 43.3 % at 2 and 4 mM Si applications, respectively.

Table 5.2. Effect of Si nutrition on root and shoot Cd concentration of wheat cultivars

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Iqbal-2000</th>
<th>Lasani-2008</th>
<th>Inqalab-91</th>
<th>Sehar-2006</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>92 d</td>
<td>123 a</td>
<td>106 bc</td>
<td>107 bc</td>
<td>107</td>
</tr>
<tr>
<td>Si 2 mM</td>
<td>79 (-13.1) e</td>
<td>120 (-2.2) a</td>
<td>108 (1.8) bc</td>
<td>108 (1.7) bc</td>
<td>104</td>
</tr>
<tr>
<td>Si 4 mM</td>
<td>81 (-11.5) e</td>
<td>110 (-10.4) b</td>
<td>105 (-1.5) bc</td>
<td>103 (-3.6) c</td>
<td>100</td>
</tr>
<tr>
<td>Si 6 mM</td>
<td>79 (-13.3) e</td>
<td>111 (-9.5) b</td>
<td>107 (0.84) bc</td>
<td>110 (3.0) bc</td>
<td>102</td>
</tr>
<tr>
<td>Mean</td>
<td>83</td>
<td>116</td>
<td>107</td>
<td>107</td>
<td></td>
</tr>
</tbody>
</table>

LSD Cultivar Treatment Cult × Treat
3.30 3.38 6.76

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Iqbal-2000</th>
<th>Lasani-2008</th>
<th>Inqalab-91</th>
<th>Sehar-2006</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>40.6</td>
<td>37.9</td>
<td>51.2</td>
<td>47.4</td>
<td>44.3 a</td>
</tr>
<tr>
<td>Si 2 mM</td>
<td>29.9 (-26.3)</td>
<td>29.2 (-22.9)</td>
<td>41.0 (-19.9)</td>
<td>40.7 (-14.2)</td>
<td>35.2 b</td>
</tr>
<tr>
<td>Si 4 mM</td>
<td>23.0 (-43.3)</td>
<td>24.4 (-35.5)</td>
<td>34.2 (-33.3)</td>
<td>36.2 (-23.6)</td>
<td>29.5 c</td>
</tr>
<tr>
<td>Si 6 mM</td>
<td>25.9 (-36.2)</td>
<td>26.1 (-31.2)</td>
<td>34.8 (-32.1)</td>
<td>36.1 (-23.8)</td>
<td>30.7 c</td>
</tr>
<tr>
<td>Mean</td>
<td>29.9 b</td>
<td>29.4 b</td>
<td>40.3 a</td>
<td>40.1 a</td>
<td></td>
</tr>
</tbody>
</table>

LSD Cultivar Treatment Cult. × Treat.
1.73 1.70 NS

Values (means with % increase or decrease over control in parenthesis) for each of RCd and SCd followed by different letter(s) are significantly different from each other at α = 0.05.

5.4.6. Root to shoot Cd translocation
There was a decrease in Cd translocation from roots to shoots with Si application in all the cultivars irrespective of whether it was LSCd or HSCd one (Fig. 5.4). Maximum decrease in Cd translocation index (CdTI) was recorded at 4 mM Si beyond that there was no further decrease in CdTI. Among cultivars, LSCd Iqbal-2000 showed the maximum decrease in Cd TI (35.9 %) while it remained minimum for Sehar-2006 (21.1%) at 4 mM Si application.
5.4.7. Concentration of Si in roots and shoots of wheat cultivars
The concentration of Si (mg g\(^{-1}\) dry matter) both in roots and shoots of Cd stressed wheat cultivars increased with Si application but no increase was observed beyond 4 \(mM\) application (Table. 5.4). Concentration of Si in roots (RSi) ranged between 7.5 to 19.2 while that of roots (SSi) was 13.5 to 27.3 mg g\(^{-1}\). However, RSi was less than that of SSi and LSCd cultivars had significantly higher Si both in roots and shoots compared to HSCd cultivars (Fig 5.5). The ratio of RSi to SSi was higher in LSCd cultivars compared to HSCd cultivars (Fig. 5.5).

5.4.8. Relationship of Cd concentration and translocation with transpiration rate and root Si concentration
Keeping in view the differential effect of Si application on transpiration rate and RSi of LSCd and HSCd cultivars and their role in Cd absorption by plants, relationship of Cd concentration, and translocation with transpiration rate and RSi was examined (Fig. 5.6). The correlation depicted that SCd, RCd and CdTI in LSCd cultivars Iqbal-2000 and Lasani-2008 were not related to transpiration (Fig. 5.6 a). However, in HSCd cultivars Inqlab-91 and Sehar-2000 RCD showed no relationship with transpiration rate whereas SCd had a significant negative correlation (p < 0.001) with transpiration rate (Fig 5.6 a, b). Similarly, CdTI also showed
significant negative correlation with transpiration rate (0.031 and 0.016 for Inqlab-91 and Sehar-2006, respectively) in HSCd cultivars (Fig. 5.6b).

Except Lasani-2008, SCd in Cd stressed wheat cultivars showed significant negative correlation with RSi (p ≤ 0.024) (Fig. 5.7 a). In Lasani-2008, the relationship although existed but it was weak (p = 0.065). The RCd was negatively correlated with RSi (p = 0.02) only in Lasani-2008 while other cultivars showed no relationship among these traits (Fig. 5.7 a). Only in Iqbal-2000 CdTI showed significant negative correlation with RSi while rest of the cultivars did not show any relation between these attributes (Fig. 5.7 b).

Table 5.3 Silicon concentration in roots and shoots of Cd stressed wheat cultivars

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Iqbal-2000</th>
<th>Lasani-2008</th>
<th>Inqlab-91</th>
<th>Sehar-2006</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root Si (mg g⁻¹ DM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Si 2 mM</td>
<td>13.4</td>
<td>11.8</td>
<td>6.1</td>
<td>7.5</td>
<td>9.7 b</td>
</tr>
<tr>
<td>Si 4 mM</td>
<td>18.8</td>
<td>14.9</td>
<td>9.3</td>
<td>10.6</td>
<td>13.4 a</td>
</tr>
<tr>
<td>Si 6 mM</td>
<td>19.2</td>
<td>16.1</td>
<td>9.0</td>
<td>10.9</td>
<td>13.8 a</td>
</tr>
<tr>
<td>Mean</td>
<td>17.1 a</td>
<td>14.3 b</td>
<td>8.1 d</td>
<td>9.7 c</td>
<td></td>
</tr>
</tbody>
</table>

| LSD Cultivar Treatment Cult. × Treat. | 1.27 | 1.10 | NS |
| Shoot Si (mg kg⁻¹ DM) | | | |
| Treatment | Iqbal-2000 | Lasani-2008 | Inqlab-91 | Sehar-2006 | Mean |
| Si 2 mM | 19.3 | 17.4 | 12.8 | 13.5 | 15.7 |
| Si 4 mM | 26.8 | 24.3 | 18.2 | 19.1 | 22.1 |
| Si 6 mM | 27.2 | 26.1 | 18.0 | 20.0 | 22.8 |
| Mean | 24.4 a | 22.6 b | 16.3 c | 17.5 c |

Values for Si concentration in roots and shoot followed by different letters are significantly different from each other at α = 0.05.

5.5. Discussion

The study aimed at evaluating the effect of Si nutrition on growth response, antioxidant activities and Cd concentration in roots and shoots of Cd stressed cultivars differing in shoot Cd concentrating abilities. Two HSCd cultivars (Iqbal-2000 and Lasani-2008) and two LSCd cultivars (Inqlab-91 and Sehar-2006) used in this study were selected out of 15 cultivars based upon low and high concentration of Cd in shoots, respectively. The results are discussed in the light of existing literature on subject matter as follows:
The bars followed by different letters in each set of column are significantly different from each other at α = 0.05 (LSD: SSi = 0.815; LSD RSi = 0.647).

5.5.1. Growth and Cd concentration of Si treated wheat cultivars

Addition of Si to Cd treated nutrient solution enhanced SDM and RDM of HSCd cultivar Sehar-2006. Silicon addition at 4 mM Si seems the optimum level since beyond this dry matter did not increase further. More interestingly, Si did not improve the SDM of HSCd cultivar Inqlab-91 which had been proved SDM-tolerant to the same Cd stress level during initial screening study. In LSCd cultivars, Si enhanced dry matter was either non-significant or increase was very low compared to HSCd cultivars.

Application of Si significantly decreased RCd concentration only in LSCd cultivars while that of SCd in both the HSCd and LSCd cultivars. Similar to root and shoot DM, Si application at 4 mM appeared as the optimum level for decreasing Cd concentration in wheat cultivars except for RCd concentration of Lasani-2008 which did not decrease beyond 2 mM Si application. Although treatment level varied, Si induced increase in DM and decrease in Cd concentration has also been reported for Cd stressed wheat (Rizwan et al., 2012), rice (Zhang et al., 2008), maize (Lukačová et al., 2013; da Cunha et al., 2008) peanut (Shi et al., 2010) and brassica (Song et al., 2009) plants. The improvement in dry matter of HSCd sensitive cultivars with Si application could be regarded as the reverse of Cd toxicity.
Fig. 5.6. Cadmium concentration and CdTI of Cd stressed wheat cultivars as a function of transpiration rate.
Fig. 5.7. Cadmium concentration and CdTI of Cd stressed wheat cultivars as a function of Si concentration in roots.
However, contrary to our results, for *Brassica chinensis* L. and peanut, the improvement in dry matter and decrease in Cd concentration was higher for Cd-tolerant cultivars compared to sensitive ones. In our study decrease in Cd concentration was concomitant with an increase in dry matter (except SDM of Inqlab-91) that confirm the findings of Rizwan *et al.* (2012) who attributed Si induced decrease in SCd to growth dilution effect. However, in Inqlab-91, Si induced decrease in SCd was independent of increase in dry matter as it remained unaffected by both the Cd (Study 1) and Si treatments (in present studies). It implies that Si induced decrease in Cd concentration was not related to growth dilution; a fact which remained un-elucidated in the studies of Rizwan *et al.* (2012).

Also the significantly decreased Cd translocation from root to shoot and low RCd concentration (LSCd cultivars only) suggests that Si decreased both Cd absorption and translocation independent of enhancement in growth. These results are consistent with earlier studies which reported that Si decreased the absorption and translocation of Cd in rice (*Shi et al.* 2005a) and maize (*Liang et al.*, 2005). A number of mechanisms have been suggested by which Si could decrease absorption and translocation of Cd in plants. According to *Shi et al.* (2005b), heavy deposition of Si in the vicinity of root endodermis could block the bypass flow of Cd and restrict the apoplasmic transport to xylem. Silicon could also co-precipitate Cd in vacuoles thereby decreasing membrane lipid peroxidation via stimulating antioxidant system (*Liang et al.*, 2007). *Wang et al.* (2000) reported that cell wall-bound silica has a strong affinity for Cd. Thus, elevated concentration of Si in Si-accumulating plants, such as wheat, could significantly inhibit apoplastic Cd translocation by trapping Cd in cell wall. Another main reason might be the Si enhanced Ca concentration in plants which, owing to its chemical similarity, could compete with Cd for absorption by plant roots and translocation within plants (*Anderson and Nilsson*, 1974). In present studies, significant negative correlation of RSi with CdTI in Iqbal-2000 revealed that Si deposition in roots decrease Cd translocation by either of the above mechanism. However, decrease in SCd despite no relationship between RSi and CdTI in Cd stressed wheat cultivars (except Iqbal-2000) suggest that Si induced decrease in SCd seems due to decrease in Cd absorption as well as translocation in wheat cultivars in addition to growth dilution effect. The effect of Si on Cd accumulation both in roots and shoots also varied with the cultivar. It was reported by *Lukačová Kuliková and Lux* (2010) that Si (5 mM) treated plants showed higher decrease in root and shoot Cd content (43 and 35 %,
respectively) in Reduta while lowest in Almansa (12 %) and Valintena (6 %) hybrids of maize grown under 100 uM Cd stress. Cadmium concentrations in shoots and roots of LSCd cultivars did not follow the same trend as SCd decreased while RCd remained unaffected with Si application. Similar results were also reported for hydroponically Si supplemented rice (Liu et al., 2009; Shi et al., 2005a; Zhang et al., 2008), Brassica chinensis (Song et al., 2009) and peanut (Shi et al., 2010). Moreover, the results of this study for LSCd contradict while that of HSCd cultivars are in agreement with the studies reporting increase in both RSi and SSi following Si application (Vaculík et al., 2009).

5.5.2 Silicon concentration in roots and shoots of wheat cultivars
Wheat cultivars like other Si accumulating crops such as rice had more Si in shoot compared to roots indicating that Si has been actively absorbed by roots (Takahashi et al., 1990). Similarly, active absorption of Si by wheat has also been suggested by Rains et al. (2006). However, Si concentration both in roots and shoots of wheat cultivars did not increase beyond 4 mM Si application which indicated that saturation indices of these cultivars for Si lay somewhere between 2 to 4 mM application rate. The same could also be concluded from the effect of Si on activities of antioxidant enzymes and dry matter wherein these parameters did not respond beyond either 2 or 4 mM Si application. Additionally, in present studies lower RSi and SSi in Cd sensitive HSCd cultivars compared to LSCd cultivars was recorded which is explained by decreased Si concentration in roots and shoots with increasing Cd concentration in rice. Regarding difference between SSi and RSi, the results of the present studies confirmed the finding of Rizwan et al. (2012) who reported higher SSi than RSi in wheat.

5.5.3 Gas exchange in wheat cultivars as affected by Si nutrition
In general, our results showed that Si treated sensitive HSCd wheat cultivars showed higher improvement in chlorophyll contents and photosynthesis while decrease in stomatal conductance and transpiration rate. Conversely, Cd stress had higher negative effect on chlorophyll contents and photosynthesis in sensitive HSCd cultivars compared to tolerant LSCd cultivars. Moreover, improvement or reduction in gas exchange attributes in Si treated plants was limited to 4 mM Si application. Thus, Si improved chlorophyll contents and photosynthesis could be regarded as the alleviation of Cd toxicity in Cd stressed plants.
Although at different treatment levels, Si induced improvement in chlorophyll content and photosynthesis has also been reported in Cd stressed maize (Lukačová et al., 2013) and rice (Nwugo and Huerta, 2008). Plants absorb Si in monosilicic acid form that is mainly stored in silica cells of leaf forming a Si-cuticle double layer (Mitani et al., 2005). Thus, improved cell wall thickness modified stomatal action and reduces cuticular transpiration thereby decreases excessive transpiration (Hattori et al., 2007). Unaffected or improved photosynthesis accompanied by decrease in stomatal conductance consequently leads to improved WUE as has been previously reported for Si treated rice (Nwugo and Huerta, 2008) and maize (Gao et al., 2006). Since Cd stress has shown decrease in the activity of RUBISCO in barley (Stiborova, 1988) and of FBP and GAPDH in garden pea (Chugh and Swahney, 1999), Si induced inhibition in Gs without affecting photosynthesis in HSCd cultivars seemed to be associated with Si triggered improvement in activities of antioxidant and Calvin cycle enzymes. However, these findings are contradictory to some earlier studies which have shown positive effect of Si on stomatal conductance (Ma and Takahashi, 2002b) and transpiration. Nwugo and Huerta (2008) has explained this contradiction as the difference in Si nutrition level used in these studies since in their studies Si induced reduction in stomatal conductance without inhibiting A was limited up to 0.2 mM Si application. It could be suggested that Si treated plants decreased stomatal conductance along with increased or sustained photosynthetic rate as a mechanism of inhibiting Cd translocation from roots to shoots.

A significant negative correlation between transpiration rate and SCd was observed in HSCd cultivars Inqlab-91 and Sehar-2006 (Fig 5.6a) which showed that these cultivars adopted the mechanism of reduced transpiration to lower down Cd concentration and toxicity in shoots in the presence of Si.

### 5.5.4 Antioxidant activity and MDA contents

The Cd stress in plant cells produces ROSs which are scavenged by enzymatic or non-enzymatic antioxidants. The Si treatment enhanced the activities of CAT, APX, GPX and SOD antioxidant enzymes in Cd stressed wheat cultivars. Generally, the increase was higher in LSCd cultivars compared to HSCd cultivars. Additionally, the optimum level of Si enhancing antioxidant activity was either 4 mM (CAT, GPX and SOD) or 2 mM (CAT) indicating that the optimum level of Si lay in between these treatments. However, the resultant increase in
antioxidant activity with Si treatment alleviated lipid peroxidation only in HSCd sensitive cultivars as indicated by decreased MDA concentration in leaves. Toxic concentration of Cd in plants could produce too many reactive oxygen radicals to be scavenged by these enzymes resulting in antioxidant starved plants. Thus, Si inhibited Cd uptake and translocation could increase the activities of antioxidant enzymes indirectly. In addition to reduced uptake and translocation, Si induced alteration of subcellular partitioning of Cd in favour of cell wall bound fraction would have decreased the proportion of toxic free Cd ions in cell organelles with a consequence of increase in antioxidant activity. A higher decrease in LSCd tolerant cultivars compared to HSCd sensitive cultivars also suggest that Si could reduce the antioxidant activity in plants by decreasing tissue Cd concentration. Similarly, Shi et al. (2010) attributed the higher Cd tolerance and antioxidant activity in Luhua-11 peanut cultivar to the Si induced increase in cell wall bound Cd fraction. However, our results are contradictory to the findings of Song et al. (2009) who showed higher increase in the activities of antioxidant enzymes in tolerant, low Cd cultivars compared to sensitive, high Cd cultivars of Chinese cabbage. In present studies, if we consider LSCd cultivars tolerant to 15 uM Cd as non-stressed plants, our findings are in line with the results of Shi et al. (2010) who reported no effect of Si treatments in non-Cd stressed peanut plants. In conclusion, the increase in activities of antioxidant enzymes and alleviation of lipid peroxidation in Si treated plants could be regarded as related to decrease in tissue Cd concentration.

5.6. Conclusions

The growth response to Cd stress and Cd concentration both in roots and shoots of Cd stressed wheat cultivars confirmed the results of the screening study, i.e. the Cd concentration was low in LSCd while high in HSCd cultivars. Silicon decreased Cd concentration in shoots by decreasing Cd absorption as well as root to shoot translocation. Higher retention of Cd in roots of both LSCd and HSCd cultivars while decrease in excessive transpiration rate only in HSCd cultivars with Si application seems among the major mechanisms suppressing Cd translocation to shoots. The HSCd sensitive cultivars when supplemented with Si, showed improvement in growth and physiological response which suggest that it was the reverse of Cd stress on plant growth. Higher increase in antioxidant activity with corresponding higher decrease in SCd in LSCd cultivars suggested that improvement in antioxidant activity was associated with lowering Cd concentration in tissue.
OPTIMIZATION OF SILICON NUTRITION TO MINIMIZE CADMIUM ACCUMULATION IN WHEAT GRAINS

6.1. Abstract
The effect of Si application on Cd immobilization in soil and concentration in low and high shoot-Cd (HSCd and LSCd, respectively) cultivars was evaluated in a greenhouse pot experiment. Based on initial screening study; selected LSCd cultivars (Iqbal-2000 and Lasani-2008) and HSCd cultivars (Inqlab-91 and Sehar-2006) were grown on artificially Cd contaminated (10 mg Cd kg\(^{-1}\)) soil. Three levels of Si (50, 100 and 150 mg kg\(^{-1}\) soil) as calcium silicate (CaSiO\(_3\)) were applied. None of the wheat cultivars showed any toxicity symptom or growth retardation to the applied Cd stress. Silicon supply to Cd treated plant did not improve root and shoot dry matter of wheat cultivars. However, grain yield of wheat cultivars increased at the highest dose of applied Si (Si\(_{150}\)). Significantly low in plant available soil Cd was recorded with Si\(_{150}\) application without any change in soil pH. Silicon application not only caused a linear decrease in Cd contents of shoots and grain but also its translocation from roots to shoots and grains. Decrease in shoot Cd concentration was higher in HSCd cultivars whereas grain Cd concentration showed higher decrease in LSCd cultivars. As a conclusion, Si decreased Cd concentration in wheat cultivars by both decrease in plant available soil Cd and its translocation from roots to shoots. Application of Si at 150 mg kg\(^{-1}\) proved an optimum level of Si that caused significantly lower Cd concentration in wheat grains.

6.2. Introduction
Accumulation of Cd to phytotoxic levels may cause significant growth inhibition and yield loss. Plants have evolved different mechanisms to avoid Cd uptake and accumulation in grains including exclusion at root level, compartmentalization of Cd and production of stress proteins (Clemens \textit{et al.}, 2006). Cadmium absorption, translocation and toxicity in plants is also affected by interaction with other mineral nutrients such as Si (Liang \textit{et al.}, 2007).
Although Si is not essential element for plants, but role of Si is so significant for too many plant species under so many situations that it has been categorized as a ‘quasi-essential’ element (Epstein and Bloom, 2005). In wheat and many other members of the Poaceae family, Si is actively absorbed by roots (Rains et al., 2006; Currie and Perry, 2007). Silicon could decreases plant available fraction of Cd in soil by co-precipitating with it and also by increasing soil pH (da Cunha and do Nascimento, 2008; Chen et al., 2000; Liang et al., 2005). Owing to the ability of silicate ions (SiO$_3^{2-}$) to get protonated and increase the pH of resulting solution, most of silicate amendments are alkaline in nature (Dwivedi et al., 2007; Kumpiene et al., 2007; Rijkenberg and Depree, 2010; Singh et al. 2008). However, ability of SiO$_3^{2-}$ to get protonate and affect the pH could depend upon whether reaction of soil is acidic or alkaline. Additionally, Si application at higher doses could promote polymerization of SiO$_3$-slag which is considered as potential metal chelating agent (Sommer et al., 2006).

Within plant body, Si could decrease the translocation of Cd within plants (Liang et al., 2005; Nwugo and Huerta, 2008; Lukacova et al., 2013) through deposition in root endodermis which strengthen casparian band resulting in restricted bypass flow of Cd from roots to shoots. It can also co-precipitate with Cd as Cd-silicate in vacuole which could again limit the Cd transport to edible parts as observed in rice (Shi et al., 2005a).

Liang et al. (2005) and Chen et al. (2000) have shown that Si decreased soil pH and available Cd in soil and its concentration in plants. However, in contradiction da Cunha et al. (2008) did not report any change in soil pH associated with decrease in Cd concentration in plant tissues. So far, most of the work conducted in this regard has been focused on the response of crops to Si applied in highly weathered acid soils wherein the chemical reaction occurring to buffer the change in pH would be quite different to that of alkaline soils which has not been investigated yet. Studies addressing the Si-mediated suppression of metal translocation within plants were mostly conducted in hydroponics and also the Cd concentrating ability and/ or tolerance of plant species has not been considered in these investigations. In this context, present work aimed at studying the effects of Si application on soil pH, AB-DTPA extractable soil Cd and Cd concentration in four wheat cultivars differing in Cd accumulating ability and its tolerance under alkaline soil.
6.3. Materials and Methods

6.3.1. Experimental treatments and design
Artificially Cd contaminated soil was used for the study. The study comprised of two factors: three levels of Si (50, 100 and 150 mg kg\(^{-1}\) soil) along with uncontaminated control, and four wheat cultivars contrasting in shoot Cd accumulation. Cultivar Iqbal-2000 and Lasani-2008 were LSCd while HSCd cultivars were Inqlab-91 and Sehar-2006. The experiment was arranged in factorial design comprising of three replications.

6.3.2. Soil preparation and seed sowing
Soil (surface 20 cm) in bulk was collected from agricultural farm of University of Agriculture, Faisalabad. Soil was air dried, ground, sieved through 2 mm sieve and mixed thoroughly. In polythene lined iron tubs, soil was impregnated with Cd (10 mg Cd kg\(^{-1}\) soil) using CdCl\(_2\).H\(_2\)O and equilibrated for a period of 60 days at field capacity. Then, soil was filled in polythene lined glazed pots (10 kg soil pot\(^{-1}\)) and Si (50, 100 and 150 mg kg\(^{-1}\)) as calcium silicate (CaSiO\(_3\)) was mixed in soil of respective pots. Un-contaminated control was also maintained to compare the toxic effect of Cd on plants. Basal dose of 100, 80 and 50 mg N, P and K kg\(^{-1}\) of soil was applied in solution as urea, single superphosphate (SSP) and potassium sulphate (KSO\(_4\)), respectively. Whole of the P and K and one third of N was applied at sowing while remaining N was applied at 30 and 50 days of sowing in equal splits. After mixing CaSiO\(_3\) and fertilizer solution, soil was equilibrated at field capacity for an additional period of two weeks. An uncontaminated control containing no Cd and Si was also maintained for each cultivar. Six seeds of wheat were sown in each pot and later on at two leaf stage three seedlings pot\(^{-1}\) were retained up to maturity. The uprooted seedlings were crushed and mixed thoroughly into the same pot. The plants were irrigated daily water to field capacity using deionized.

6.3.4. Harvesting and sample preparation
Plants were harvested at physiological maturity and oven dried to a constant weight at 65 ±5°C in a forced air oven for 74 h. Grains were separated from spikes and straw and grain yield was recorded. The straw and grain samples were ground in stainless steel mills and stored in labeled polythene bags for the determination of Cd and Si. Post-harvest soil samples were taken from
pots with the help of stainless steel soil sampling tube, air dried and passed through a 2 mm sieve for analysis of AB-DTPA extractable Cd. Roots were separated from soil by forced water washing of post-harvest soil.

6.3.5. Plant analysis
Determination of Cd was carried out as described in section 4.3.3. Silicon in grain and straw was determined following the method described in section 5.3.4.

6.3.6. Soil analyses
Physical and chemical properties of original soil such as textural class, EC_e, pH_s, organic matter, CaCO_3, total metals and available Si were determined (Table 6.1). The soil after wheat harvest was analyzed for AB-DTPA extractable Cd. Description of methods used for physical and chemical analysis of soil is as follow:

6.3.6.1. Particle size analysis
Hydrometer method (Bouyoucos, 1967) was followed for particle size analysis. Forty gram of the air dry soil was taken in a 400 mL beaker, 40 mL of 2 % sodium hexametaphosphate [(NaPO_3)_6] solution was added, mixture was transferred to dispersion cup and stirred for 10 minutes. The contents of dispersion cup were washed into 1000 mL graduated cylinder having 1L capacity within 36± 2 cm height. Hydrometer was placed in cylinder and made the volume up to 1000 mL with distilled water. Hydrometer was removed and contents of cylinder were shaken manually by means of a metal plunger. When uniform suspension was obtained, plunger was taken out and after 4 minutes hydrometer reading (HR_1) was recorded. Shaking procedure was repeated after removing hydrometer with minimum of disturbance and second hydrometer reading (HR_2) was recorded after 2 h. Since hydrometer is calibrated at temperature of 67 °F (20 °C), the HR_1 and HR_2 were corrected for temperature variation (For each degree above 20°C, added a factor of 0.3 to the reading and for each degree less than 20°C, subtracted a factor of 0.3 from the reading to get corrected hydrometer reading) and designated as CHR_1 and CHR_2, respectively.
Calculations involved are:

\[
\text{Silt + clay (\%)} = (\text{CHR}_1) \times \left( \frac{100}{\text{weight of soil}} \right)
\]

\[
\text{Clay (\%)} = \left( \frac{\text{CHR}_2}{\text{weight of soil}} \right) \times \left( \frac{100}{\text{weight of soil}} \right)
\]

\[
\text{Silt (\%)} = \% \left( \text{silt + clay} \right) - (\% \text{ clay})
\]

\[
\text{Sand (\%)} = 100 - \% \left( \text{silt + clay} \right)
\]

Soil textural class was determined using USDA textural triangle.

Table 6.1 Properties of soil used for the experiment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Textural class</td>
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<td>Loam</td>
</tr>
<tr>
<td>Sand</td>
<td>%</td>
<td>37.38</td>
</tr>
<tr>
<td>Silt</td>
<td>%</td>
<td>42.10</td>
</tr>
<tr>
<td>Clay</td>
<td>%</td>
<td>20.54</td>
</tr>
<tr>
<td>pHs</td>
<td></td>
<td>8.05</td>
</tr>
<tr>
<td>EC\text{e}</td>
<td>dS m\text{-1}</td>
<td>2.11</td>
</tr>
<tr>
<td>SAR</td>
<td>(mmol L\text{-1})\text{1/2}</td>
<td>7.09</td>
</tr>
<tr>
<td>CEC</td>
<td>%</td>
<td>7.51</td>
</tr>
<tr>
<td>OM</td>
<td>%</td>
<td>0.86</td>
</tr>
<tr>
<td>CaCO\text{3}</td>
<td>%</td>
<td>7.33</td>
</tr>
<tr>
<td>AB-DTPA extractable Cd</td>
<td>mg kg\text{-1}</td>
<td>0.125</td>
</tr>
<tr>
<td>AB-DTPA extractable Zn</td>
<td>%</td>
<td>0.472</td>
</tr>
<tr>
<td>Total Cd</td>
<td>%</td>
<td>6.42</td>
</tr>
<tr>
<td>Total Zn</td>
<td>%</td>
<td>93.47</td>
</tr>
</tbody>
</table>

6.3.6.2. \textbf{pH and electrical conductivity (EC\text{e})}

Soil (350 g) was soaked in distilled water and allowed to stand over-night. Soil saturated paste was prepared and pH of paste (pHs) was recorded with the help of Sensodirect-200 pH meter after calibrating it with buffer solutions of pH 4.00 and 8.00. Extract from soil saturated paste was obtained by applying positive pressure with the help of filter press. The electrical conductivity of extract (EC\text{e}) was recorded with the help of SensoDirecct Cond. 200 conductivity meter after calibrating it with 0.01 N KCl solution.

6.3.6.3. \textbf{Cation exchange capacity (CEC)}

Five g portions of soil were saturated with 1\text{N CH}_3\text{COONa} buffered to pH 8.2, washed thrice with ethanol and finally extracted with 1\text{N CH}_3\text{COONH}_4 (pH 7.0). Sodium in the extract was determined with Jenway PFP-7 flame photometer keeping Na\text{+} filter in place (Method 19 of U.S. Salinity Lab. Staff, 1954).
The CEC was calculated by the formula:

$$\text{CEC (cmol}_c \text{ kg}^{-1}) = \left[ \frac{\text{Na (mmol}_c \text{ L}^{-1}) \times 100 \times 100}{1000 \times \text{soil wt (g)}} \right]$$

### 6.3.6.4. Organic matter

Soil organic matter was determined following the method described by Walkly-Black (Jackson, 1962). One gram of soil was swirled in 10 mL of 1.0 \( N \) \( \text{K}_2\text{Cr}_2\text{O}_7 \) and 20 mL of concentrated \( \text{H}_2\text{SO}_4 \). After 30 minutes, the mixture was diluted to about 250 mL with distilled water and titrated against 0.5 \( M \) ferrous ammonium sulphate \([(\text{NH}_4)_2\text{SO}_4.\text{FeSO}_4.6\text{H}_2\text{O}]\) in the presence of 10 mL \( \text{H}_3\text{PO}_4 \) and 15 drops of diphenylamine as an indicator to dull green endpoint. Organic matter (%) was calculated as follow:

\[
\text{OM (\%)} = \left[ (V_{\text{blank}} - V_{\text{sample}}) \times M \times 0.69 \right] / \text{Wt. of soil (g)},
\]

where

- \( V_{\text{blank}} \): Volume (mL) of \((\text{NH}_4)_2\text{SO}_4.\text{FeSO}_4.6\text{H}_2\text{O}\) used to titrate blank
- \( V_{\text{sample}} \): Volume (mL) of \((\text{NH}_4)_2\text{SO}_4.\text{FeSO}_4.6\text{H}_2\text{O}\) used to titrate sample
- \( M \): Molarity of \((\text{NH}_4)_2\text{SO}_4.\text{FeSO}_4.6\text{H}_2\text{O}\) solution
- 0.69 = 0.003 x 100 x (100/74) x (100/58), where
- 0.003 = me wt. of carbon
- 100 = To convert OM in %
- 100/58 = Factor to convert carbon to OM
- 100/74 = Recovery factor for carbon

### 6.3.6.5. Calcium carbonate (lime)

Lime contents of soil were determined by its neutralization with standard \( \text{HCl} \) (FAO, 1974). One gram of air-dry soil was treated with 1:1 \( \text{HCl} \) in 250 mL Erlenmeyer flask and left the mixture overnight. Added distilled water (100 mL) and 2-3 drops of phenolphthalein indicator in the flask. Un-reacted acid was determined by titration against 1 \( N \) \( \text{NaOH} \).

Lime was calculated by the formula:

\[
\% \text{lime} = \left[ (10 \times N_{\text{HCl}}) - (R \times N_{\text{NaOH}}) \times 0.05 \times 100 \right] / \text{Wt}
\]

where

- \( N_{\text{HCl}} \): Normality of \( \text{HCl} \)
- \( N_{\text{NaOH}} \): Normality of \( \text{NaOH} \)
- \( R \): Volume of \( \text{NaOH} \) used in back titration
- 0.05 = \( \text{CaCO}_3 \) neutralized by 1 mL of \( \text{HCl} \)
- Wt = weight of soil sample (g)
6.3.6.6. Ammonium bicarbonate-DTPA (AB-DTPA) extractable Cd
The AB-DTPA extracting solution was prepared by dissolving 79.06 g NH₄HCO₃ and 1.97 g of DTPA in a liter volume of solution. The pH of the solution was adjusted to 7.60 with the help of HCl. Soil (10 g) was placed in a 250 mL Erlenmeyer flask, added 20 mL of freshly prepared extracting solution, shook on a reciprocating shaker at 180 cycles per minute for 15 minutes by keeping flasks open (Soltanpour, 1985). The mixture was filtered and metals were determined with atomic absorption spectrophotometer (Model Thermo Electron S –Series).

6.3.6.7. Total metals
For total Cd concentration in soils, method described by Amacher (1996) was followed. A sample weighing 1.0 g of air-dried soil was taken in 50 mL pyrex conical flask, added 10 mL concentrated HNO₃ and placed overnight. Next morning, heated the flask, cooled and then added 1 mL of HNO₃ and 4 mL of HClO₄ and heated at 200 ºC till fumes of HClO₄ appeared. Sample was cooled and then heated to 70 ºC for one hour after adding 5 mL of 1:10 HCl. Allowed the sample to cool and made volume up to 50 mL with 1% HCl. After filtration through Whatman No. 42 filter paper, filtrate was stored in plastic bottles. Metal ions were determined with the help of atomic absorption spectrometer (Model Thermo S –Series).

6.3.8. Statistical analysis
The data regarding plant dry matter, Cd and Si accumulation, soil pH and AB-DTPA extractable Cd post-harvest soil in response to Si application were statistically analyzed following ANOVA technique and LSD test was applied to differentiate the treatment differences (Steel and Torrie, 1996) using Statistix Version 8.1 software package.

6.4. Results
6.4.1. Plant growth and dry matter
Root dry matter (RDM) and straw and grain yield were significantly different among the cultivars but exposure to Cd₁₀ stress did not show negative effect on any of these growth attributes (Table 6.2). Additionally, no symptom of visual toxicity could be seen on leaves during the course of plant development. Application of Si to Cd₁₀ contaminated soil tended to increase RDM but effect was non-significant over the range of tested Si doses (Table 6.2).
Table 6.2 Effect of Si application to Cd contaminated soil on root dry matter and grain and straw yield of Cd stressed wheat cultivars

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Iqbal-2000</th>
<th>Lasani-2008</th>
<th>Inqlab-91</th>
<th>Sehar-2006</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root dry matter (g pot⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.82</td>
<td>1.95</td>
<td>1.77</td>
<td>1.69</td>
<td>1.81</td>
</tr>
<tr>
<td>Cd₁₀</td>
<td>1.79</td>
<td>1.93</td>
<td>1.58</td>
<td>1.58</td>
<td>1.72</td>
</tr>
<tr>
<td>Cd₁₀Si₅₀</td>
<td>1.84</td>
<td>2.07</td>
<td>1.54</td>
<td>1.66</td>
<td>1.78</td>
</tr>
<tr>
<td>Cd₁₀Si₁₀₀</td>
<td>2.02</td>
<td>2.27</td>
<td>1.59</td>
<td>1.62</td>
<td>1.88</td>
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<tr>
<td>Cd₁₀Si₁₅₀</td>
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<td>2.39</td>
<td>1.87</td>
<td>1.66</td>
<td>2.01</td>
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<tr>
<td>Mean</td>
<td>1.87 a</td>
<td>2.06 a</td>
<td>1.62 b</td>
<td>1.64 b</td>
<td></td>
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<table>
<thead>
<tr>
<th>LSD</th>
<th>Cultivar</th>
<th>Treatment</th>
<th>Cult. × Treat.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>NS</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Straw yield (g pot⁻¹)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>15.2</td>
<td>18.5</td>
<td>16.1</td>
<td>13.1</td>
<td>15.7</td>
</tr>
<tr>
<td>Cd₁₀</td>
<td>15.7</td>
<td>17.7</td>
<td>15.6</td>
<td>13.5</td>
<td>15.6</td>
</tr>
<tr>
<td>Cd₁₀Si₅₀</td>
<td>16.1</td>
<td>17.7</td>
<td>15.5</td>
<td>14.2</td>
<td>15.9</td>
</tr>
<tr>
<td>Cd₁₀Si₁₀₀</td>
<td>16.7</td>
<td>18.1</td>
<td>15.7</td>
<td>14.4</td>
<td>16.2</td>
</tr>
<tr>
<td>Cd₁₀Si₁₅₀</td>
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<td>17.0</td>
<td>15.9</td>
<td>13.3</td>
<td>15.7</td>
</tr>
<tr>
<td>Mean</td>
<td>16.1 b</td>
<td>17.8 a</td>
<td>15.8 b</td>
<td>13.7 c</td>
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<table>
<thead>
<tr>
<th>LSD</th>
<th>Cultivar</th>
<th>Treatment</th>
<th>Cult. × Treat.</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
<td>NS</td>
</tr>
</tbody>
</table>

<table>
<thead>
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<th>Grain yield (g pot⁻¹)</th>
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<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>C</td>
<td>7.25</td>
<td>8.42</td>
<td>7.13</td>
<td>9.07</td>
<td>7.97 b</td>
</tr>
<tr>
<td>Cd₁₀</td>
<td>6.98</td>
<td>8.12</td>
<td>6.96</td>
<td>8.53</td>
<td>7.65 b</td>
</tr>
<tr>
<td>Cd₁₀Si₅₀</td>
<td>6.84</td>
<td>8.15</td>
<td>7.43</td>
<td>8.81</td>
<td>7.81 b</td>
</tr>
<tr>
<td>Cd₁₀Si₁₀₀</td>
<td>7.22</td>
<td>8.35</td>
<td>7.49</td>
<td>8.85</td>
<td>7.98 b</td>
</tr>
<tr>
<td>Cd₁₀Si₁₅₀</td>
<td>7.85</td>
<td>8.49</td>
<td>8.02</td>
<td>9.25</td>
<td>8.40 a</td>
</tr>
<tr>
<td>Mean</td>
<td>7.24 c</td>
<td>8.31 b</td>
<td>7.41 c</td>
<td>8.90 a</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LSD</th>
<th>Cultivar</th>
<th>Treatment</th>
<th>Cult. × Treat.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>NS</td>
</tr>
</tbody>
</table>

Means sharing different letter(s) are significantly different from each other at α = 0.05.

Similarly, straw yield remained unaffected by Cd₅₀ contamination as well as by application of Si to contaminated soil. Unlike RDM and straw yield, grain yield increased with application of Si to Cd₁₀ contaminated soil but increase was significant only at the highest treatment dose (Si₁₅₀). The maximum increase in grain yield (15.2 %) was recorded for Inqlab-91 followed by Lasani-2008 (12.5%), Sehar-2006 (8.4%) and Iqbal-2000 (4.6%). Except Inqlab-91, grain yield of Si₁₅₀ treated wheat cultivars was statistically similar to that from uncontaminated soil. In Inqlab-91, Si₁₅₀ treated Cd stressed plants had significantly higher grain yield compared to those grown under uncontaminated soil condition.
None of the agronomic traits including tillers (number pot\textsuperscript{-1}), plant height (cm) and spike length (cm) showed any negative response to Cd\textsubscript{10} contamination or enhancement with supplementation of Si nutrition in any cultivar (data not presented).

6.4.2. Cadmium concentration in roots, straw and grains of wheat cultivars

The effect of Si application on root Cd concentration (RCd) was non-significant; however, the LSCd cultivars had higher RCd concentration compared to HSCd cultivars (Fig. 6.1a). The maximum RCd (mg kg\textsuperscript{-1}) was recorded in Iqbal-2000 (5.82) followed by Lasani-2008 (5.80), Sehar-2006 (5.62) and the minimum was in Inqlab-91 (5.30). Contrarily, significantly lower shoot and grain Cd concentrations (SCd and GCd, respectively) were recorded in LSCd cultivars compared to HSCd cultivars under non-Si treated Cd\textsubscript{10} contaminated soils with the exception of non-significant difference in SCd between Iqbal-2000 (4.75 mg kg\textsuperscript{-1}) and Inqlab-91 (5.13 mg kg\textsuperscript{-1}) (Fig 6.1b-c). Lower SCd was observed in plants grown under Si treated Cd\textsubscript{10} amended soil compared to those with no Si application (Fig. 6.1 b). The decrease in SCd concentration was linear with the Si doses applied and the highest decrease was recorded at Si\textsubscript{150} application. However, Si application caused higher decrease in SCd in HSCd cultivars compared to LSCd cultivars and resultant SCd at Si\textsubscript{150} was statistically similar among the two groups of cultivars. The SCd concentration in no-Si treated Cd\textsubscript{10} amended soil was 18.9 and 17.7 \% higher for Iqbal-2000 and Lasani2008 while 26.4 and 27.4\% for Inqlab-91 and Sehar-2006 compared with Si\textsubscript{150} supplied plants.

Similar to Cd concentration in straw, there was linear decrease in its concentration in grain showing maximum decrease at Si\textsubscript{150} application for both the groups of cultivars. However, unlike SCd, the decrease in GCd concentration was higher in LSCd cultivars (24.8 and 19.6 \% for Iqbal-2000 and Lasani-2008, respectively) compared to HSCd cultivars (12.4 and 15.1 \% for Inqlab-91 and Sehar-2006, respectively). Consequently, there was significantly higher GCd in HSCd cultivars Inqlab-91 (2.46 mg kg\textsuperscript{-1}) and Sehar-2006 (2.26 mg kg\textsuperscript{-1}) than LSCd cultivars Iqbal-2000 (1.80 mg kg\textsuperscript{-1}) and Lasani-2008 (1.29 mg kg\textsuperscript{-1}) at Si\textsubscript{150} application. However, GCd was statistically at par among HSCd cultivars but significantly lower in LSCd cultivar Lasani-2008 than its colleague Iqbal-2000. Comparing the Cd concentration among roots, shoots and grains, it was observed that RCd was significantly higher than SCd, and SCd than GCd for all the cultivars (Fig. 6.1).
Fig. 6.1. Effect of Si application on Cd concentration in roots (RCd), shoots (SCd) and grains (GCd) of wheat cultivars grown in soil receiving Cd at 10 mg kg\(^{-1}\) soil.
Bars sharing different letters are significantly different from each other at \(p \leq 0.05\) (LSD: cultivar × treatment; RCd = NS, SCd = 0.468, GCd = 0.243)
6.4.3. Cadmium enrichment and translocation factors as affected by Si nutrition

Cadmium enrichment factor of shoot (SEF) and grain (GEF) was calculated as the ratio of Cd concentration in plant tissue to its concentration in soil. Higher shoot and root Cd enrichment factors were observed for HSCd cultivars compared to LSCd cultivars (Fig. 6.2 a-b). The SEF was 1.154 and 1.147 for Iqbal-2000 and Lasani-2008 while it was 1.387 and 1.474 for Inqlab-91 and Sehar-2006, respectively in Cd treated no Si amended soil. Application of Si caused linear decreased in SEFs for both LSCd and HSCd cultivars and maximum decrease was recorded at Si$_{150}$ application. Moreover, the decrease in SEFs was higher in HSCd cultivars than LSCd cultivars.

Likewise, considerable higher root to GEFs of Cd was recorded in HSCd cultivars Inqlab-91 (0.760) and Sehar-2006 (0.713) than Iqbal-2000 (0.582) and Lasani-2008 (0.713) in Cd amended no Si treated soil (Fig. 6.2b). Application of Si caused linear decrease in GEF of Cd in all the cultivars and maximum decrease was recorded at Si$_{150}$ application. However, contrary to SEF, decrease in GEF was higher in LSCd compared to HSCd cultivars.

Higher root to shoot Cd translocation index (RSTI) was observed in HSCd cultivars compared to LSCd cultivars (Fig. 6.2 c). The RSTI was 0.79 and 0.76 for Iqbal-2000 and Lasani-2008 while it was 0.91 and 1.00 for Inqlab-91 and Sehar-2006, respectively. Application of Si decreased RSTI in both LSCd and HSCd cultivars and maximum decrease was recorded at Si$_{150}$ application. However, the decrease in RSTI at Si$_{150}$ application was slightly higher in HSCd cultivars Inqlab-91 (20.9 %) and Sehar-2006 (25.2 %) than LSCd cultivars Iqbal-2000 (14.1 %) and Lasani-2008 (17.1 %).

Similar to RSTI, considerable higher root to grain Cd translocation index (RGTI) was recorded in HSCd cultivars Inqlab-91 (0.50) and Sehar-2006 (0.48) than Iqbal-2000 (0.41) and Lasani-2008 (0.28) (Fig. 6.2 d). Application of Si decreased RGTI of Cd in all the cultivars but effect was dependent on applied Si dose. At Si$_{50}$ application (Si$_{50}$), no decrease in RGTI was observed for HSCd cultivars while at Si$_{100}$ only Inqlab-91 did not show any change in root to grain Cd translocation. At Si$_{150}$ application, the highest decrease in RGTI was recorded and decrease was very low in HSCd cultivars than LSCd ones. Inqlab-91 showed 3.6 and 3.2 times lower RGTI than Iqbal-200 and Lasani-2008 while the corresponding decrease values were 1.6 and 1.2 for Sehar-2006 at Si$_{150}$ application.
Fig. 6.2. Effect of Si application on Cd enrichment and translocation factors in shoots and grains of wheat cultivars.

### 6.4.3. Silicon concentration in roots, shoots and grain of wheat cultivars

Although there was a little decrease, but Si concentration in roots, shoots and grains of wheat cultivars (RSi, SSi and GSi, respectively) statistically remained unaffected by soil applied Cd$_{10}$ (Fig. 6.3 a-c) in no-Si treated soil. Addition of Si to Cd$_{10}$ treated soil significantly (p $< 0.05$) affected high RSi of wheat cultivars (6.3a). The increase in RSi (mg g$^{-1}$ DM) was linear with Si doses applied and it was the maximum at the highest dose of applied Si (Si$_{150}$) for all the cultivars. The range of RSi was 1.79 to 7.86 for Iqbal-2000, 1.81 to 8.41 for Lasani-2008, 1.50 to 6.32 for Inqlab-91 and 1.38 to 6.58 g mg$^{-1}$ DM for Sehar-2006 from Si$_{0}$ to Si$_{150}$ application rate. Moreover, LSCd cultivars had significantly higher RSi compared to HSCd cultivars at either level of Si supplementation.
Fig. 6.3 Effect of Si application on Si concentration in roots (RSi), shoots (SSi) and grains (GSi) of wheat cultivars grown in soil receiving Cd at 10 mg kg\(^{-1}\). Bars sharing different letters are significantly different from each other at \(\alpha = 0.05\) (LSD: \(\text{RCd} = 0.792, \text{SCd} = 0.468, \text{GCd} = 0.243\)).

At Si\(_{150}\) application, among HSCd cultivars RSi was statistically at par (\(p \leq 0.05\)) while it differed significantly among LSCd cultivars being higher in Lasani-2008. The SSi (mg g\(^{-1}\) DM)
also remained unaffected with Cd application to soil and it increased linearly with increasing soil applied Si. The range of SSi was 4.62 to 17.30 for Iqbal-2000, 4.10 to 17.23 for Lasani-2008, 4.31 to 16.94 for Inqlab-91 and 4.98 to 16.94 mg g\(^{-1}\) DM for Sehar-2006 from Si\(_0\) to Si\(_{150}\) application. However, the SSi was statistically similar among all the wheat cultivars in all the treatments.

Similar to RSi and SSi, GSi increased linearly with increasing soil application of Si and it was statistically similar among wheat cultivars at the lowest level of applied Si (Si\(_{50}\)). Onward to Si\(_{100}\) application, GSi differed significantly among the cultivars (p < 0.05). The GSi was not related to inherent shoot Cd concentrations of the cultivars. The higher GSi was observed in Iqbal-2000 and Inqlab-91 while lower in Lasani-2008 and Sehar-2006. At Si\(_{150}\) application, GSi (mg g\(^{-1}\) DM) of Lasani-2000 (11.8) was statistically similar to both of the Iqbal-2000 (12.7) and Sehar-2006 (11.1). Comparing Si concentration among roots, shoots and grains, it was observed that the highest Si concentration was found in shoots followed by grains and least in roots (Fig. 6.3).

6.4.3. Soil pH and post-wheat harvest AB-DTPA extractable Cd

The pH was statistically similar among the post-harvest soils sown with LSCd and HSCd cultivars and also within the members of these two groups of cultivars (Data not shown). Similarly, pH values were also similar among the Cd contaminated and un-contaminated soil pots receiving no Si and sown with either of the cultivars. AB-DTPA extractable Cd in no-Si treated soil (Si\(_0\)) was higher in pots sown with LSCd cultivars compared to those sown with HSCd cultivars; however, the pots sown with Lasanai-2008 differed non-significantly from HSCd cultivars (Table 6.3). Moreover, the AB-DTPA extractable Cd was also statistically at par within LSCd and/or HSCd cultivars. The values of AB-DTPA extractable Cd were 4.127, 3.83, 3.70 and 3.73 mg kg\(^{-1}\) soil for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively in Cd\(_{10}\) contaminated pots with no Si addition. Silicon amended Cd treated post-harvest soils had lower AB-DTPA extractable Cd than no-Si amended soil and the difference (%) is referred as immobilization of Cd in soil. The immobilization of Cd with Si\(_{50}\), Si\(_{100}\) and Si\(_{150}\) application was 0.52, 5.53 and 21.23 % for Iqbal-2000; 1.15, 6.32 and 16.68 % for Lasani-2008; 2.38, 9.69 and 19.68 % for Inqlab-91 and 3.53, 8.50 and 16.13 % for Sehar-2006, respectively. However, the effect was significant only at the highest dose of Si (Si\(_{150}\)
application. As the data depict, the maximum Cd immobilization was recorded in soil sown with Iqbal-2000 while it was minimum for Sehar-2006 and Lasani-2008. Following Si$_{150}$ induced immobilization of Cd in soil, AB-DTPA extractable Cd was similar in soils sown with either of the cultivars.

Table 6.3. Effect of Si application on AB-DTPA extractable Cd in post-harvest soil

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Iqbal-2000</th>
<th>Lasani-2008</th>
<th>Inqlab-91</th>
<th>Sehar-2006</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd$_{10}$</td>
<td>4.12</td>
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<td>3.73</td>
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</tr>
<tr>
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<td>3.60 b</td>
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<td>3.47 bc</td>
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LSD

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<th>LSD</th>
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<th>Treatment</th>
<th>Cult. × Treat.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.180</td>
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<td>0.184</td>
<td>NS</td>
</tr>
</tbody>
</table>

Means sharing different letters are significantly different from each other at α = 0.05.

### 6.5 Discussion

#### 6.5.1 Growth response of wheat cultivars to soil applied Cd

Cadmium is toxic heavy metal and it can effect plant growth through a number of mechanisms including nutritional and water imbalance (Gong et al., 2005) and production of reactive oxygen species (Qadir et al., 2004; Dong et al., 2006). However, in present study exposure to Cd$_{10}$ stress did not affect shoot, root and grain biomass as well as agronomic traits including tiller production, plant height and spike length of LSCd as well as HSCd cultivars (Table 6.2). Additionally, symptoms of any visual toxicity could not be observed on leaves during the course of plant development. Despite well documented severe phyto-toxicity of Cd (Sarwar et al., 2010; Saifullah et al., 2013), a number of plant species have also been reported to contain significant amounts of Cd without any visual toxicity and/or growth retardation.

The Cd concentrating ability of plants without showing any visual toxicity is exhibited by many food crops. Garrett et al. (1998) reported that soil Cd up to 3.8 mg kg$^{-1}$ did not caused any phyto-toxicity in wheat wherein wheat grain accumulated up to 0.50 mg Cd kg$^{-1}$. Similarly, Yu et al. (2006) reported that more than 50 % of 43 tested rice cultivars produced similar or more grain yield at higher levels of soil Cd (75.69 to 77.55 mg kg$^{-1}$) compared to
uncontaminated soil. Non-significant correlation between Cd tolerance and concentration in wheat inbred lines (Ci et al., 2010) suggested that these traits are independent of each other. Stevens and McLaughlin (2006) reported that plants could concentrate considerable Cd in harvestable parts that is not toxic for them but could be harmful for humans. In our study, the case was even worse wherein GCd in wheat cultivars was much higher than the safe consumption level for humans which is 0.1 mg Cd kg\(^{-1}\) of grain (WHO, 1989).

The absence of toxicity at high level of soil Cd confirms the occurrence of this phenomenon in natural contaminated low-Cd soil where growth retardation is more unlikely. Ultimately, farmers would not be able to get any prediction of grain Cd contents toxic to humans and animals based on crop growth. Moreover, Cd level in food that could harm human reaches well before any toxicity to plants, therefore, solving the former problem will ultimately result in the solution of later.

6.5.2 Growth response of wheat cultivars to Si nutrition

A number of possible mechanisms are proposed by which Si can increase resistance of plants under Cd stress which is a major among the major heavy metal soil pollutants. With the application of Si to Cd\(_{10}\) contaminated soil, root dry matter and straw yield tended to increase but increase was non-significant (Table 6.2). However, grain yield of all the wheat cultivars increased significantly with application of Si\(_{150}\) to Cd\(_{10}\) contaminated soil. Since root, shoot and grain biomass of wheat cultivars did not affected by Cd contamination, the increase in grain yield of wheat could not be regarded as the alleviation of Cd toxicity instead beneficial effect of Si nutrition. Although beneficial effect of Si on growth of plants under stress conditions are more evident, however, beneficial effect has also been reported under normal growth conditions by many researchers (Parveen and Ashraf, 2010; Tahir et al., 2006; Al-aghahary et al., 2004). The effect of Si on growth of Cd-unstressed plant might be due to influences on the absorption and transport of several mineral elements that help perform many functions in plants (Epstein and Bloom, 2005, Ma and Takahashi, 2002, Hammond et al., 1995).
6.5.3 Effect of Si on concentration and translocation of Cd in wheat cultivars

The differential Cd uptake among plant species could be exploited to produce low Cd food which is a low cost and environmental friendly strategy. The variation in Cd uptake from soil by roots and translocation to shoots and grain could be responsible for the variable concentration of Cd in grain. To define Cd uptake potential of the roots, generally bio-concentration factor also termed as enrichment factors (BCF/EF) have been used. In present study, higher grain and straw Cd EFs and lower RCd was recorded for HSCd cultivars compared to LSCd cultivars (Fig. 6.2 a, d). Similarly, Jamali et al. (2009) reported higher bio-concentration factors of various heavy metals including Cd for grains of wheat varieties TJ-83 and Mehran- 89 compared to Anmol and Abadgar grown on the same agricultural plots. In addition to lower EFs, there was also lower root to shoot and to grain translocation of Cd in LSCd cultivars under non-Si treated, Cd10 contaminated soil (Fig. 6.2 c, d). These findings are in line with that of Chan and Hale (2004) who reported a high grain Cd accumulating durum wheat cultivar Kyle to be lower in root-Cd accumulation than a lower grain-Cd accumulating cultivar Arcola. Similarly, Hart et al. (1998) and Greger and Löfstedt (2004) found that different Cd concentration in grains of bread and durum wheat cultivars was related to variations in the transfer from root to shoot.

The application of Si150 significantly decreased SCd and GCd concentration in wheat cultivars but RCd remained unaffected (Table 6.2). The decrease in SCd and GCd was associated both with decrease in EFs and Cd translocation from roots to shoots as well as to grain (Fig. 6.2). The decrease in straw and grain EFs indicate lower uptake of Cd from soil. Silicon could immobilize Cd in soil as Cd-silicate which is one of the mechanisms that reduce Cd uptake (da Cunha et al., 2008; Li et al., 2008). The Si induced decrease in Cd uptake could also be due to enhanced production of antioxidant that helped maintain integrity of plasma membrane inhibiting indiscriminate Cd uptake (Tian et al., 2011). Silicon is reported to improve Ca status of plant tissue (Hammond et al. 1995) which is not only a key element in maintaining plasma membrane integrity but it also compete with Cd for uptake and translocation (Andreson and Nilsson, 1974). Putwattana et al. (2010) have reported decrease in Cd concentration and whole plant accumulation and lowest transfer factor of Cd in Sweet basil (Ocimum basilicum) with application of Si fertilizer (10 and 20 %) to Cd contaminated soil (20 mg kg⁻¹ soil).
However, the effect of Si on G Cd concentration was dependent on the Si dose applied and the Cd accumulating ability of wheat cultivars. Higher decrease in G Cd was observed in LSCd cultivars with the application of Si compared to HSCd cultivars. These results are in line with the findings of Lukačová Kuliková and Lux (2010) who reported higher Si induced depression in root and shoot Cd concentration (43 and 35 %, respectively) for Reduta compared to Almansa (12 %) and Valintena (6 %) maize hybrids grown under 100 uM Cd stress.

6.5.4 Post-harvest soil pH and available Cd

The resultant pH of Si amended soil would depend upon the initial pH of soils. The decrease in available Cd was reported to be associated with increased pH of Si treated soil (Chen et al., 2000 and Liang et al., 2005). As the soils used in the above mentioned studies were acidic in reaction (pH), therefore, it was thought that pH induced adsorption of Cd would only be possible in acid soils. Contrarily, in present study a linear decrease in AB-DTPA extractable Cd was noted without any increase in pH of post-wheat harvest soil treated with CaSiO₃ (Table 6.3). The contradiction regarding effect of Si on soil pH could be explained by different level and/ or sources of Si applied and reaction (pH) of the soils used. For example, increase in soil pH was reported for Si doses as high as 400 mg kg⁻¹ by Liang et al. (2005). Our results confirmed the findings of da Cunha et al. (2008) who reported that pH of post-maize harvest soil (pH 4.90) remained unaffected followed by application of CaSiO₃ up to 200 mg kg⁻¹ but proportion of Cd decreased in exchangeable fraction. Similarly, it has been found that plant available Cd lowered to 18.8% by Si fertilizer (Li et al., 2008) and silicate slag was found more effective in decreasing the exchangeable soil Cd (Cheng and Hseu, 2002). It was reported by Dietzel (2000) and Sommer et al. (2006) that besides increase in pH, silicate addition to soil promotes polymerization of silicate composts which is a potential ligands to complex heavy-metal in soil. Thus, the present study suggested that reactions governing Si-mediated immobilization of metals in alkaline soil are independent of soil pH.

6.6. Conclusions

The wheat cultivars did not show any toxicity symptom or growth retardation to soil-applied Cd stress despite a considerable Cd concentration in different plant tissues. Silicon application at 150 mg kg⁻¹ soil proved an optimum level to immobilize Cd in soil, decrease Cd absorption
by roots and its translocation from roots to shoots and grains. Moreover, Cd immobilization in soil with applied Si was not related to increase in soil pH. It is concluded that concentration of Cd in wheat grains can be minimized by soil application of Si but application dose could differ with soil type and plant species.
7.1. Abstract

It is well documented that Zn is competing cation for Cd absorption and translocation in plants. Silicon has also been shown to restrict Cd accumulation in plants. Since Zn and Cd are chemically similar, Si otherwise can either weaken or strengthen the antagonism between them, which has not been studied yet. Therefore, sole and combined application of Zn (10 mg kg\(^{-1}\)) and Si (150 mg kg\(^{-1}\)) to Cd contaminated normal soils was evaluated for their effect on Cd and Zn concentration grain of previously selected of low– and – high shoot Cd cultivars (Iqbal-2000 and Lasani-2008) and high shoot Cd (HSCd; Inqlab-91 and Sehar-2006) cultivars grown on artificially Cd contaminated soil (10 mg kg\(^{-1}\)) soil. On normal soil, application of Zn significantly improved both grain and straw yield while Si enhanced only grain yield. Zinc induced increase in grain and straw yield was more pronounced in high-shoot-Cd cultivars owing to their efficient Zn uptake and translocation abilities. Applying Si in combination with Zn did not show significant improvement in grain yield and decrease in Cd concentration of grain over Zn and/ Si application alone. Sole application of Zn as well as Si decreased Cd concentration in grain while for shoot Cd only Si did so. The Zn-induced decrease in Cd concentration in grain (GCd) was related to decrease in shoot to grain Cd translocation whereas Si decreased Cd by lowering AB-DTPA extractable Cd in soil as well as depressing shoot to grain Cd translocation that could also be attributed to decreased competitive interaction between Zn and Cd transport resulting from depressed Cd translocation.

7.2. Introduction

In plants, Cd inhibits absorption and translocation of nutrients such as N (Hernandez et al., 1996), Zn (Yang et al., 1996) and Fe (Alcantara et al., 1994), interact with water balance (Gong et al., 2005) and is involved in the production of reactive oxygen species (ROS). In human
body, critical organ for long-term Cd exposure is the kidney, where Cd principally affects renal tubular function. In extreme cases chronic toxicity of Cd leads to pulmonary emphysema (shortness of breath) and bone fractures; Itai-Itai disease (Yeung and Hsu, 2005).

Plant species with low Cd absorption and translocation could be cultivated to obtain Cd-safe food. However, low Cd accumulating plant species and/or cultivars could be inherently low yielding and/ or susceptible to attack by some pest which may restrict their cultivation. On the other hand, mineral nutrients may operate to affect Cd absorption and translocation through various intrinsic and extrinsic mechanisms. Extrinsic mechanisms refer to the changes in soil/growth medium, for example co-precipitation of Cd in soils and intrinsic mechanisms involved the changes in the physiological behavior of plants where by it reduces Cd uptake and translocation. Moreover, low Cd accumulating behavior of plants may get improved or a cultivar accumulating significant amount of Cd may also show low accumulation with the optimization of certain plant nutrients.

In this regard, Si and Zn have gained much attention in minimizing Cd accumulation. Silicon is beneficial plant nutrient and despite poor understanding of the mechanism involved it is known to reduce metal accumulation in plants. Silicon deposition in root endodermis strengthens the casparian band and restricts the transport of metal in bypass flow from roots to shoots (Shi et al., 2005a). Co-precipitation of metal with Si as Cd-silicate in plants is another mechanism by which Cd transport could be limited to the edible parts (Shi et al., 2005a). On the other hand, Zn is chemically similar to Cd and might act as competitive ion for Cd absorption from soil by roots (Das et al., 1997) and as well as interactive with Cd within the transport system of plants (Hart et al., 2002). It is also an integral part of various antioxidant enzymes of plants and under optimum supply Cd can’t replace it in such enzymes to produce oxidative stress. Studies reveal that effects of Zn on Cd accumulation are not consistent and may differ with plant species and Zn and Cd concentration in soil. Moreover, owing to chemical similarities between Cd and Zn, Si is also reported to decrease Zn concentration and toxicity in Zn-stressed plants exposed to its toxic concentration. On the other hand, some reports confirmed enhancement of Zn concentration in plants receiving Si nutrition under optimum (Rizwan et al., 2012) or toxic Zn supplies (Song et al., 2011). Furthermore, solitary effect of Zn and/ or Si on Cd has been studied so far and investigations elaborating their interactive effect on Cd uptake and translocation have not been studied yet. Keeping in view
the above facts, a greenhouse pot experiment was conducted to evaluate the effect of solitary or combined application Zn and Si on growth and Cd and Zn concentration of wheat cultivars contrasting in shoot Cd concentration.

7.3. Materials and Methods

7.3.1. Experimental treatments and design

The experiment comprised of four treatments viz.: Contaminated control (C, 10 mg Cd kg\(^{-1}\) soil); Zn at 10 mg kg\(^{-1}\) soils (Zn), Si at 150 mg kg\(^{-1}\) (Si); Zn and Si together (Zn + Si). Two LSCd (Iqbal-2000 and Lasani-2008) and two HSCd cultivars (Inqlab-91 and Sehar-2006) were used in this study. The experiment was arranged in factorial design with three replications.

7.3.2. Application of treatments and sowing of wheat

Soil was prepared and contaminated with Cd (10 mg Cd kg\(^{-1}\) soil) as described in 6.3.2 of Chapter 6. The Cd contaminated soil was filled in polythene lined glazed pots (10 kg soil pot\(^{-1}\)). Zinc (10 mg kg\(^{-1}\)) as zinc sulphate heptahydrated (ZnSO\(_4\).7H\(_2\)O) and/or Si (150 mg kg\(^{-1}\)) as calcium silicate (CaSiO\(_3\)) were homogenized in soil of respective pots. Basal dose of 100, 80 and 50 mg N, P and K kg\(^{-1}\) of soil was applied in solution as urea, single superphosphate (SSP) and potassium sulphate (KCl), respectively. Whole of the P and K and one third of N was applied at sowing while remaining N was applied at 30 and 50 days of sowing in equal splits. After mixing CaSiO\(_3\) and fertilizer solution, soil was equilibrated at field capacity for an additional period of two weeks. Six seeds of wheat cultivars were sown in each pot which later on thinned to three seedlings pot\(^{-1}\) and retained up to maturity. The uprooted seedlings were crushed and mixed thoroughly into respective pots. The plants were irrigated daily using deionized water to field capacity. The rest of the experimental procedure (fertilization, irrigation, harvesting and sample preparation) was same as described in Chapter 6.

7.3.3. Harvesting and sample preparation

Plants were harvested at physiological maturity and air dried. Samples were oven dried for 72 h at 65±5 °C in a forced air oven. Grains were separated from spikes and straw and grain yield was recorded. The straw and grain samples were ground in stainless steel grinding mills and stored in labeled polythene bags for the determination of Cd and Si. Post-harvest soil samples
were taken from pots with the help of stainless steel tube, air dried and passed through a 2 mm sieve for analysis of AB-DTPA extractable Cd and Zn.

7.3.4. Plant analysis

7.3.4.1. Cadmium and micronutrient determination
Determination of Cd and Zn was carried out as described in section 4.3.3 of the chapter 4.

7.3.4.2. Silicon determination
Silicon in grain and straw was determined following method described in section 5.3.4 of chapter 5.

7.3.5. Soil Analysis
Details of the methods used for physicochemical properties of soil such as textural class, ECe, pHs, organic matter, CaCO₃, total soil metals, post-harvest soil Cd, available Si have been described in section 6.3.6 (Table 6.1).

7.3.6. Statistical analysis
The data regarding plant biomass and Cd and Zn concentrations in straw, grain and soil in response to Si and Zn application were statistically analyzed by two-way ANOVA (Steel et al., 1996). The statistical significance was compared at 0.05 probability using Statistix Version 8.1 software package.

7.4. Results

7.4.1. Grain and straw yield
The data regarding grain and straw yield of wheat cultivars are presented in Fig. 7.1. Grain yield of wheat cultivars increased significantly with the application of Zn (10 mg kg⁻¹) and Si (150 mg kg⁻¹) alone (Zn and/or Si) or together (Zn + Si) to Cd contaminated (10 mg kg⁻¹) Zn deficient soil (Fig. 7.1 a). The improvement in grain yield with Zn application was higher in HSCd cultivars Inqlab-91 and Sehar-2006 (37.7 and 18.7 %, respectively) compared to 13.9 and 11.8 % that was recorded for LSCd cultivars Iqbal-2000 and Lasani-2008, respectively. Similar to Zn, application of Si to wheat cultivars increased grain yield of wheat cultivars; however, the improvement was higher and significant only in LSCd cultivars. It was improved by 10.7, 7.1, 2.7 and 4.2 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006,
respectively with the application of Si. Increase in grain yield of LSCd with Zn+ Si treatment was statistically at par with either of Zn and/or Si applied alone. Whereas in HSCd cultivars, Zn+Si induced increase in grain yield was statistically similar to that of sole applied Zn while significantly higher than sole application of Si. Overall, improvement in grain yield (%) with Zn+Si treatment compared to control remained higher for HSCd cultivars Inqlab-91 (46.5) and Sehar-2006 (25.9) than LSCd cultivars Iqbal-2000 (19.8) and Lasani-2008 (14.1). In Zn+Si treatment, the grain yield corresponding to Zn (difference in grain yield improvement between Zn+Si and Si alone treatment) over Zn application alone was higher for HSCd cultivars Inqlab-91 (43.8) Sehar-2006 (21.8) than LSCd cultivars Iqbal-2000 (9.1) and Lasani-2008 (7.0). Although there was no substantial difference, improvement (%) in grain yield corresponding to Si in Zn+Si treatment (difference in grain yield improvement between Zn+Si and Zn alone treatment) over Si application alone was higher in HSCd cultivars Inqlab-91 (8.8) and Sehar-2006 (7.2) than LSCd cultivars Iqbal-2000 (5.8) and Lasani-2008 (2.3).

Similar to grain yield, alone (Zn) or Si-combined application of Zn (Zn + Si) to Cd contaminated soil deficient in plant available Zn improved straw yield of wheat cultivars compared to control (Fig. 7.1 b). The improvement in straw yield with Zn application remained higher for HSCd cultivars compared to LSCd cultivars of which Lasani-2008 showed non-significant increase. It increased by 11.6 and 6.19 % for LSCd cultivars Iqbal-2000 and Lasani-2008, respectively over control. In case of HSCd cultivars Inqlab-91 and Sehar-2006, improvement in straw yield over control was 17.6 and 14.7 %, respectively.

Although it tended to increase, but application of Si could not improve straw significantly. It improved in Si treatment by 2.38, 1.81, 2.73 and 5.99 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively. Likewise grain yield, improvement in straw yield (% increase) with Zn+Si treatment over control remained higher for HSCd cultivars Inqlab-91 (21.4) and Sehar-2006 (24.6) than LSCd cultivars Iqbal-2000 (18.6) and Lasani-2008 (9.3). Similar to Zn application alone, the improvement in straw yield (% increase) corresponding to Zn in Zn+Si treatment over Zn application alone was higher for HSCd cultivars, Inqlab-91 (18.6) Sehar-2006 (18.7) than LSCd cultivars, Iqbal-2000 (16.2) and Lasani-2008 (7.5). However, improvement in straw yield (% increase) corresponding to Si in Zn+Si treatment over Si application alone was not related to the shoot Cd accumulating abilities of the wheat cultivars. The Si induced improvement in straw yield with Zn+Si over Si
application alone was 6.98, 3.15, 3.78 and 9.91 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively.

![Fig. 7.1. Grain and straw yield of wheat cultivars as affected by Si and Zn application to Cd contaminated Zn deficient soil. Bars sharing different letter(s) are significantly different from each other at p ≤ 0.05 (LSD: GBM = 1.576; SBM = 2.818). Error bars indicates standard error (SE).](image)

**7.4.2. Cadmium concentration in grains and straw**

Wheat cultivars concentrated significant amount of Cd in grains (GCd) which was related to their inherent shoot Cd concentrating abilities; that is, LSCd cultivars had lower while HSCd cultivar had higher GCd (Fig. 7.2a). Moreover, GCd concentration was statistically similar between the members of LSCd and/ or HSCd cultivars. The GCd was 2.66, 2.38, 4.41 and 4.17 mg kg⁻¹ for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively.
The GCd of wheat cultivars decreased significantly with Zn (10 mg kg\(^{-1}\)) and Si (150 mg kg\(^{-1}\)) application alone (Zn and/ or Si) or together (Zn + Si) to Cd contaminated (10 mg kg\(^{-1}\)) Zn deficient soil (Fig. 7.1 a). The depression in GCd (%) in Zn treated plants was higher for HSCd cultivars Inqlab-91 (30.8) and Sehar-2006 (33.1) compared to LSCd cultivars Iqbal-2000 (23.2) and Lasani-2008 (20.7). However, contrary to Zn, Si treated plants of LSCd cultivars showed substantially higher decrease in GCd compared to HSCd cultivars. The decrease in GCd with Si treatment was 40.8, 37.6, 25.9 and 21.8 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively. The depressing effect of Zn and/ or Si on GCd was statistically similar between members of LSCd and/ or HSCd cultivars. Combined application of Zn and Si caused further decrease in GCd concentration over control compared to the sole application of either of two. The decrease (%) in GCd with Zn+Si treatment over control remained 47.6 and 45.9, 40.2 and 44.6 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively. Partitioning of Zn+Si treatment effect into Zn and/ or Si as described above revealed that it was low for both of nutrients than in Zn and/ or Si alone treatments and summed up to a higher value. For example, in LSCd cultivars, Si induced decrease in Zn+Si treatment lowered to 24.4 and 25.2 % from 40.8 and 37.6 % with Zn alone for Iqbal-2000 and Lasani-2008, respectively. Similarly, in HSCd cultivars Inqlab-91 and Sehar-2006 Zn decreased GCd by 30.8 and 33.1 in Zn alone treatment which declined to 14.3 and 22.8 % in Zn+Si treatment. In LSCd cultivars, GCd with Zn+Si treatment was statistically at par with Si alone while significantly higher than Zn application alone. However, in HSCd cultivars, the opposite was true with non-significant difference between Zn+Si and Zn treatment for GCd.

Likewise GCd, considerable Cd concentration was observed in straw (SCd) of wheat cultivars so that LSCd cultivars had lower while HSCd cultivar had higher SCd (Fig. 7.2b). Moreover, GCd concentration was statistically similar between the members of LSCd and/ or HSCd cultivars. The SCd was 6.15, 6.51, 8.03 and 8.56 mg kg\(^{-1}\) for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively. The SCd was 2.50 times higher than GCd in LSCd cultivars while corresponding value was 1.93 for HSCd cultivars.

The application of Zn did not influence while Si decreased SCd concentration in wheat cultivars. The HSCd cultivars Inqlab-91 and Sehar-2006 showed significant decrease of 17.9 and 18.2 %, respectively in SCd which was higher than corresponding decrease for LSCd cultivars Iqbal-2000 (14.8) and Lasani-2008 (12.9). Moreover, Si induced decrease in SCd
over control in Lasani-2008 was non-significant. Combined application of Zn and Si (Zn+Si) caused statistically similar decrease in SCd to that of Si application alone that was also higher in HSCd cultivars. The decrease in SCd was 15.9, 10.4, 18.6 and 16.9 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively. Partitioning of Zn+Si treatment effect in to Zn and/ or Si as described above revealed that effect of Zn in Zn+Si treatment was also non-significant. Moreover, decrease in SCd corresponding to Si in Zn+Si treatment was statistically similar to that in Si application alone. It was 18.5, 3.2, 16.6 and 14.6 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively.

7.4.3. Zinc concentration in grains and straw

Under Cd contaminated Zn deficient soil, LSCd cultivars had lower while HSCd cultivars had higher Zn concentration in grains (GZn) (Fig. 7.3a). The GZn was 28.7, 25.2, 36.9 and 41.4 mg kg\(^{-1}\) for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively. Moreover, GZn concentration in LSCd cultivars Iqbal-2000 and Sehar-2006 was statistically higher than Lasani-2008 and Inqlab-91, respectively.

Application of Zn to Cd contaminated soil significantly enhanced GZn in both group of cultivars; however, the increase (%) was higher for HSCd cultivars Inqlab-91 (30.8) and Sehar-2006 (33.6) compared to LSCd cultivars Iqbal-2000 (22.5) and Lasani-2008 (27.3). Similarly, Si application to Zn deficient Cd contaminated soil significantly enhanced GZn by 9.6 and 12.7 % for LSCd cultivars Iqbal-2000 and Lasani-2008, respectively. The GZn concentration also tended to increase in Si treated plant of HSCd cultivars Inqlab-91 (6.93) and Sehar-2006 (5.83) but effect was non-significant. Applying Zn in combination with Si (Si+Zn) to LSCd cultivars increased GZn concentration further to that with sole application of either of the two. The increase (% over control) in GZn with Zn+Si treatment remained 36.6 and 39.4 % for Iqbal-2000 and Lasani-2008, respectively. In HSCd cultivars Inqlab-91 and Sehar-2006 the respective increase in GZn with Zn+Si treatment was 41.6 and 40.0 % which was statistically similar to that with Si and higher than Zn application alone. In all the treatments, GZn concentration was higher in Iqbal-2000 than Lasani-2008 while it was statistically similar between Inqlab-91 and Sehar-2006.
Fig. 7.2. Cadmium concentration in grain and straw of wheat cultivars as affected by Si and Zn application to Cd contaminated Zn deficient soil. Bars sharing different letter(s) are significant among each other at $\alpha = 0.05$ (LSD: G\text{Cd} = 0.433; S\text{Cd} = 0.871). Error bars indicates standard error (SE).

Contrary to G\text{Zn}, Zn concentration in straw (S\text{Zn}) of wheat cultivars was higher in L\text{SCd} cultivars whereas lower in H\text{SCd} cultivars in control as well as Zn and/ or Si treated soil (Fig 7.3b). Moreover, the mean S\text{Zn} in L\text{SCd} cultivars was 2.32 times higher than their G\text{Zn} and the corresponding value for H\text{SCd} cultivars was 1.29. The S\text{Zn} was 59.9, 64.7, 52.1 and 48.3 mg kg\textsuperscript{-1} for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively. The application of Zn significantly increased whereas Si did not affect S\text{Zn} in L\text{SCd} as well as H\text{SCd} cultivars. Furthermore, the increase in S\text{Zn} was not related to the shoot Cd concentrating abilities of the wheat cultivars.
Fig. 7.3. Zinc concentration in grains and straw of wheat cultivars as affected by Si and Zn application to Cd contaminated Zn deficient soil. Bars sharing different letter(s) are significantly different from each other at \( p \leq 0.05 \) (LSD: \( \text{GZn} = 2.811; \text{SZn} = 8.506 \)). Error bars indicates standard error (SE).

The maximum increase (%) in SZn was recorded for Sehar-2006 (42.1) whereas it was minimum for Iqbal-2000 (29.2). The SZn did not improve in Si treated plants of LSCd cultivars whereas it tended to increase in HSCd Inqlab-91 (6.57) and Sehar-2006 (7.90) that was non-significant. Applying Si along with Zn (Zn+Si) did not affect SZn concentration compared to sole Zn application in both the LSCd and HSCd cultivars. The increase in SZn with Zn+Si application was 28.4, 35.7, 28.9 and 32.9 for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively.
7.4.4. Silicon concentration in grains and straw

Silicon concentration (mg g\(^{-1}\)) in grains (GSi) was statistically similar among all the wheat cultivars and it was 3.07, 2.61, 3.16 and 2.44 mg g\(^{-1}\) for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively (Fig. 7.4a). Application of Zn increased GSi in all the cultivars, however, the increase (%) was significant only in HSCd cultivars Inqlab-91 (59.4) and Sehar-2006 (61.4) which was higher than Iqbal-2000 (39.7) and Lasani-2008 (30.4). Silicon addition significantly increased GSi concentration in all the cultivars and increase was not related to their Cd concentrating abilities. The GSi in Si treated plants was 12.3, 9.9, 11.2 and 10.2 mg g\(^{-1}\) for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively which correspond to 301, 282, 254 and 319 % increase over no Si treated plants. The combined application of Zn and Si (Zn+Si) further improved GSi over Si application alone in all the cultivars. Moreover, improvement in GSi with Zn+Si compared to Si alone was higher in HSCd cultivars Inqlab-91 (92.7) and Sehar-2006 (152) than LSCd cultivars Iqbal-2000 (44.2) and Lasani-2008 (66.6).

Likewise GSi, Si concentration (mg g\(^{-1}\)) in straw (SSi) was statistically similar among all the wheat cultivars and it was 4.71, 4.19, 4.36 and 5.14 mg g\(^{-1}\) for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively (Fig. 7.4b). Moreover, the mean SSi of wheat cultivars was 1.38 to 2.11 times higher than GSi. Application of Zn to Cd contaminated Zn deficient soil increased SSi in all the cultivars and increase (%) was significant in both of the HSCd cultivars Inqlab-91 (54.7) and Sehar-2006 (46.2). The improvement in SSi in LSCd Iqabl-2000 (26.8) and Lasani-2008 (39.3) was lower than HSCd cultivars and significant only in Lasani-2000. Silicon addition significantly increased GSi concentration in all the cultivars and increase was not related to their Cd concentrating abilities. The GSi in Si treated plants was 14.9, 16.1, 15.4 and 17.1 mg g\(^{-1}\) for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively which correspond to 216, 284, 252 and 232 % increase over that in no-Si and/ or Zn treated plants. The combined application of Zn and Si (Zn+Si) further improved GSi over Si application alone in all the cultivars. Moreover, improvement in GSi with Zn+Si compared to Si alone was higher in HSCd cultivars Inqlab-91 (66.7) and Sehar-2006 (58.4) than LSCd cultivars Iqbal-2000 (33.8) and Lasani-2008 (47.7).
Fig. 7.4. Silicon concentration in grains (a) and straw (b) of wheat cultivars as affected by Si and Zn application to Cd contaminated Zn deficient soil.

Bars sharing different letter(s) are significantly different from each other at p ≤ 0.05 (LSD: GSi = 1.717; SSi = 1.383). Error bars indicates standard error (SE).

7.4.5. AB-DTPA extractable Cd and Zn in post-harvest soil

The maximum AB-DTPA extractable soil Cd (mg kg⁻¹) was observed in control pots receiving no Zn and/or Si. Moreover, it was higher in soil sown with LSCd cultivars Iqbal-2000 (4.16) and Lasani-2008 (4.41) than HSCd cultivars Inqlab-91 (3.15) and Sehar-2006 (3.27). Application of Zn had no effect on AB-DTPA extractable Cd in soil sown with either of the cultivars. The AB-DTAP extractable Cd in Zn treated soil sown with Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006 was 4.41, 3.96, 3.21 and 3.16 mg kg⁻¹, respectively. Application of Si significantly decreased (conversely immobilized) AB-DTAP extractable Cd in soil sown with either of the cultivars. The decrease in AB-DTPA extractable Cd ranged between 9.14
(Inqlab-91) to 14.5 (Lasani-2008). The combined application of Zn and Si (Zn+Si) did not further immobilize Cd in soil over Si application alone in soils sown with either of the cultivars. The AB-DTAP extractable Cd in Zn+Si treated soil sown with Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006 was 3.80, 3.44, 2.79 and 2.80 mg kg\(^{-1}\), respectively.

The AB-DTPA extractable soil Zn in no-Si and/or no-Zn treated Cd contaminated soil sown with LSCd cultivars Iqbal-2000 (0.80) and Lasani-2008 (0.77) was statistically similar to that with HSCd cultivars Inqlab-91 (0.88) and Sehar-2006 (0.85) (Fig 7.5b). Application of Zn significantly increased AB-DTPA Zn in soils sown with either of the cultivars. The resultant AB-DTPA extractable Cd followed by addition of Zn to soil was 1.83, 1.57, 2.07 and 2.72 mg kg\(^{-1}\) for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively which correspond to 103 to 221 % increase. Application of Si alone (Si) or in combination with Zn (Zn+Si) did not influence AB-DTPA extractable Zn in soil sown with either of the cultivars. Thus, AB-DTPA extractable Zn was statistically similar between Si and control as well as Zn and Zn+Si treatments.

7.4.6. Shoot to grain Cd translocation index

The maximum shoot to grain translocation index (CdGTI) was recorded in Cd contaminated control soil (Fig. 7.6). Moreover, CdGTI was lower for LSCd cultivars Iqbal-2000 (0.411) and Lasani-2008 (0.368) than that for HSCd cultivars Inqlab-91 (0.551) and Sehar-2006 (0.484). Application of Zn depressed Cd translocation from shoot to grain and depression in Cd translocation was higher in HSCd cultivars Inqlab-91 (29.5) and Sehar-2006 (31.4) than LSCd cultivars Iqbal-2000 (25.9), Lasani-2008 (13.1). Although effect was more pronounced in HSCd cultivars, application of Si decreased CdGTI in all the cultivars. Following Si induced decrease of 32.6, 28.7, 9.7 and 4.3 % in CdGTI, it was 0.302, 0.262, 0.498 and 0.462 for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively. The combined application of Zn and Si (Zn+Si) further decreased CdGTI over Si for LSCd cultivars Iqbal-2000 (12.3) and Lasani-2008 (26.4) and no effect in Si treated HSCd cultivars was observed. While over Zn treatment alone, in both LSCd and HSCd cultivars, Cd translocation decreased with Zn+Si treatment. However, depression in Cd shoot to grain translocation was higher in HSCd cultivars Inqlab-91 (17.0) and Sehar-2006 (28.8) than LSCd cultivars Iqbal-2000 (6.7) and Lasani-2008 (10.8).
Fig. 7.5. AB-DTPA extractable Cd (SoCd) and Zn (SoZn) in post wheat harvest soil as affected by Si and Zn application to Cd contaminated soil. Bars sharing different letters(s) are significantly different from each other at p ≤ 0.05 (LSD: AB-DTPA Cd = 0.382; AB-DTPA Zn = 0.314). Error bars indicates standard error (SE).

7.5. Discussion

7.5.1. Grain and straw yield
Zinc deficiency limits the grain yield of crops in many parts of the world (Cakmak, 2008) and it is an important nutritional problem in a large number of countries. Application of Zn significantly increased grain and straw yield of wheat cultivars and even more so in HSCd cultivars (Fig. 7.1). Likewise, Kutman et al. (2011) reported 35 % improvement in wheat grain yield grown under soil severely deficient in DTPA extractable Zn (0.1 mg kg\(^{-1}\)) supplied with Zn at 5 mg kg\(^{-1}\).
The higher increase in yield of HSCd could be due to efficient Zn uptake and translocation by these cultivars. In this regard, Maqsood et al. (2009) reported Inqlab-91 and Sehar-2006 to be Zn-efficient while Iqbal-2000 as Zn-inefficient among the tested 12 wheat genotypes. This result indicates that Zn induced improvement in biomass is related to Zn use efficiency of plant species. Earlier results on Zn deficiency confirmed that wheat grain yield is more sensitive to Zn deficiency than straw yield (Yilmaz et al., 1997; Kutman et al., 2010). Consistently, positive effect of Zn on grain yield was more pronounced than that on straw dry matter in present study (Fig. 7.1). The Zn deficiency-induced impairment in the development of reproductive organs is the reason behind sensitivity of grain yield to Zn deficiency (Cakmak and Engels, 1999).

Contrary to Zn, Si application to Zn-deficient Cd contaminated soil enhanced only grain yield of the wheat cultivars and improvement was higher in LSCd cultivars. The improvement in grain biomass with Si under Zn supply (Zn+Si) was non-significant compared to Si application alone. The improvement in grain yield of Si treated plants under Zn-deficient soil could be due to Si-induced improvement in Zn uptake. It was reported by da Cunha et al. (2009) that Si application up to 150 mg kg⁻¹ resulted in higher Zn accumulation by maize...
plants. Secondly, the effect of Si on growth of Cd-unstressed plant might be due to influences on the absorption and transport of several mineral elements that help perform many functions in plants (Epstein and Bloom, 2005, Ma and Takahashi, 2002). Although beneficial effect of Si on growth of plants under stress conditions are more evident, still it has been reported under normal growth conditions by many researchers (Parveen and Ashraf, 2010; Tahir et al., 2006; Al-aghabary et al., 2004).

Since in previous study, no decline in root, shoot and grain biomass or toxicity symptoms on leaf of all the wheat cultivars could be seen under the employed Cd stress, the increase in straw and grain yield of wheat could not be regarded as the alleviation of Cd toxicity instead of beneficial effect of Zn and/or Si nutrition.

7.5.2. Cadmium concentration in grains and straw

Uptake of Cd have been shown to depend upon Zn contents of soil and under low Zn contents plants may accumulate higher levels of Cd even from marginal contaminated soil to exceed the regulatory levels. In present study, application of Zn to Zn-deficient plants significantly decreased Cd concentration in grains of wheat cultivars while no change in SCd was observed (Fig. 7.2). This result agrees with the finding of Oliver et al. (1994) reporting 50% decrease in grain Cd concentration with Zn application at 5 kg ha\(^{-1}\) to wheat grown on marginally or severely Zn-deficient soils. Moreover, Zn-induced decrease in GCd was much more pronounced for HSCd cultivars than LSCd cultivars that could be attributed to the higher uptake and translocation of Zn (Maqsood et al., 2009) by the former cultivars which efficiently competed with Cd uptake and translocation. These findings are in line with the results of Hart et al. (2002) who attributed the competitive interaction during uptake and transport between Cd and Zn to the existence of a common transport system on the plasma membranes. However, Zn was also shown to interfere with phloem-mediated Cd transport in durum wheat inhibiting re-mobilization of Cd from leaves to grain while plant starts senescence (Cakmak et al., 2000). Since Zn did not affect available Cd in soil and decreased Cd translocation from straw to grain (Fig. 7.6) without altering SCd, thus Zn-induced decrease in GCd could exclusively be attributed to competitive transport interaction between Zn and Cd in plants.

This study confirmed the findings of our earlier work (Study 4) showing that Si application at 150 mg kg\(^{-1}\) soil significantly decreased SCd as well as GCd concentration in
wheat cultivars (Fig. 7.2). The Si induced decrease in Cd uptake could be due to enhanced production of antioxidant that helped maintain integrity of plasma membrane inhibiting indiscriminate Cd uptake (Tian et al., 2011). Moreover, Si is reported to improve Ca status of plant tissue (Hammond et al., 1995) which is not only a key element in maintaining plasma membrane integrity but it also compete with Cd for uptake and translocation (Andreson and Nilsson, 1974). The pronounced effect of Si on SCd and GCd concentration of LSCd cultivars than HSCd cultivars might be associated with higher deposition of Si in roots as observed in Study 4. The decrease in SCd indicates lower uptake from soils while decline in GCd could be attributed to both lower uptake as well as translocation from straw to grain (Fig. 7.6). The decrease in plant available soil Cd in Si treated pots (Fig. 7.5) confirms the above assumption. In line to our findings, da Cunha et al. (2008) and Li et al. (2008) reported that immobilization of Cd in soil as Cd-silicate has been reported as one of the mechanisms that reduced Cd uptake.

7.5.3. Zinc and Si concentration in grains and straw

Application of Zn significantly increased Zn concentration in grains (GZn) as well as straw (SZn) of all the wheat cultivars with even much more pronounced effect in HSCd cultivars (Fig. 7.3). Higher concentration of Zn in grains and straw of HSCd cultivars Inqlab-91 and Sehar-2006 than Iqbal-2000 confirmed the finding of Maqsood et al. (2009) who reported the former cultivars to be efficient in uptake and shoot to grain translocation of Zn. The HSCd cultivars with higher GZn concentration retained less Zn in straw compared to LSCd cultivars. Consistently Imtiaz et al. (2010) reported that Zn-efficient cultivars from Pakistan including Bakhtawar, Gatcher S61, Wilgoyne, and Madrigal accumulated lesser amounts of Zn in their shoots than inefficient cultivars Durati, Songlen, Excalibur, and Chakwal-86. The wheat cultivars efficient in shoot to grain Zn translocation showed higher decrease in SCd and GCd which suggests that Zn-efficient wheat cultivars more efficiently excluded Cd from grains and straw. It was also confirmed by higher decrease in translocation index of Cd in Zn-efficient HSCd wheat cultivars concentration (Fig. 7.6). Moreover, no toxic effect of increasing tissue Zn on grain and straw yields was observed. The GZn ranged between 25.2–39.3 mg kg\(^{-1}\) for LSCd cultivars while 36.9 to 57.9 for HSCd cultivars. Although Zn concentration required to obtain 90 to 97.5 % relative grain yield is in the range of 25–40 μg Zn g\(^{-1}\) of wheat grains (Cakmak, 2008) but concentration between 50–100 μg Zn g\(^{-1}\) DM could be achieved without
any significant yield loss (Marschner, 1995). Therefore, little higher Zn application than that required to obtain maximum relative grain yield would help plants further to exclude Cd from edible parts.

In this study, Si supplementation to Zn deficient plants significantly increased Zn concentration both in straw and grains. It was also reported by da Cunha et al. (2009) that Si application up to 150 mg kg⁻¹ resulted in higher Zn accumulation by maize plants even under high Zn supply. Similarly, improved grain Zn concentration in Si treated wheat plants was observed by Rizwan et al. (2012). The Si-induced increase in GZn and SZn could be the indirect consequence of decrease in Cd translocation that competes Zn for transport within plants instead of affecting available Zn in soil (Fig. 7.5b). Although beneficial effect of Si on growth of plants under stress conditions are more evident, still these have been reported under normal growth conditions by many researchers (Parveen and Ashraf, 2010; Tahir et al., 2006; Al-aghabary et al., 2004). Contrasting results reporting no significant effect (Masarovic et al., 2012) or decrease in Zn concentration of Si treated plants (Gu et al., 2012) also exist. But, it would be worth mentioning that these studies attempted Si-induced alleviation of Zn toxicity in Zn-stressed plants unlike deficient or optimum Zn supplied wheat plants in this study.

Likewise Zn, Si application significantly increased Si concentration in straw and grains as it has been reported for wheat and other cereals in many earlier findings (Tahir et al., 2006; da Cunha et al., 2009; Parveen and Ashraf, 2010). Silicon concentration in wheat cultivars was not related to their Cd concentrating abilities. Moreover, Zn supplied wheat plants grown with or without Zn on Cd contaminated soils showed further enhanced GSi and SSi with even higher increase in HSCd cultivars that could be related to higher Zn use efficiency of these cultivars as discussed above. Higher Si concentration both in roots and shoots of Zn supplied Sorghum bicolor plants have been reported by Masarovic et al. (2012). Others have shown either no effect (Kaya et al., 2009) or reduced Si concentration (Song et al., 2012) in plants supplied with extensively high toxic Zn level. These contrasting reports seem depending on the extent of Zn toxicity and physiological response of plant species used in these studies.

7.5.4. AB-DTPA extractable Zn and Cd in post-harvest soil
Owing to similar geochemistry of Zn and Cd (Mengal and Kerkby, 2001), Cd adsorption and/or desorption was expected to be affected by Zn addition to Zn deficient soil but no such
interaction between Zn and Cd was observed for AB-DTPA extractable Cd in soil (Fig. 7.5a). However, confirming the results of Study 4, Si addition to soil significantly decreased AB-DTPA extractable Cd in post-wheat harvest soil. It was reported by Dietzel (2000) and Sommer et al. (2006) that silicate addition to soil promotes polymerization of silicate composites which is potential ligands to complex heavy-metal in soil. Our results confirmed the findings of da Cunha et al. (2008) who reported proportion of exchangeable soil Cd decreased in soil (pH 4.90) following application of CaSiO$_3$ up to 200 mg kg$^{-1}$. Similarly, it has been found that plant available Cd lowered to 18.8% by Si fertilizer (Li et al., 2008) and silicate slag was much effective in decreasing the exchangeable soil Cd (Cheng and Hseu, 2002). Combining Zn with Si (Zn+Si) did not show any change in AB-DTPA extractable Cd in soil compared to Si application alone. Thus, the present study suggested that not Zn but Si could decrease plant uptake of Cd by lowering its plant available concentration in soil.

As it could be suspected from higher GZn and SZn, significant increase in AB-DTPA extractable Zn concentration in post-wheat harvest soil was observed (Fig 7.5b). However, despite increased GZn and SZn, no increase in AB-DTPA extractable Zn was observed in Si and/or Zn+Si treated soil over control and/or Zn addition alone suggesting its increased translocation from roots to shoots and then grain resulting from lowered Cd translocation.

7.5. Conclusions
Application of both Zn and Si improved grain and straw yield of wheat cultivars. The combined application of Zn and Si decreased Cd concentration in soil and consequently in grains and straw of wheat cultivars without affecting Zn concentration in grain and straw. Zinc decreased grain Cd concentration by lowering its translocation from shoot to grain and was depending on Zn uptake and translocation efficiency of wheat cultivars. While Si induced decrease in grain Cd was owing to its ability to minimize plant available Cd in soil and lowering its translocation from shoots to grain. Moreover, Si application to Cd contaminated soil strengthened the competitive effect of Zn on Cd accumulation in grains by enhanceing root Zn absorption and its translocation within plants.
CHAPTER 8

CADMIUM ACCUMULATION BY WHEAT IN RESPONSE TO SILICON AND ZINC APPLICATION TO SALT-AFFECTED SOILS

8.1. Abstract
The role of Si to minimize Cd concentration in edible parts of plants is also well documented. However, owing to chemical similarities between Cd and Zn, Si is also expected to affect Zn uptake and translocation in plants and studies on this aspect are lacking. Moreover, presence of excessive salts in soil could also dilute the antagonism between Zn and Cd. Therefore, sole and combined application of Zn (10 mg kg\(^{-1}\)) and Si (150 mg kg\(^{-1}\)) to Cd contaminated saline and sodic soils was evaluated for their effect on Cd and Zn concentration in grains of previously selected of low- (Iqbal-2000 and Lasani-2008) and high- shoot Cd cultivars (HSCd; Inqlab-91 and Sehar-2006) grown on artificially Cd contaminated (10 mg kg\(^{-1}\)) saline and sodic soils. Application of Zn and/or Si significantly improved both grain and straw yield. Zinc induced increase in grain and straw yield was more pronounced in high-shoot-Cd cultivars owing to their efficient Zn uptake and translocation abilities. Moreover, the degree of improvement in yield was higher than the normal soil as it was observed in Chapter 7. Applying Si in combination with Zn did not showed significant improvement in grain yield and decrease in Cd concentration of grain over Zn and/ Si application alone. Sole application of Zn as well as Si decreased Cd concentration in grain while for shoot Cd only Si did so. The Zn-induced decrease in G\(_{Cd}\) was related to decrease in shoot to grain Cd translocation whereas Si decreased Cd by lowering AB-DTPA extractable Cd in soil as well as by depressing shoot to grain Cd translocation that could also be attributed to decrease competitive interaction between Zn and Cd resulting from depressed Cd translocation.

8.2. Introduction
Since many plants can accumulate significant amounts of Cd in edible parts without any toxicity or yield loss (Stevens and McLaughlin, 2006), its concentrations should be minimized to allowable dietary threshold limits. Consumption of Cd containing food for prolonged
periods may result in accumulation to such higher level that causes disorders like pulmonary emphysema (shortness of breath) and bone fractures; Itai-Itai disease (Yeung and Hsu, 2005). Among soil factors, soil salinity could shift the soil-solution chemical equilibrium in favour of more soluble Cd species like CdCl$_2^0$ and CdCl$^+$ thereby enhancing its availability to plants (Weggler-Beaton et al., 2000). The lower adsorbing ability of these species to soil than free Cd$^{2+}$ ions increases Cd mobility at the soil-root interface. Moreover, these complexes can enhance transport of Cd across plasma membrane which results in increased soil-plant transfer of Cd under salinity. It was shown that combined stress of NaCl and Cd caused higher plasma membrane permeability and enhanced production of oxygen radicals and H$_2$O$_2$ in comparison to Cd and NaCl treatments alone in wheat (Muhling and Lauchli, 2003). Thus, the problem of Cd accumulation in edible parts of plants could become even more severe when plants are exposed to salinity stress (Shafi et al., 2009, 2010) which is the case in countries like Pakistan where approximately 26 % of the total irrigated land is salt-affected (Pakistan Bureau of Statistics, 2010) and untreated waste effluent of poor quality (high EC, SAR and/or ESP) is being use to irrigate the crops (Ghafoor et al., 2008).

Cadmium affects uptake of many essential nutrients by plants (Sun et al., 2007). On the other hand, application of some beneficial plant nutrients may operate to affect Cd uptake and translocation through various intrinsic and extrinsic mechanisms. In this regard, Si and Zn have gained much attention in minimizing Cd accumulation. Silicon deposition in root endodermis strengthens the casparian band and restricts the transport of metal in bypass flow from roots to shoots (Shi et al., 2005a). Co-precipitation of metal with Si as Cd-silicate in plants is another mechanism by which Cd transport could be limited to the edible parts (Shi et al., 2005a). Indirectly, Si can also lower Cd uptake by alleviating specific ion effect through reduced Na uptake as a result of its immobilization in soil (Gong et al., 2003; Liang et al., 2003).

Zinc is chemically similar to Cd and might act as competitive ion for Cd absorption from soil by roots (Das et al., 1997) and as well as interactive with Cd within the transport system of plants (Hart et al., 2002). It is also an integral part of various antioxidant enzymes of plants and under optimum supply Cd could not replace it in such enzymes to produce oxidative stress. Studies revealed that effects of Zn on Cd accumulation are not consistent and may differ with Zn application level and soil characteristics including salt concentration.
Redondo-Go´mez et al. (2011) reported that the addition of NaCl to growth medium enhanced the accumulation of Zn in tillers of Spartina densiflora. Moreover, owing to chemical similarities between Cd and Zn, Si is also reported to decrease Zn concentration and toxicity in Zn-stressed plants exposed its toxic concentration. Contrastingly, some reports confirmed enhancement of Zn concentration in plants receiving Si nutrition under optimum (Rizwan et al. 2012) or toxic Zn supplies (Song et al. 2011). To the best of knowledge, despite the large number of studies investigating the role of Zn and Si on Cd accumulation in grain, interactive role of these nutrients has not been studied yet. Therefore, the present greenhouse pot experiment aimed at the effect of solitary or combined application of Zn and Si on growth and Cd and Zn concentration of wheat cultivars contrasting in shoot Cd cultivars on saline and sodic soils.

8.3. Materials and Methods

8.3.1. Experimental treatments and design
Saline and Sodic soils deficient in plant available Zn were collected, prepared and artificially contaminated with Cd at 10 mg kg$^{-1}$ as described under section 6.3.2 of Chapter 6. The experiment comprised of five treatments for both of the soils viz: uncontaminated control (C); Contaminated control (CC); Zn at 10 mg kg$^{-1}$ to contaminated soils (CC+Zn), Si at 10 mg kg$^{-1}$ to contaminated soils (CC+Si) and addition of both Zn and Si to contaminated soils (CC+Zn+Si). The same four genotypes of wheat differing Cd accumulation in shoot (Inqlab and Sehar high accumulating and Lasani and Iqbal low accumulating) were used. The, experiment followed CRD comprising of three replications. The rest of the experimental procedure was same as described in section 7.3.1.

8.3.2. Application of treatments and sowing of wheat
Application of treatments and sowing of wheat cultivars was carried out the same way as described in section 7.3.2.

8.3.3. Harvesting and sample preparation
Harvesting, sample collection and preparation was carried out the same way as described in section 7.3.3.
8.3.4. Plant analysis

8.3.4.1. Cadmium and Zn determination
Concentration of Cd and Zn in straw and grains was determined following the procedure described in section 4.3.3 of chapter 4.

8.3.4.2. Silicon determination
Silicon was determined in grain and straw following method described in section 5.3.3 of chapter 5.

8.3.5. Soil Analysis
In addition to soil analysis described under section 6.3.6 of chapter 6, following additional chemical properties of saline and sodic soils were analyzed:

8.3.5.1. Soluble ions

8.3.5.1.1. Calcium and Magnesium \((Ca^{2+} + Mg^{2+})\)
Saturation extract was titrated against 0.01 \(N\) EDTA (disodium) solution to a blue colour end point using eriochrome black T indicator in the presence of NH\(_4\)OH + NH\(_4\)Cl buffer solution for the determination of Ca\(^{2+}\) + Mg\(^{2+}\) (Method 7 of U.S. Salinity Lab. Staff, 1954).
\[
Ca^{2+} + Mg^{2+} (\text{mmol L}^{-1}) = \frac{(\text{EDTA used (ml)} x 0.01 x 1000)}{\text{volume of extract (mL)}}
\]

8.3.5.1.2. Sodium and Potassium \((Na^+ + K^+)\)
Soluble Na\(^+\) and K\(^+\) were determined with Jenway PFP-7 flame photometer having Na\(^+\) or K\(^+\) filter in place. The instrument was calibrated with a series of Na\(^+\) or K\(^+\) (0-20 ppm) standard solutions (Method 10a and 11a of U.S. Salinity Lab. Staff, 1954). Then emission of test samples was recorded and Na\(^+\) or K\(^+\) was calculated as under:
\[
Na^+ / K^+ (\text{mmol L}^{-1}) = \frac{[Na^+ / K^+ (\text{mg L}^{-1}) x DF]}{\text{Eq. wt of Na}^+/ K^+}
\]
8.3.5.1.3. Carbonates (CO$_3^{2-}$)
Carbonates were determined by titrating 10 mL of saturation extract against 0.01 $N$ H$_2$SO$_4$ to a colorless end point using phenolphthalein as indicator (Method 12 of U.S. Salinity Lab. Staff, 1954). Concentration of carbonates was calculated by using formula:

$$\text{CO}_3^{2-} \ (\text{mmol} \ \text{L}^{-1}) = [(2 \times R_1) \times 0.01 \times 1000] / \text{Aliquot taken (mL)}$$

8.3.5.1.4. Bicarbonates (HCO$_3^-$)
After the determination of CO$_3^{2-}$, the same aliquot was titrated against 0.01 $N$ H$_2$SO$_4$ to a pinkish yellow endpoint using methyl orange as an indicator and bicarbonates were determined as follows (Method 12 of U.S. Salinity Lab. Staff, 1954);

$$\text{HCO}_3^- \ (\text{mmol} \ \text{L}^{-1}) = [(R_2 - R_1) \times 0.01 \times 1000] / \text{Aliquot taken (mL)}$$

Where $R_1$ = mL of H$_2$SO$_4$ used for CO$_3^{2-}$ and $R_2$ = mL of H$_2$SO$_4$ used for HCO$_3^-$

8.3.5.1.5. Chlorides (Cl$^-$)
Aliquot after CO$_3^{2-}$ and HCO$_3^-$ determination were titrated against 0.01 $N$ AgNO$_3$ solution to a brick red end point using potassium chromate (K$_2$CrO$_4$) as an indicator for the determination of Cl$^-$ (Method 14 of U.S. Salinity Lab. Staff, 1954).

$$\text{Cl}^- \ (\text{mmol} \ \text{L}^{-1}) = [0.01 \ N \text{AgNO}_3 \text{used (mL)} \times 0.01 \times 1000] / \text{aliquot taken (mL)}$$

8.3.5.1.6. Sulphate (SO$_4^{2-}$)
These were determined by difference, i.e.

$$\text{SO}_4^{2-} = \text{TSS} - (\text{CO}_3^{2-} + \text{HCO}_3^- + \text{Cl}^-),$$

where concentration of all ions expressed in mmol$_c$ L$^{-1}$.

8.3.5.1.7. Sodium Adsorption Ratio (SAR)
The SAR was calculated by the following formula:

$$\text{SAR (mmolL}^{-1})^{1/2} = \text{Na}^+ / [(\text{Ca}^{2+} + \text{Mg}^{2+}) / 2]^{1/2},$$

Here concentration of all ions is expressed in mmol$_c$ L$^{-1}$ (Method 20b of U.S. Salinity Lab. Staff, 1954).
8.3.6. Statistical analysis
The data regarding plant biomass and Cd and Zn concentrations in straw, grains and soil in response to Si and Zn application were statistically analyzed by two-way ANOVA (Steel et al., 1996). The statistical significance was compared at 0.05 probability using Statistix Version 8.1 software package.

8.4. Results
8.4.1. Grain yield
The contamination of saline and/or sodic soil with Cd (10 mg kg\(^{-1}\) soil) did not cause any adverse effect on grain yield of all of the cultivars, however, it increased significantly (p \(\leq\) 0.05) with the application of Zn (Zn) to Cd contaminated (10 mg kg\(^{-1}\)) Zn deficient salt-affected soils (Fig. 8.1a and b). The Zn induced improvement in grain yield was higher in HSCd cultivars compared to LSCd cultivars on both the soils. Zinc application increased grain yield (over CCd treatment) by 13.4 and 21.4 % on saline soils (Fig. 8.1a) while 22.3 and 21.9 % on sodic soil (Fig. 8.1b) for LSCd cultivars Iqbal-2000 and Lasani-2008, respectively. In case of HSCd cultivars, Inqlab-91 and Sehar-2006, improvement in grain yield was 31.1 and 24.3 % on saline soil (Fig. 8.1a) while 27.8 and 29.1 % on sodic soil (Fig. 8.1b), respectively.

Similarly, application of Si (Si) to significantly increased grain yield of wheat cultivars subjected to combined stress of salts and Cd stressed; however, the improvement was higher in Lasani-2008 and Inqlab-91 compared to rest of the cultivars on both the soils. The improvement in grain yield with Si application was 19.2, 26.6, 32.2 and 15.9 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively on saline soils (Fig. 8.1a). On Sodic soil, Si enhanced grain yield was 12.8, 30.1, 26.9 and 21.9 for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively (Fig. 8.1b). Except cultivar Iqbal-2000 on sodic soils, wheat cultivars showed significantly higher increase in grain yield with the combined application of Zn and Si than the alone application of either of these nutrients. The increase in grain yield of Iqbal-2000 with Zn+Si treatment on sodic soils was statistically similar to that with Zn and/or Si alone application. The increase in grain yield under Zn+Si treatment remained higher for Lasani-2008 and Inqlab-91 on both the soils. The improvement in grain yield with Zn+Si application was 40.6, 50.8, 58.4 and 38.3 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively on saline soils (Fig. 8.1a). On Sodic soil (Fig. 8.1b), Si enhanced grain yield
was 26.7, 42.9, 46.9 and 38.9 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively. It depicts that enhancement in grain yield with Zn+Si treatment was higher than their additive effect from Zn and Si treatments for HSCd cultivars Inqlab-91 and Sehar-2006 whereas reverse was true for LSCd cultivars on saline soils. On sodic soil, Zn+Si enhanced grain yield was higher than their additive effect from Zn and Si treatments in all the cultivars.

**8.4.2. Straw yield**

The contamination of saline and/or sodic soils with Cd (10 mg kg⁻¹ soil) did not have any adverse effect on straw yield of all the cultivars under study (Fig. 8.1c and d). However, the straw yield of wheat cultivars increased significantly (p ⩽ 0.05) with the application of Zn (Zn) to Cd contaminated (10 mg kg⁻¹) Zn deficient salt affected soils (Fig. 8.1c and d). The Zn induced improvement in straw yield was higher in HSCd cultivars compared to LSCd cultivars on both the soils. Zinc application increased straw yield by 7.9 and 6.4 % on saline soils (Fig. 8.1c) while 16.7 and 7.1 % on sodic soil (Fig. 8.1d) for LSCd cultivars Iqbal-2000 and Lasani-2008, respectively. In case of HSCd cultivars Inqlab-91 and Sehar-2006, improvement in straw yield was 13.6 and 10.6 % on saline (Fig. 8.1c) while 23.3 and 24.2 % on sodic-soil (Fig. 8.1d), respectively. Similarly to Zn, application of Si (Si) significantly increased straw yield of salt and Cd stressed wheat cultivars (CCd treatment); however, the improvement was higher in Lasani-2008 and Inqlab-91 compared to rest of cultivars on both of soils. The improvement in straw yield with Si application was 19.2, 26.6, 32.2 and 15.9 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively on saline soils (Fig. 8.1c).

On Sodic soil, Si enhanced straw yield was 12.8, 30.1, 26.9 and 21.9 for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively (Fig. 8.1d). The enhancement in straw yield with combined application of Zn and Si was higher than alone application of either of these nutrients. Increase in straw yield on Zn+Si treatment remained higher for Lasani-2008 and Inqlab-91 on both the soils. The improvement in straw yield with Zn+Si application was 40.6, 50.8, 58.4 and 38.3 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively on saline soils. On Sodic soil, Si enhanced straw yield was 26.7, 42.9, 46.9 and 38.9 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively. The enhancement in straw yield with Zn+Si treatment was not considerably higher and/or lower than additive effects from their sole application.
8.4.3. Cadmium concentration in wheat grains

The Cd concentration in wheat grains (GCd) from both the soils was in accordance with the results of initial studies; that is, LSCd and HSCd cultivars stood low and high in GCd, respectively (Fig. 8.2a and b). However, the difference in GCd between LSCd and HSCd cultivars was narrow compared that on non-saline soil or hydroponics. The application of Zn (Zn) to Cd contaminated (10 mg kg^{-1}) Zn deficient salt-affected soils decreased GCd in wheat cultivars with even higher effect on HSCd cultivars. In Zn treated plants, GCd decreased by 8.5 and 11.6 % on saline soil (Fig. 8.2a) while 12.6 and 14.3 % on sodic soil (Fig. 8.2b) for LSCd cultivars Iqbal-2000 and Lasani-2008, respectively. In case of HSCd cultivars Inqlab-91 and Sehar-2006, the decrease in GCd was 14.8 and 16.5 % on saline soil (Fig. 8.2a) while 17.9 and 20.4 % on sodic soil (Fig. 8.2b), respectively.

Similarly, Si application (Si) to wheat cultivars significantly decreased GCd in salts stressed Cd treated plants but decrease was higher in Lasani-2008 and Inqlab-91 on both the soils. The decrease in GCd with Si application was 11.1, 20.6, 23.9 and 19.3 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively on saline soils (Fig. 8.2a). On sodic soil, Si depressed GCd by 8.8, 18.3, 14.9 and 11.1 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively (Fig. 8.2b). Although Zn+Si treatment decreased GCd further to their sole application, but the effect was non-significant in Sehar-2006 and Iqbal-2000 compared to both Zn and/ or Si treatments on saline soil.

The decrease was higher in Lasani-2008 and Inqlab-91 on both the soils and it was statistically similar with Si treatment in Inqlab-91 on saline soil. The depression in GCd with Zn+Si application was 19.6, 32.2, 38.7 and 35.6 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively on saline soils (Fig. 8.2a). On Sodic soil (Fig. 8.2b), Si decreased GCd by 22.0, 39.3, 28.9 and 24.4 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively.

8.4.4. Cadmium concentration in straw

Likewise GCd, lower and higher concentration of Cd was observed in straw (SCd) of LSCd and HSCd cultivars, respectively and difference in SCd between these two groups of cultivars was narrow compared that on normal soil or in hydroponics as observed in earlier studies (Fig. 8.2c and d).
Fig. 8.1. Effect of Zn and Si application on yield of grain (a and b on saline and sodic soil, respectively) and straw (c and d on saline and sodic soil, respectively) of wheat cultivars grown in soil receiving Cd at 10 mg kg\(^{-1}\).

Bars following different letters are significantly different from each other at \(\alpha = 0.05\) (LSD treatment × cultivars: Saline soil a = 1.06; b = 0.92; c = 1.85; d = 2.48)
Fig. 8.2. Effect of Zn and Si application on Cd concentration in grains (a and b on saline and sodic soil, respectively) and straw (c and d on saline and sodic soil, respectively) of wheat cultivars grown in soil receiving Cd at 10 mg kg\(^{-1}\).

Bars following different letters are significantly different from each other at \(\alpha = 0.05\) (LSD treatment \(\times\) cultivars: a = 0.154; b = 0.210; c = 0.364; d = 0.617)
On saline soil, application of Zn decreased SCd in all the cultivars; however, the decrease (%) was significant only in HSCd cultivars Inqlab-91 (12.4) and Sehar-2006 (13.3) being higher than Iqbal-2000 (6.4) and Lasani-2008 (9.1) (Fig. 8.2c). Similar response to Zn application was observed on sodic soil except the significant effect on Lasani-2008 in addition to HSCd cultivars. The Zn induced decrease in SCd on sodic soil was 10.4, 11.9, 16.0 and 18.4 for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively (Fig. 8.2d).

Silicon application (Si) to wheat cultivars also significantly decreased SCd in salts stressed Cd treated plants (except Iqbal-2000) with even higher decrease in Lasani-2008 and Inqlab-91 on both the soils. The decrease in SCd with Si application was 10.4, 21.3, 13.7 and 7.4 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively on saline soil (Fig. 8.2c). On sodic soil, Si decreased SCd was 15.2, 23.0, 18.5 and 12.8 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively (Fig. 8.2d). Except Iqbal-2000 on saline soil, combined application of Zn and Si decreased SCd significantly compared to CCd treatment and decrease was higher in Lasani-2008 and Inqlab-91 on both the soils. On saline soil, Zn+Si treatment decreased SCd further to their sole application but the effect was non-significant than sole applied Si in Lasani-2008 and Zn in Sehar-2006 (Fig. 8.2c). On the other hand, Zn+Si induced decrease was significantly higher than Zn alone in all the cultivars but it was higher than Si only in Iqbal-2000 and Lasani-2008 on sodic soil (Fig. 8.2d). The depression in SCd with Zn+Si application was 16.7, 30.4, 26.1 and 20.7 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively on saline soil. On sodic soil, Si decreased SCd by 24.7, 33.4, 34.1 and 28.9 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively.

8.4.5. Zinc concentration in grains

The Zn concentration in grains (GZn) of HSCd cultivars was significantly higher (p < 0.05) than LSCd cultivars in all the treatments on both soils (Fig. 8.3a and b). Moreover, contamination of salt-affected soils (C treatment) with Cd (CCd) decreased GZn in all the cultivars; however, the effect was significant effect only in HSCd cultivars on saline soil (Fig. 8.3a and b). The decrease in GZn with Cd addition to soil was 7.63, 8.7, 8.8 and 11.6 for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively on saline soil (Fig.
8.3a). On sodic soil (Fig. 8.3b), with CCd treatment GZn decreased by 11.0, 10.6, 3.7 and 9.9 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively compared to control. As expected, application of Zn to Cd contaminated saline and sodic soils caused a significant increase in GZn with even more pronounced effect in HSCd cultivars. Zinc application improved GZn by 25.2 and 29.7 % on saline soils (Fig. 8.3a) while 35.2 and 28.4 % on sodic soil (Fig. 8.3b) in LSCd cultivars Iqbal-2000 and Lasani-2008, respectively. In case of HSCd cultivars Inqlab-91 and Sehar-2006, improvement in GZn was 36.8 and 40.5 % on saline (Fig. 8.3a) while 41.0 and 43.9 % on sodic soil (Fig. 8.3b), respectively.

Silicon application (Si) to wheat cultivars also significantly improved GZn in salts stressed Cd treated plants (except Iqbal-2000) with even higher decrease in Lasani-2008 and Inqlab-91 on both the soils. The improvement in GZn in Si treated plants was 14.4, 17.7, 15.3 and 12.3 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively on saline soils (Fig. 8.3a). On sodic soil, Si enhanced GZn by 12.1, 17.2, 19.5 and 12.2 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively (Fig. 8.3b). Except Iqbal-2000 on sodic soil and Sehar-2006 on both the soils, combined application of Zn and Si (Zn+Si) improved GZn significantly compared to sole applied Zn or Si and improvement was higher in Lasani-2008 and Inqlab-91 on both the soils. The cultivar Iqbal-2000 showed similar GZn between Zn and Zn+Si treatment on sodic soil (Fig. 8.3b) while the same was observed for Sehr-2006 on both the soils (Fig. 8.3a and b). The improvement in GZn with Zn+Si application was 41.1, 46.9, 72.1 and 67.1 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively on saline soil (Fig. 8.3a). On Sodic soil (Fig. 8.3b), Zn+Si enhanced GZn by 22.5, 25.5, 39.4 and 33.4 % for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively.

8.4.6. Zinc concentration in straw

Contrary to GZn, concentration of Zn in straw (SZn) of LSCd cultivars was significantly higher (p < 0.05) than HSCd cultivars in all the treatments on both soils (Fig. 8.3c and d). Although contamination of salt-affected soils (C treatment) with Cd (CCd) decreased GZn in all the cultivars; however, the effect was significant effect only in Inqlab-91 on saline
The decrease in SZn with Cd addition to soil was 4.9, 7.71, 11.9 and 0.1% for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively on saline soil (Fig. 8.3c). On sodic soil, in CCd treatment SZn decreased by 7.0, 8.8, 7.6 and 6.2% for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively compared to control (Fig. 8.3c). Zinc application to Cd contaminated saline and sodic soils caused a significant increase in SZn but contrary to GZn increase was higher in LSCd cultivars. Zinc application improved SZn by 24.7 and 37.2% on saline soils (Fig. 8.3c) while 35.5 and 31.2% on sodic soil (Fig. 8.3d) in LSCd cultivars Iqbal-2000 and Lasani-2008, respectively. In case of HSCd cultivars Inqlab-91 and Sehar-2006, improvement in GZn was 33.4 and 28.6% on saline (Fig. 8.3c) while 38.2 and 43.2% on sodic soil (Fig. 8.3d), respectively.

Silicon addition to soil (Si) also improved SZn of wheat cultivars although the increase was non-significant in Iqbal-2000 on sodic and while in Sehar-2000 on both the soils. Moreover, the improvement in SZn was higher decrease in Lasani-2008 and Inqlab-91 on both the soils. The improvement in SZn in Si treated plants was 14.4, 17.7, 15.3 and 12.3% for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively on saline soils (Fig. 8.3c). On sodic soil, Si enhanced GZn by 7.9, 15.2, 25.9 and 12.3% for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively (Fig. 8.3d). Although combined application of Zn and Si (Zn+Si) improved SZn of wheat cultivars further to alone applied Zn but increase was significant only in Lasani-2008 on saline soil. The improvement in GZn with Zn+Si application was 31.1, 59.9, 38.9 and 26.7% for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively on saline soils. On sodic soil, Zn+Si enhanced GZn was 43.9, 43.4, 50.4 and 53.9% for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively.

8.4.7. AB-DTPA extractable Cd and Zn concentration in post-harvest soil

The maximum AB-DTPA extractable soil Cd was observed in control pots receiving no Zn and/ or Si (Fig. 8.7). Application of Zn tended to decrease AB-DTPA extractable Cd in soil but effect was non-significant for both the soils sown with either of the cultivars. Application of Si significantly decreased AB-DTPA extractable Cd in soil (conversely immobilized Cd in soils) on both the soils. Moreover, followed by Si induced immobilization of Cd in soil, the AB-DTPA extractable in soil was statistically similar
among all the cultivars. The decrease in AB-DTPA extractable Cd ranging between 21.2 and 26.5 on saline (Fig. 8.7a) while 22.0 and 28.7 on sodic soils for Lasani-2008 and Inqlab-91, respectively (Fig. 8.7b). The combined application of Zn and Si (Zn+Si) did not further immobilize Cd in soil over Si application alone in soils sown with either of the cultivar. The decrease in AB-DTAP extractable Cd in Zn+Si treated soil sown corresponding to Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006 sown soil was 23.7, 27.8, 22.7 and 22.6 % on saline while 28.4, 31.3, 31.4 and 32.2 % on sodic soil, respectively.

Application of Zn significantly increased AB-DTPA Zn in soils sown with either of the cultivars. On saline soil, the resultant AB-DTPA extractable Cd followed by addition of Zn to soil was 1.91, 1.63, 2.06 and 1.90 mg kg\(^{-1}\) for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively corresponding to 100 - 134 % increase over control. While on sodic soil, AB-DTPA extractable Zn in Zn-treated pots was 4.15, 3.68, 3.52 and 4.11 mg kg\(^{-1}\) for Iqbal-2000, Lasani-2008, Inqlab-91 and Sehar-2006, respectively corresponding to 477 - 681 % increase over control. Application of Si alone (Si) or in combination with Zn (Zn+Si) did not influenced AB-DTPA extractable Zn in soil sown with either of the cultivars. Thus, AB-DTPA extractable Zn was statistically similar between Si treated and control as well as Zn and Zn+Si treatments.

### 8.5. Discussion

#### 8.5.1 Grain and straw yield

Cadmium is toxic heavy metal and it can effect plant growth through a number of mechanisms including nutritional and water imbalance (Gong et al., 2005) and production of reactive oxygen species (Qadir et al., 2004; Dong et al., 2006). However, contaminating salt-affected soils with Cd (10 mg kg\(^{-1}\)) did not influence straw and grain yield of the wheat cultivars as it was observed on normal soils in study 5 and 6. Similarly, Kang et al. (2007) reported no further decrease in shoot length of both salt tolerant and sensitive salt-stressed soybean cultivars with the addition of Cd to soil. Such Cd accumulating ability without showing any visual toxicity is exhibited by many food crops. Garrett et al. (1998) reported that soil Cd up to 3.8 mg kg\(^{-1}\) did not caused any phyto-toxicity in wheat wherein wheat grain accumulated up to 0.50 mg Cd kg\(^{-1}\).
Fig. 8.3. Effect of Zn and Si application on Zn concentration in grains (a and b on saline and sodic soil, respectively) and straw (c and d on saline and sodic soil, respectively) of wheat cultivars grown in soil receiving Cd at 10 mg kg\(^{-1}\). Bars following different letters are significantly different from each other at \(\alpha = 0.05\) (LSD treatment × cultivars: a = 3.75; b = 2.66; c = 10.66; d = 7.96)
Fig. 8.4. Effect of Zn and Si application on concentration of AB-DTPA extractable Cd (a and b on saline and sodic soil, respectively) and Zn (c and d on saline and sodic soil, respectively) in post-harvest soil.
Bars following different letters are significantly different from each other at $\alpha = 0.05$ (LSD treatment × cultivars: a = 0.389; b = 0.597; c = 0.307; d = 0.426)
Non-significant correlation between Cd tolerance and concentration in wheat inbred lines (Ci et al., 2010) suggested that these traits are independent of each other. Stevens and McLaughlin (2006) reported that plants could concentrate considerable Cd in harvestable parts that is not toxic for them but could be harmful for humans as observed in this study wherein GCd in wheat cultivars was much higher than safe consumption level for humans (0.1 mg Cd kg\(^{-1}\) of grain) advised by WHO (1989). Application of Zn to Zn-deficient, salt-affected soils significantly increased grain and straw biomass of wheat cultivars and even more so in HSCd cultivars (Fig. 8.1 and 8.2).

Zinc deficiency is reported to limit the grain yield of crops in many parts of the world (Cakmak, 2008) and it is an important nutritional problem in a large number of countries. Likewise, Kutman et al. (2011) reported 35% improvement in wheat grain yield grown on soil severely deficient in DTPA extractable Zn (0.1 mg kg\(^{-1}\)) supplied with Zn at 5 mg kg\(^{-1}\). The higher increase in yield of HSCd cultivars could be related to efficient Zn uptake and translocation by these cultivars. It has already been screened out by Maqsood et al. (2009) that Inqlab-91 and Sehar-2006 were Zn-efficient compared to Iqbal-2000 which was Zn-inefficient among the tested 12 wheat genotypes. It employed that Zn induced improvement in biomass was related to Zn use efficiency of plant species. It was also observed that positive effect of Zn on grain yield was more pronounced than that on straw dry matter in present study (Fig. 8.2). It is in consistent with the earlier findings on Zn deficiency confirming that wheat grain yield is more sensitive to Zn deficiency than that of straw (Yilmaz et al., 1997; Kutman et al., 2010). The higher sensitivity of grain yield to Zn deficiency is due to Zn deficiency-induced impairment in the development of reproductive organs (Cakmak and Engels 1999).

In previous studies on hydroponics and normal soil, Si did not or could only enhance grain biomass of wheat cultivars. Contrarily, on salt-affected soils, soil applied Si enhanced both straw and grain yield and improvement was much more pronounced than the hydroponic or normal soil culture (Fig. 8.2-3). It is confirmatory to the established fact that although some beneficial effect of Si on plant growth are notable on normal growth conditions, but the benefits are much more evident on stress conditions (Parveen and Ashraf, 2010; Tahir et al., 2006; Al-Aghabary et al., 2004). The improvement in straw and grain biomass of Si treated plants on Zn-deficient soil could be due to Si-induced improvement in Zn uptake. Likewise these results, it was reported by da Cunha et al. (2009) that Si application up to 150 mg kg\(^{-1}\) resulted in
higher Zn accumulation by maize plants. Secondly, the effect of Si on growth of Cd-unstressed plant might be due to influences on the absorption and transport of several mineral elements that help perform many functions in plants (Epstein and Bloom, 2005, Ma and Takahashi, 2002).

Since no decline in root, shoot and grain biomass or toxicity symptoms on leaf of all the wheat cultivars could be seen on the employed Cd stress, the increase in straw and grain yield of wheat could not be regarded as the alleviation of Cd toxicity instead beneficial effect of Zn and/or Si nutrition. Moreover, the improvement in straw and grain yield was higher in Lasani-2008 and Inqlab-91 depicting that it was not related to Cd concentrating abilities of the cultivar. Instead, higher sensitivity to excess salts by Lasani-2008 and Inqlab-91 compared to the other cultivars could explain this contrasting response behavior. Kanwal et al. (2011) reported Sehar-2006 to be tolerant whereas Lasani-2008 and Inqlab-91 as sensitive wheat cultivars to NaCl stress as assessed from their response to chlorophyll and gas exchange attributes. In another study, Iqbal-2000 was categorized among tolerant wheat cultivars producing higher grain yield than the sensitive cultivars including Lasani-2008 on a highly saline sodic soil (EC 7.5 dS m\(^{-1}\) and ESP 16\%). Thus, higher improvement in biomass of these cultivars stood as Si induced reverse of salt-toxicity.

8.5.2 Cadmium concentration in grains and straw

Uptake of Cd have been shown to depend on Zn contents of soil and on low Zn contents plants may accumulate higher levels of Cd even from marginal contaminated soil to exceed the regulatory levels. In present study, application of Zn to Zn-deficient plants lowered Cd concentration in grains and straw of wheat cultivars (Fig. 8.2-3). This result agrees with the finding of Oliver et al. (1994) who reported 50% decrease in grain Cd concentration with Zn application at 5 kg ha\(^{-1}\) to wheat grown on marginally or severely Zn-deficient soils. Moreover, Zn-induced decrease was much more pronounced for HSCd cultivars than LSCd cultivars that could be attributed to the higher uptake and translocation of Zn (Maqsood et al. 2009) by the former cultivars which efficiently competed with Cd uptake and translocation. These findings are in line with the results of Hart et al. (2002) who attributed the competitive interaction during uptake and transport between Cd and Zn to the existence of a common transport system on the plasma membranes. Also, Zn was shown to interfere with phloem-mediated Cd transport.
in durum wheat inhibiting re-mobilization of Cd from leaves to grain around senescence (Cakmak et al., 2000). Since Zn depressed Cd concentration in straw as well as grain (Fig. 8.7 and 8.8), it confirmed that Zn-induced decrease in SCd and GCd could be both due to restricted uptake from soil and translocation to shoots.

The results confirmed the findings of our earlier study (Study 5) that Si application (150 mg kg⁻¹ soil) significantly decreased SCd as well as GCd concentration in wheat cultivars (Fig. 8.3 and 8.4). The Si induced decrease in Cd uptake could be due to enhanced production of antioxidant that helped maintain integrity of plasma membrane inhibiting indiscriminate Cd uptake (Tian et al., 2011). Moreover, Si is reported to improve Ca status of plant tissue (Hammond et al. 1995) which is not only a key element in maintaining plasma membrane integrity but it also compete with Cd for uptake and translocation (Andreson and Nilsson, 1974). The pronounced effect of Si in depressing SCd and GCd concentration of Lasani-2008 and Inqlab-2000 compared to the other cultivars might be associated with higher sensitivity to salt-stress that was reversed by Si nutrition. Kanwal et al. (2011) reported Sehar-2006 to be tolerant whereas Lasani-2008 and Inqlab-91 as sensitive wheat cultivars to NaCl stress as assessed from their response to chlorophyll and gas exchange attributes. In another study, Iqbal-2000 was categorized among tolerant wheat cultivars producing higher grain yield than the sensitive cultivars including Lasani-2008 on a highly saline sodic soil (EC 7.5 dS m⁻¹ and ESP 16 %). The decrease in plant available soil Cd (Fig 8.7) as well as translocation of Cd from roots to shoots (Fig. 8.9) confirms the involvement of both decreased uptake and translocation in lowering tissue Cd in in Si treated plants. In line to these findings, da Cunha et al., (2008) and Li et al., (2008) reported that immobilization of Cd in soil as Cd-silicate has been reported as one of the mechanisms that reduce Cd uptake.

In saline soil, followed by Zn induced decrease in GCd, the pattern of Cd accumulation remained unchanged among cultivars whereas among Si and Zn+ Si treated plants, GCd in Inqlab-91, a HSCd cultivar, was statistically similar to that of LSCd cultivars (Fig. 8.3a). Moreover, The Zn and Si treated plants showed similar GCd except Inqlab-91 where it was lower in Si treated plants. It employees that application these essential nutrients could decrease Cd concentration in edible parts of high Cd cultivars comparable to that of low Cd accumulating ones.
8.5.3. Zinc concentration in grains and straw

Contamination of salt-affected soils with Cd tended to decrease Zn concentration in all the wheat cultivars (Fig. 8.3) which might be due to competitive interaction between these two elements for uptake from soil and translocation to shoot and grains. Application of Zn significantly increased Zn concentration in grains (GZn) as well as straw (SZn) of all the wheat cultivars with even much more pronounced effect in HSCd cultivars (Fig. 8.3) on both the soils. Higher concentration of Zn in grains of HSCd cultivars Inqlab-91 and Sehar-2006 than Iqbal-2000 confirmed the finding of Maqsood et al. (2009) who reported the former cultivars to be efficient in uptake and shoot to grain translocation of Zn. The HSCd cultivars with higher GZn concentration retained less Zn in straw compared to LSCd cultivars. The wheat cultivars efficient in shoot to grain Zn translocation showed higher decrease in SCd and GCd which suggest that Zn-efficient wheat cultivars more efficiently excluded Cd from grains and straw. Moreover, no toxic effect of increasing tissue Zn on grain and straw yields was observed. The GZn ranged between 14.7 to 49.9 mg kg\(^{-1}\) for LSCd cultivars while 21.9 to 67.1 for HSCd cultivars. Although Zn concentration required to obtain 90 to 97.5 % relative grain yield is in the range of 25–40 μg Zn g\(^{-1}\) of wheat grains (Cakmak, 2008) but concentration between 50–100 mg Zn kg\(^{-1}\) DM could be achieved without any significant yield loss (Marschner, 1995). Therefore, little higher Zn application than that required to obtain maximum relative grain yield would help plants further to exclude Cd from edible parts.

In general, Si supplementation to Zn deficient plants significantly increased Zn concentration both in straw and grains. The similar result was reported by da Cunha et al. (2009) that Si application up to 150 mg kg\(^{-1}\) resulted in higher Zn accumulation by maize plants even on high Zn supply. Similarly, improved grain Zn concentration in Si treated wheat plants was observed by Rizwan et al. (2012). The Si-induced increase in GZn and SZn could be the indirect consequence of decrease in Cd translocation that compete Zn for transport within plants instead of affecting available Zn in soil (Fig. 8.4a and b). Contrasting results reporting no significant effect (Masarovic et al., 2012) or decrease in Zn concentration of Si treated plants (Gu et al., 2012) also exist. But, it would be worth mentioning that these studies attempted Si-induced alleviation of Zn toxicity in Zn-stressed plants unlike deficient or optimum Zn supplied wheat plants as in this study.
8.5.4 AB-DTPA extractable Zn and Cd in post-harvest soil

Being similar in geochemistry to Cd (Mengal and Kirkby, 2001), Zn was expected to affect Cd adsorption and/or desorption in Zn deficient soil but no such effect was observed for AB-DTPA extractable Cd in soil (Fig. 8.4a and b). On the other hand, confirmatory to the results of 4th study, AB-DTPA extractable Cd significantly decreased in post-wheat harvest soil. It was reported by Dietzel (2000) and Sommer et al. (2006) that silicate addition to soil promotes polymerization of silicate composts which is potential ligands to complex heavy-metal in soil. Our results confirmed the findings of da Cunha et al. (2008) who reported that following application of CaSiO3 up to 200 mg kg⁻¹, proportion of exchangeable soil Cd decreased in soil (pH 4.90). Similarly, it has been found that plant available Cd lowered to 18.8% by Si fertilizer (Li et al., 2008) and silicate slag was much effective in decreasing the exchangeable soil Cd (Cheng and Hseu, 2002). Combined application of Zn and Si (Zn+Si) did not further immobilized Cd in soil compared to Si application alone. Thus, the present study suggested that not Zn but Si could decrease plant uptake of Cd by lowering its plant available concentration in soil. It could be suspected from higher Zn in grain and straw of wheat cultivars that significant increase in AB-DTPA extractable Zn would have been occurred following Si addition to soil. However, no such increase in AB-DTPA extractable Zn was observed in Si and/or Zn+Si treated soil (Fig. 8.4c and d) suggesting its increased translocation from roots to shoots and then grain as a consequence of lowered Cd translocation.

8.5. Conclusions

Application of both Zn and Si improved grain and straw biomass of wheat cultivars in both of the saline and sodic soils. Addition of both Zn and Si Zn suppressed Cd concentration in grains and straw of wheat by lowering uptake and translocation within plants. However, Si did not affect Zn concentration in either of the wheat cultivars on both the soils. Zinc decreased grain Cd concentration by lowering its translocation from shoot to grain and this effect was more pronounced on cultivars those are efficient in Zn translocation from shoots to grains. Moreover, the degree of improvement in yield by Zn was higher than the normal soil. The Si induced decrease in GCd was owing to its ability to minimize plant available Cd in soil and lowering its translocation from shoots to grain. Moreover, Si induced improvement in dry matter yield and suppression in Cd concentration was higher in salt-sensitive wheat cultivars.
SUMMARY

Among the most toxic heavy metals, cadmium (Cd) is of special concern because of its high mobility in soil-plant system and toxicity to living organisms at very low concentration range. The most likely origins of Cd in soils are untreated sewage water irrigation, application of contaminated phosphate-fertilizers and atmospheric deposition. In plants, Cd inhibits uptake and translocation of nutrients such as nitrogen, zinc and iron, interacts with water balance and is involved in the production of reactive oxygen species (ROS).

Variation in mineral uptake by plant has been recognized for many years and there are now several examples exploiting these genetic differences to improve food safety. The Cd contamination has caused concern sufficient to exploit genetic variability in plants to produce low Cd food from Cd contaminated soils those are wide spread. Low Cd in cereal grains could also be achieved through optimizing plant nutrients in soil those may limit Cd absorption from soil and translocation within the plants. In this regard, Si and Zn have gained much attention in minimizing Cd in plants. Silicon is beneficial plant nutrient and inspite of the poor understanding of mechanisms involved, it is known that Si could decrease metal accumulation. On the other hand, Zn is chemically similar to Cd and might act as competing cation for Cd absorption from soil by roots as well as have antagonistic interaction with Cd within the transport system of plants.

For this PhD research project, cultivating low-Cd plant species and optimum application of zinc (Zn) and silicon (Si) nutrients was hypothesized as a possible solution to avoid Cd accumulation in wheat cultivars. For this, 15 cultivars of wheat approved for general cultivation in Pakistan were grown with four levels of Cd stress (0, 15, 30 and 30 µM) for two weeks to study differences in Cd concentration in their shoots and roots. Although root and shoot dry matter was negatively affected in most of the cultivars even at the lowest rate of Cd application but some cultivars did not show any toxic effect. Shoot and root Cd concentration showed significant differences among cultivars. Cultivar Lasani-2008 and Iqbal-2000 had the lowest while Sehar-2006 and Inqlab-91 were containing the highest concentration of Cd in their shoots. Both absorption by roots and translocation to shoots appears to play role in regulating differential Cd concentration in shoots of wheat cultivars. There was no relationship between relative dry matter and Cd concentration in roots and shoots suggesting that cultivars
with low tissue Cd but higher tolerance could be selected for utilizing as parent material for the development of low Cd cultivars with all other desired traits.

Plant species differing in shoot Cd accumulation could differ in root zone acidification potential and production of antioxidant enzymes. Therefore, based upon screening study, selected low and high shoot Cd cultivars identified in screening study were evaluated for their ability to modify the root zone pH and effect on the production of antioxidant enzymes under Cd stress of 15 µM for two weeks (Chapter 4). The growth response to Cd stress and Cd concentration both in root and shoot of wheat cultivars confirmed the results of initial screening study i.e. the Cd concentration was low in shoots of cultivars Iqbal-2000 and Lasani-2008 while high in Inqlab-91 and Sehar-2006. Wheat cultivars tended to induce a higher mean daily decrease in pH of Cd contaminated nutrient solution (0.199 units) than with no Cd (0.177 units). The decrease in pH of uncontaminated nutrient solution was lower (0.141 units) for Lasani-2008 compared to rest of the cultivars. Under Cd stress, activities of antioxidant enzymes catalase (CAT) and ascorbate peroxidase (APX) decreased, superoxide dismutase (SOD) remained unaffected and whereas that of guaiacol peroxidase (GPX) improved significantly. The decrease in activity of CAT and APX and improvement in GPX was higher in low-shoot-Cd. Lipid peroxidation (malondialdehyde content) significantly increased in all the cultivars under Cd stress and it was higher in high-shoot-Cd cultivars. It is concluded that decrease in pH was not related to shoot Cd concentration of the cultivars. However, low Cd concentration in low-shoot-Cd was related to sustained or higher activity of antioxidant enzymes which was not observed for high-shoot-Cd cultivars.

It was observed in above study that low concentration shoot was related to higher antioxidant activity. On the other hand, Si has been reported to increase antioxidant production in plants. Therefore, in other study, effect of Si application on growth, antioxidant activity and Cd concentration in two each of low- and high-shoot Cd cultivars was evaluated (Chapter 5). In low-shoot-Cd cultivars, Si enhanced dry matter was either non-significant or increase was lower compared to high-shoot-Cd cultivars. Silicon did not improve the shoot and root dry matter of Silicon treated sensitive high-shoot-Cd cultivars showed higher improvement in chlorophyll contents and photosynthesis while decrease in stomatal conductance and transpiration rate. Thus, Si improved chlorophyll contents and photosynthesis could be regarded as the alleviation of Cd toxicity in Cd stressed plants. Improvement or
reduction in gas exchange attributes of Si treated plants was limited to 2 or 4 mM Si application. The Si treatment enhanced the activities of CAT, APX, GPX and SOD antioxidant enzymes and depressed MDA contents in Cd stressed wheat cultivars. Generally, the increase (Antioxidants) or decrease (MDA) was higher in low-shoot-Cd cultivars. Application of Si decreased Cd concentration in roots of only low-shoot-Cd cultivars while that in shoots of both the low- and –high-Cd cultivars. The decrease in SCd concentration was due to both the decrease uptake and less translocation from roots to shoots. Higher retention of Cd in roots of both low- and –high shoot Cd cultivars while decrease in excessive transpiration only in high-shoot-Cd cultivars with Si application proved to be the mechanisms suppressing Cd translocation to shoots.

In another study, Si application on alkaline calcareous soil was optimized for immobilization of Cd and translocation within plant (Chapter 6). Based upon initial screening study, selected low–shoot–Cd cultivars Iqbal-2000 and Lasani-2008 and high–shoot–Cd cultivars Inqlab-91 and Sehar-2006 were grown in artificially contaminated (10 mg kg\(^{-1}\)) soil with three levels of Si (50, 100 and 150 mg kg\(^{-1}\) soil) as calcium silicate (CaSiO\(_3\)). None of the wheat cultivars showed any toxicity symptom or growth retardation to the applied Cd stress. Silicon supply to Cd treated plant improved only grain yield at the highest dose of applied Si (Si\(_{150}\)). Significant decrease in plant available soil Cd was observed with Si\(_{150}\) application without any change in soil pH. Decrease in shoot Cd concentration was high-shoot-Cd cultivars whereas Cd concentration grain showed higher decrease in in LSCd cultivars. As a conclusion, Si decreased Cd concentration in wheat cultivars by both decrease in plant available soil Cd and its translocation from roots to shoots. Moreover, application of Si at 150 mg kg\(^{-1}\) proved to be the optimum level of Si that significantly lowered Cd concentration in wheat grains.

The role of Zn in minimizing Cd concentration in edible parts of plants is also well documented. However, owing to chemical similarities between Cd and Zn, Si is also expected to affect Zn uptake and translocation in plants and studies on this aspect are lacking. Therefore, sole and combined application of Zn (10 mg kg\(^{-1}\)) and Si (150 mg kg\(^{-1}\)) to Cd contaminated normal (Chapter 7) and salt-affected (Chapter 8) soils was evaluated for their effect on Cd and Zn concentration grain of previously selected of low– and – high shoot Cd cultivars (Iqbal-2000 and Lasani-2008) and high shoot Cd (HSCd; Inqlab-91 and Sehar-2006) cultivars grown on artificially Cd contaminated soil (10 mg kg\(^{-1}\)) soil. On normal soil, application of Zn
significantly improved both grain and straw yield while Si enhanced only grain yield. Zinc induced increase in grain and straw yield was more pronounced in high-shoot-Cd cultivars owing to their efficient Zn uptake and translocation abilities. Applying Si in combination with Zn did not showed significant improvement in grain yield and decrease in Cd concentration of grain over Zn and/ Si application alone. Sole application of Zn as well as Si decreased Cd concentration in grain while for shoot Cd only Si did so. The Zn-induced decrease in Cd concentration in grain was related to decrease in shoot to grain Cd translocation whereas Si decreased Cd by lowering AB-DTPA extractable Cd in soil as well as depressing shoot to grain Cd translocation. Silicon induced improvement in grain yield and decline in Cd concentration was higher for salt-affected than normal soil and also for salt-sensitive compared to –tolerant wheat cultivars.

Overall, project revealed that growing low-Cd cultivars on Cd contaminated soils is better option to obtain Cd free grain food. Moreover, application of Zn and Si can further improve grain food being low in Cd content.
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