WHEAT (*Triticum aestivum* L.) RESPONSE TO EXOGENOUS SELENIUM SUPPLY UNDER DROUGHT STRESS

By

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To

The Controller of Examinations
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We the supervisory committee, certify that the contents and form of thesis submitted by Mr. Fahim Nawaz, Regd. Number 2003-ag-1820, have been found satisfactory and recommend that it be processed for evaluation by the External Examiner(s) for the award of the degree.

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(Fahim Nawaz)

Dedicated to my parents, brothers and sisters

DECLARATION

I hereby declare that the contents of the thesis "Wheat (*Triticum aestivum* L.) response to exogenous selenium supply under drought stress" are the product of my own research and no part has been occupied from any published sources (except the references, standard mathematical or genetic model/ equations/formula/ protocol etc). I further declare that this work has not been submitted for the award of any other diploma/degree. The university may take action if the information provided is found inaccurate at any stage. In case of any default, the scholar will be proceeded against as per HEC plagiarism policy.

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ABSTRACT

Premise of the research-Selenium (Se) has become an element of interest to many biologists because of its physiological and toxicological importance. The identification of effective Se dose and application method is crucial for better understanding of Se role in crop plants under drought stress. The present study, therefore, was planned to evaluate the response of water-stressed wheat (*Triticum aestivum* L.) to exogenous Se supply. The study was carried out at the Department of Crop Physiology, University of Agriculture, Faisalabad-Pakistan and Stress Physiology Laboratory, Salinity and Environmental Division, Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad-Pakistan. **Methodology**-A series of laboratory, wire / greenhouse, lysimeter and field experiments were conducted for this study. In laboratory experiments, fifteen local wheat genotypes were screened out for their response to PEG-6000 induced water stress of -0.5 MPa at germination and seedling stage. Wire / greenhouse experiments were conducted using one drought tolerant (Kohistan-97) and one sensitive (Pasban-90) genotype, selected from laboratory experiments, to determine appropriate rates for three methods viz. seed priming (75 µM), fertigation (7.35 µM) and foliar spray (7.06 µM) of Se helpful in improving drought tolerance in wheat plants subjected to water stress at seedling stage. The optimum rates determined in screen house experiments of each method of Se application were tested for appropriate method and application time (vegetative or reproductive growth stage) in lysimeter and field experiments. **Pivotal results-**Drought stress significantly reduced growth, water relations, gas exchange and yield attributes of both wheat genotypes. However, exogenous Se supply was observed to be helpful in improving the drought tolerance potential and yield through maintenance of turgor, increased accumulation of osmolytes and enhancement in enzymatic activity of water-stressed wheat plants. The supplemental Se supply significantly improved Se and potassium (K) concentration in shoot and grain, whereas phosphorous (P), magnesium (Mg), zinc (Zn), iron (Fe) and calcium (Ca) contents in shoot were reduced by Se supply. The grain Mg and Fe concentration increased while grain P concentration reduced by exogenous Se supply. Non-significant effect of Se supply was recorded on grain Zn concentration. **Conclusion**- The cultivation of drought tolerant wheat genotypes is essential to obtain economical crop yield under water stress conditions as wheat genotype Kohistan-97 (drought tolerant) was more successful in the maintenance of physiological, biochemical and yield attributes than Pasban-90 (drought sensitive). Selenium application through fertigation @ 7.35 µM and foliar spray @ 7.06 µM at tillering stage was found effective under both normal and water deficit conditions.

LIST OF ABBREVIATIONS

Abbreviation	Description
PI	Promptness Index
EI	Emergence Index
MET	Mean Emergence Time
GSI	Germination Stress Tolerance Index
PHSI	Plant Height Stress Tolerance Index
RLSI	Root Length Stress Tolerance Index
DMSI	Dry Matter Stress Tolerance Index
SFSI	Shoots Fresh Weights Stress Tolerance Index
RFSI	Roots Fresh Weights Stress Tolerance Index
Ψ_w	Water Potential
Ψ_s	Osmotic Potential
Ψ_p	Turgor Potential
RWC	Relative Water Contents
Pn	Net CO_2 Assimilation Rate
E	Transpiration Rate
g_s	Stomatal Conductance
TSS	Total Soluble Sugars
TSP	Total Soluble Proteins
TFA	Total Free Amino acids
CAT	Catalase activity
POX	Peroxidase Activity
APX	Ascorbate Peroxidase Activity
Se	Selenium
Fe	Iron
Zn	Zinc
P	Phosphorous
K	Potassium
Mg	Magnesium
Ca	Calcium
BY	Biological Yield
GY	Grain Yield
HI	Harvest Index

CHAPTER-1 INTRODUCTION

Climate change has emerged as one of the most complex challenges of the 21st century and has become an area of interest in the past few decades. The developing countries like Pakistan have become extremely vulnerable to the impacts of climate change. The scarcity of water is the serious concern for food security of the country and climate change has aggravated the risks of extreme events like droughts in the country (Government of Pakistan, 2011-12). The precipitation in the country is likely to decrease in near future that would adversely affect the agricultural productivity of major crops. The arid and semi-arid regions of the country are at greater risk because these regions are already facing acute shortage of water. Furthermore, an increasing frequency of droughts in days ahead will make natural and cultivated vegetation more vulnerable to severe and acute shortage of water.

Drought stress is one of the major limitations to agricultural productivity around the globe (Waraich *et al.,* 2011). The recurrent droughts and temperature change would alter the biophysical relationships of crops by modifying their growing periods, scheduling of cropping seasons, altering irrigation water requirements, changing soil characteristics, and increasing the risk of drought stress, thus severely limiting the agricultural productivity (Government of Pakistan, 2010-11). Under drought, plants usually face the soil and atmospheric water deficit at different growth stages of their life cycle (Chaves *et al.,* 2002). Unlike other stress factors, drought stress develops slowly and increases with time in intensity and causes damages (Larcher, 2003). It results in impaired germination and seedling growth (Ashraf and Abu-Shakra, 1978), influences plant growth (Jamieson *et al.,* 1995; Tian and Lei 2006; Xu *et al.,* 2007), thus reducing fitness and function of plants. The severity, duration and timing of water stress are extremely important for the better understanding of plant responses to drought stress (Plaut, 2003).

Oxidative stress caused by a variety of active oxygen species formed under drought stress damage many cellular constituents such as, carbohydrates, lipids, nucleic acids and proteins which ultimately reduces plant growth, respiration and photosynthesis. The synthesis of starch is strongly inhibited even under moderate deficiency of water (Chaves, 1991) while the soluble sugar contents remain constant or even increase under stress conditions (Pinheiro *et al.,* 2001). Various enzymatic and non-enzymatic systems are found

in plants to cope with reactive oxygen species (ROS) (Giang and Huang, 2001). The increase in invertase activity due to the accumulation of simple sugars such as glucose and fructose has been observed in the leaves of the plants exposed to drought stress (Pinheiro *et al.,* 2001; Trouverie *et al.,* 2003). The mechanism of osmotic adjustment for drought tolerance by plants has been well documented. It is facilitated by compatible solutes produced at higher levels under limited water conditions (Hasegawa *et al.,* 2000; Zhu, 2001; Shao *et al.,* 2008). The accumulation of these compounds in high amounts act as osmoprotectants of membrane and prevents protein disintegration (Yancey, 1994). The accumulation of total sugars and other compatible solutes such as polyols is a characteristic feature of most plants under stress (Mohammadkhani and Heidari, 2008; Delauney and Verma, 1993).

Selenium (Se) has become an element of interest to many biologists due to its physiological and toxicological importance. It plays beneficial role in plants by enhancing growth of plants (Hartikainen and Xue, 1999), reducing damage caused by UV-induced oxidative stress (Hartikainen and Xue, 1999; Hartikainen *et al.,* 2000; Valkama *et al.,* 2003), enhancing chlorophyll contents under light stress (Seppanen *et al.,* 2003), stimulating the senesce to produce antioxidants, and improving plant tolerance to drought stress by regulating water status (Kuznetsov *et al.,* 2003; Djanaguiraman *et al.,* 2005; Ekelund and Danilov, 2001). It is known to increase superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities thus activating the protective mechanisms against oxidative stress. It is also reported to exert an antioxidant effect directed towards a decreased concentration of intracellular active oxygen species by inducing the biosynthesis of proline and peroxidase production (Kuznetsov *et al.,* 2003).

Wheat (*Triticum aestivum* L.) is a major food grain crop of Pakistan and plays a vital role in agriculture-based economy of the country. It contributes 2.7 percent to Gross Domestic Production (GDP) and 13.1 percent to value added in agriculture. The cultivated area of wheat declined by 3.6 percent in 2011-12 as compared to 2010-11 and it was cultivated on 8.805 million hectares as against 9.132 million hectares in the previous year (2010-11). More than 2 million tons of wheat is produced per annum in the country, however, its average yield of 2.75 tons ha^{-1} is much lower than many agriculturally advanced countries of the world. Wheat production decreased by 6.7 percent to 23.517 million tons in 2011-12 from 25.214 million tons in 2010-11 (Government of Pakistan, 2011-12). Many

factors contribute to low wheat yield in the country, but availability of inadequate water for crop growth or recurrent droughts contribute significantly in this regard. Moreover, irrigation system of Pakistan is not much efficient and a significant portion of water is lost before it reaches to the farmer's fields.

The specific physiological mechanisms that underlie the positive effects of Se in plants have not been clearly elucidated (Xue *et al.,* 2001). It is mostly used for the bio-fortification of crops but has also been reported to play an important role in the adjustment of plants water status under drought stress (Djanaguiraman *et al.,* 2005). Se supply favors to increase the plant biomass by increasing root and antioxidant activity (Yao *et al.,* 2009; Tadina *et al.,* 2007). There are few reports where Se application to plants under drought stress improved drought tolerance and mitigated the adverse effects of drought on total dry weight, leaf area index (LAI), relative growth rate (RGR) and crop growth rate (CGR), yield and water use efficiency in maize (Sajedi *et al.*, 2009) and rapeseed (Valadabadi *et al.,*2010). However, the literature on the role of Se in improving drought tolerance potential of wheat under drought stress is very limited. The present study, therefore, was carried out to assess the role of exogenous Se supply in alleviating the adverse effects of drought in spring wheat (*Triticum aestivum* L.). The specific objectives of the study were:

i. To investigate the effect of water stress on various physiological and biochemical attributes of wheat.
ii. To assess the effect of exogenously applied Se on growth, yield, physiological and biochemical traits of wheat grown under drought conditions.
iii. To determine appropriate rate, method and time of Se application for improving drought tolerance in wheat plants

Improving grain yield under limited water conditions has become a very sound and important applied method in arid regions of the world (Blum, 1998; Chaves and Oliverira, 2004; Zhang *et al.,* 2008). The role of selenium (Se) in plants under water stress conditions has not been well established. Although Se does not take part in various vital metabolic processes in plants, it may help the plants to reduce the damage caused by oxidative stress under various physiological stresses (Hanson *et al.,* 2003; Seppanen *et al.,* 2003). However the role of selenium in plant growth and antioxidative defense has also been questioned (Valkama *et al.,* 2003). The researchers have adopted different strategies to evaluate the role of selenium in plants under drought stress. Some of the relevant work available regarding the role of Se in alleviating adverse effect of drought stress is discussed in this chapter.

2.1. Wheat

The plants have always been a key for survival as they are vital to meet the world's food needs. They provide over 65% of food protein and more than 80 % of food energy globally. The plant products directly contribute about 82% of total world food production harvested from land resources (98%) while animals and marine products together contribute only 17% (FAO, 1988). Historically, over 3000 species of plants have been used to feed humans (Borlaug, 1981). At least 150 different species of these plants have been grown in sufficient quantities to have entered world trade. Among four major food groups of plant species; cereals, legumes, fruits and vegetables, cereal grains constitute the largest and the most important single group of foods and play a dominant role in the total world food supply.

Wheat (one of the leading cereals) has been cultivated since prehistoric times and is among the oldest man's crop. It was originally domesticated (almost 10000 years ago) in a hilly region of Southwestern Asia (Near East) called the Fertile Crescent. The area is bordered on one side by the Tigris-Euphrates basin and on the other side by the mountains of what are now Iran, Turkey, Syria and Jordan. It rapidly became the most important cereal and is still second most produced cereal in the world after maize. The modern wheat, the hexaploid (*Triticum aestivum* L.) is the most cultivated species of wheat in the world.

Wheat is an excellent health building food (Kumar *et al.,* 2011) and is an excellent source of minerals, dietary fiber, protein and B-group vitamins (Shewry, 2007; Simmonds, 1989) although nutritional composition of wheat grains might be influenced by environmental conditions. The adaptability and high yields of wheat are considered key to its dominance in temperate world, but unique properties of wheat flour dough has given it a clear advantage over other temperate crops (Kumar *et al.,* 2011). Wheat dough can be processed into a range of breads, cakes, biscuits and other baked products and processed foods like noodles and pasta. The predominant carotenoids present in wheat is Lutein (Abdel-Aal, 1993) which along with zeaxanthin, is vital for the health of human skin and eyes. The wheat bran and wheat germ are responsible for protection against heart diseases, constipation, disease of the colon called diverticulum, ischemic, appendicitis, diabetes and obesity (Kumar *et al.,* 2011).

In Pakistan, wheat is cultivated on largest acreages in almost every part of the country and is the staple diet of population of the country. Its contribution to the value added in agriculture accounts 14.4 percent and 3.0 percent to gross domestic products. The national average yields in the country are about 2.7 t ha^{-1} as against genetic yield potential of improved semi-dwarf wheat cultivars (6-8 t ha^{-1}). Though progressive farmers of irrigated area are harvesting 6 to 7 tonnes yield per hectare but farmers yield are largely dependent on rainfall in rainfed areas and ranges from 0.5-1.3 t ha^{-1} (PARC, Islamabad). About 60% yield gap exist between a potential yield and a harvested yield of wheat. Among the factors responsible of this yield gap, the shortage of water for crop production especially in rainfed areas of the country is a significant one. The present scenario suggests immediate measures to be taken not only to develop drought tolerant varieties but also to explore alternative means of alleviating adverse effects of drought stress on plants.

2.2. Drought Stress and Plant Growth

Drought stress has emerged as the single most critical threat to world food security in the recent past. It can affect plant growth by several ways. It causes impaired germination leading to poor stand establishment (Kaya *et al.,* 2006), decreases plant water relations, relative water contents and transpiration rate (Siddique *et al.,* 2001), limits the availability of energy for the assimilation of NO_3 /NH^+_4 , PO_3^{-4} and SO_2^- (Grossman and Takahashi, 2001),

reduces photosynthesis by decreasing leaf expansion, impairing photosynthetic machinery and causing premature leaf senescence (Wahid and Rasul, 2005), restricts translocation of assimilates to reproductive sinks (Asch *et al.,* 2005), reduces root and shoot biomass and root respiration rate (Liu *et al.,* 2004) and leads to the production of reactive oxygen species, such as superoxide anion radicals (O_2), hydroxyl radicals (OH), hydrogen peroxide (H_2O_2), alkoxy radicals (RO) and singlet oxygen (Munné-Bosch and Penuelas, 2003).

The effect of drought stress on various crops has been well documented. The most important problem faced by a germinating seed is the non-availability of moisture during germination. The amount of moisture in the growth medium and duration of wetting regulates the process of germination in seeds (Ashraf and Mehmood, 1990). Several researchers have reported the adverse effect of drought stress on germination and seedling growth of various crops such as sunflower (Sajjan *et al.,* 1999; El-Midaoui *et al.,* 2001), sugar beet (Sadeghian and Yavari, 2004), maize (Moussa and Abdel-Aziz, 2008), sorghum (Gill *et al.,* 2002), and kochia (Masoumi *et al.,* 2010).

The development of drought tolerant wheat genotypes is one of the important approaches to increase crop production and yield under water limiting conditions (Ashraf *et al.,* 1992). The breeding for drought tolerance is as much important as in breeding for any other objective (Dhanda *et al.,* 2004) but it is a time consuming process however, screening of existing genotypes is an efficient and rapid technique to minimize the effects of drought stress. Researchers have identified numerous drought screening techniques like measurement of root depth and density (Gregory, 1989), partitioning of root-shoot (Thornley, 1998), osmotic membrane stability of cell (Premchandra *et al.,* 1990), and germination in osmoticum (Emmerich and Hardegree, 1991) for the screening of genotypes. The simulation of drought conditions in the laboratory by using osmotic agents is an important technique for efficient screening of genotypes (Sullivan, 1971). Among different osmotic agents, PEG-6000 is recommended due to its non-toxicity and non-penetration into the seeds (Willenborg *et al.,* 2005) and there exists a strong relation between this agent and wheat emergence percentage (Thill *et al.,* 1979).

The polyethylene glycol (PEG) induced water stress of -0.4 MPa considerably reduced germination rate of wheat genotypes and the availability of soil moisture enhanced the rate of emergence of shoot (Ahmad *et al.,* 1989, Akram *et al.,* 1998). Drought stress

reduced germination percentage in both tolerant as well as sensitive genotypes of wheat. The decrease in germination percentage was recorded even at -0.1 MPa in sensitive genotypes of wheat (Singh *et al.,* 1986). The non-availability of water also affects root growth however, it is less affected than aerial parts thereby increasing the overall root to shoot ratio of plant. The weight of roots of wheat was reported to decrease significantly with non-significant reduction in their number by Ashraf (1998). It was also observed that the roots were fine and fibrous under dry than favorable moisture conditions.

Dhanda *et al.* (2004) observed considerable variation for germination percentage, seed vigor index, shoot length, root length, coleoptile length, root-to-shoot length ratio, and osmotic membrane stability in thirty diverse bread wheat genotypes. They reported seed vigor index as the most sensitive trait, followed by shoot length, germination percentage and root length. An increase in root-to-shoot length ratio was recorded under osmotic stress. In a similar study, the drought tolerance of twenty promising durum wheat genotypes was quantified by Moayedi *et al.* (2009) using physiological indices. The germination stress tolerance index (GSTI) of all genotypes was reduced under osmotic stress. They declared genotypes RASCON_39/TILO_1 and RASCON_37/BEJAH_7 as tolerant under low (-0.3 MPa) and high (-0.9 MPa) osmotic stress.

El Monayeri *et al.* (1984) recorded a significant reduction in wheat seedling growth under moisture deficit conditions. A reduction of 19-32% was observed in shoot and root length of wheat seedlings under severe water stress conditions. The relative growth rate of root decreased more significantly than shoot while drought stress increased root growth and root to shoot ratio of the seedlings. The reduction in water supply from the root to coleoptile inhibited coleoptile growth. Under limited water conditions, moderate stress at early growth stages may increase yield of wheat (Yuan *et al.,* 1999). The exposure to moderate water stress at early growth stages increased grain and biomass yield equal to or higher than well watered controls. The screening of sixteen wheat genotypes for drought tolerance at germination and seedling stages revealed genotypic variation in their response under PEG-6000 induced drought stress (Rauf *et al.,* 2006). They reported cultivar PK-18199 as drought tolerant because it attained maximum germination percentage, germination rate index, coleoptile length, and root/shoot ratio.

Drought stress significantly reduces growth of crop plants through its effects on various physiological processes in plants. A reliable and effective way to quantify plants response to drought stress is leaf water potential (Siddique and Islam, 2000). The variation in water relation characteristics among species and lines indicate their potential as drought resistant or sensitive (Sobrado and Turner, 1983). The leaf water potential decreases due to accumulation of solutes under limited water conditions and variation exists among cultivars in their response to water potential under well watered as well as drought stress conditions (Nawaz *et al.,* 2012). Sairam *et al.* (1990) reported decrease in water potential and yield of wheat at both tillering and anthesis stages however, the cultivars were found to be more sensitive to water stress at anthesis than tillering stage. Ashraf and Khan (1993) reported varietal differences among ten wheat cultivars in their response to drought stress. The results showed that M-54, Pak-15800 and Barani-83 maintained high water potential and produced more biomass than Sarsabz, Pak-81, S-232, HCWSN, V8001 and Pak-15794. Similar studies were conducted by Sharma *et al.* (1993) on two brassica cultivars. They also reported decrease in photosynthesis, water potential, osmotic potential and chlorophyll contents of both cultivars under drought stress conditions.

The leaf water potential has been reported to affect many other physiological processes such as stomatal conductance, transpiration rate, photosynthesis and nutrient uptake in plants. The non-availability of water from early or mid-grain filling until maturity reduced water potential of maize leaves that ultimately decreased the rate of photosynthesis (Ouattar *et al.,* 1987). Liang *et al.* (2002) studied the relationships among stomatal conductance, water consumption and growth rate to leaf water potential in spring wheat (*Triticum aestivum* L.). It was observed that stomatal conductance and transpiration rate decreases with decrease in leaf water potential. The water deficiency decreased osmotic regulation of wheat plants and osmotic regulation induced by drying and re-watering alternation increased water use efficiency of plants under drought stress conditions. Wang and Huang (2003) reported similar results in Kentucky bluegrass. They reported that stomatal conductance, net photosynthesis rate, relative water contents and leaf water potential declined under drought stress and found that leaf water potential and ABA synthesis were negatively correlated to each other. Nawaz *et al.* (2012) concluded from their studies that late drought stress imposed after six weeks of emergence has more deleterious

effect on the water relations, growth, yield and nutrient uptake of wheat than early drought stress imposed after three weeks of seedling emergence. The decrease in leaf water potential significantly reduced the uptake of nitrogen, phosphorous and potassium in plants under drought stress. The decrease in mineral nutrition and growth of wheat (*Triticum aestivum* L.), chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris M.*) under drought stress (Gunes *et al.,* 2007) are reported to be positively correlated with the low leaf water potential as water plays a significant role in the transfer of nitrogen from other organs to seeds (Li *et al.,* 2007).

The leaf relative water contents (RWC) are better indicators of leaf water status than water potential (Sinclair and Ludlow, 1985) and can be used for the selection of drought tolerant cultivars in wheat breeding programme (Bayoumi *et al.,* 2008). Drought tolerant genotypes maintained high RWC and moisture stress had little effect on their protoplasmic structure as compared to sensitive genotypes (Song *et al.,* 1995). There exists a highly positive correlation between RWC and photosynthetic rate (Siddique *et al.,* 2000). The ability of cultivars to maintain their turgor potential and RWC during later stages of growth play a significant role in maintenance of photosynthetic apparatus under limited soil water conditions (Xu *et al.,* 1996). The maintenance of leaf water potential, RWC and stomatal conductance under severe moisture deficit conditions can be helpful for plants to maintain photosynthesis under prolonged drought (Wang and Huang, 2003). Moaveni (2011) investigated the changes in membrane stability, chlorophyll contents and RWC of four spring wheat cultivars at flowering stage. He reported that drought stress significantly reduced all these parameters and was of the view that photosynthesis and RWC are positively correlated with each other.

Gradual exposure to limited water for longer periods of time decreases cell division and expansion that inhibits plant growth due to loss of turgor (Lawlor and Cornic, 2002). Leaf turgor maintenance is an important adaptation trait that plays a significant role in stomatal regulation and photosynthesis under drought stress (Ludlow *et al.,* 1985). Loss of turgor restricts cell expansion that ultimately reduces growth (Turner, 1986). Plants respond to decrease in water supply by actively lowering their osmotic potential, a mechanism known as osmotic adjustment that significantly contributes towards drought resistance (Blum and Sullivan, 1986; Ludlow and Muchow, 1990). The leaf osmotic potential decreases under

moisture deficit conditions and drought tolerant plants show high reduction in osmotic potential as compared to susceptible plants (Ashraf and O'Leary, 1996).

Lilley *et al.* (1996) suggested that osmotic adjustment can be used as selection criteria for desiccation tolerance of rice cultivars. Teulat *et al.* (1997) proposed the same criteria for the selection and evaluation of drought tolerance in barley and wheat genotypes. They observed that drought susceptible genotypes had lower osmotic adjustment while high yielding drought tolerant genotypes exhibited higher osmotic adjustment across contrasting environments. They reported positive correlation between RWC, leaf osmotic potential and osmotic adjustment. The drought tolerant cultivars maintain best turgor which is significantly related to better osmotic adjustment in plants (Sanchez *et al.,* 1998). Khanna (1999) observed that genotypes and species with high osmotic adjustment maintain high RWC and turgor under drought stress conditions and suggested that osmotic adjustment could be used for measuring desiccation tolerance in wheat. Fahliani and Assad (2005) reported that selection between resistant and susceptible genotypes could be made by measuring leaf water potential and osmotic potential at three developmental stages (stem elongation, booting and flowering) under moisture deficit conditions. They observed linear regression of grain yield on leaf water potential and leaf osmotic potential. Iqbal and Bano (2010) studied the effect of abscisic acid (ABA) application on four wheat cultivars under drought stress. They found marked decrease in the osmotic potential of Accession 011320 and declared it as drought susceptible and reported that ABA application resulted in higher osmotic adjustment and increased drought tolerance in the cultivars.

Shangguan *et al.* (1999) in wheat observed relationship between osmotic adjustment and photosynthesis and were of the view that osmotic adjustment plays an important role in the adjustment of photosynthetic apparatus and stomatal regulation for the maintenance of photosynthesis at low water potential. There exists a positive and significant correlation between osmotic adjustment, stomatal conductance and yield under drought stress (Gonzalez *et al.,* 1999). Blum *et al.* (1999) also reported positive correlation between osmotic adjustment and yield under pre-flowering drought stress. They concluded that differences in osmotic adjustment in wheat genotypes could be related with plant production under pre-flowering drought stress. Subbarao *et al.* (2000) reported that genotypic variation in leaf

RWC and osmotic adjustment are highly correlated and found that crop growth rate is influenced by osmotic adjustment under soil moisture deficit conditions.

Osmotic adjustment is an important plant defense mechanism, facilitated by compatible solutes produced at higher levels under stress, to protect enzyme and membrane structures by accumulation of compatible solutes. It also acts as scavenger of reactive oxygen species (Bohnert and Shen, 1999). The accumulation of these organic solutes prevent cellular dehydration and help cells to retain water in cells (Bohnert *et al.,* 1995), without deteriorating macromolecules (Pugnaire *et al.,* 1994). These compounds are accumulated in high amounts under stress and act as osmoprotectants of membrane to prevent protein disintegration (Yancey, 1994). These are classified as sugars, glycerol, amino acid (proline and glycine betaine), sugar alcohols (manitol) and other low molecular weight metabolites (Morgan, 1984). Several researchers have stated the importance of osmotic adjustment in improving the drought tolerance of plants (Hasegawa *et al.,* 2000; Zhu, 2000; Shao *et al.,* 2005). The total accumulation of solutes and the nature of accumulated solutes may vary within species and cultivars (Rhodes and Samaras, 1994).

Proline is a low molecular mass compound that accumulates in cytosol of plant cells (Voetberg and Sharp, 1991) to improve their tolerance against drastic effects of drought (Gzik, 1996; Bajji *et al.,* 2001). It not only function as an osmolyte for osmotic adjustment but also stabilizes sub-cellular structures such as membranes and proteins (Rhodes *et al.,* 1999; Ozturk and Demir, 2002), act as a protein compatible hydrotrope (Srinivas and Balasubramanian, 1995), regulates and activates multiple responses such as scavenging free radicals, and buffering cellular redox potential that help plants to combat abiotic stresses (Maggio *et al.,* 2002) and is considered as a reliable indicator for the environmental stress in plants (Claussen, 2005). It is well documented that proline accumulation under salinity or drought improves tolerance to these stresses (Aspinall and Paleg, 1982; Szegletes *et al.,* 2000; Kong *et al.,* 2001; Hsu *et al.,* 2003) and this characteristic could be used for the selection of stress tolerant cultivars (Ashraf and Haris, 2004). However, some investigators have raised questions on physiological significance of stress-induced proline accumulation and obtained contrasting results regarding its role in stress tolerance of plants (Brock, 1981; Dix and Pearce, 1981; Nadiu *et al.,* 1998; Lutts *et al.,* 2000).

Proline accumulation under stress conditions is well reported for different crops (Sivaramakrishnan *et al.,* 1998; Almansouri *et al.,* 1999). Drought stress significantly increased proline contents in both young and old leaves of sunflower (Cechin *et al.,* 2006). Similar results were reported by Mostajeran and Rahimi-Eichi (2009) in rice. They observed substantial increase in proline content of both young and old leaves under drought stress and stated that young leaves accumulated more proline than old leaves and proline accumulation was more in leaf sheath than blade under un-submerged conditions. The accumulation of free proline under water stress has also been reported in *Vigna radiate* (Hooda *et al.,* 1999) and alfalfa plants (Irigoyen *et al.,* 1992). Proline contents were higher in wheat leaves during grain filling period than pre-anthesis stage and can be used as a reliable parameter for the selection of drought tolerant genotypes (Farshadfar *et al.,* 2008). In a similar study, Qayyum *et al.* (2011) observed an increase in proline content of wheat leaves from 0.33 mg g^{-1} in control to 2.65 mg g^{-1} under osmotic stress. They were of the view that proline accumulation in stressed plants is an important adaptation for survival under field conditions (Tatar and Gevrek, 2008). Nazarli and Faraji (2011) reported that proline accumulation is related to irrigation regimes and recorded highest proline content (12 μmol g^{-1} DW) in irrigation regime of 25% field capacity (FC) while lowest value (2.4 μmol g^{-1} DW) was recorded in irrigation regime of 100% FC. Proline accumulation plays a predominant role in improving drought tolerance of wheat seedlings (Simova-Stoilova *et al.,* 2008). The degradation of proteins with instantaneous decline in their synthesis result in intensive proline accumulation in all stressed organs especially leaves and conversion of some of amino acids as arginine, glutamic and ornithine to proline also facilitate its accumulation in plants (Chaitante *et al.,* 2000).

Like proline, sugars are also important compatible solutes that play a significant role in osmoregulation under drought stress (Fallon and Phillips, 1989). The accumulation of total sugars and other compatible solutes such as polyols is characteristic feature of most plants under stress (Mohammadkhani and Heidari, 2008; Delauney and Verma, 1993). They are involved in the activation and regulation of nitrate reductase (NR) activity (Kaiser and Huber, 2001; Kaiser *et al.,* 2002; Iglesias-Bartolome *et al.,* 2004; Lillo *et al.,* 2004), enhance the transcription of NR genes (Sivasanker *et al.,* 1997; Klein *et al.,* 2000; Larios *et al.,* 2001) and regulate the activity of enzymes at post translational level (Carpenter and Gowe, 1988

and Wolkers *et al.,* 1998). Clifford *et al.* (1998) observed significant role of sugars in osmotic adjustment of *Ziziphus mauritiana* under limited water conditions while Patakas *et al.* (2002) reported contradictory results in grapevine plants and stated non-significant differences in sugar contents of water stressed and unstressed plants.

The production of soluble sugars under drought stress may be the result of amylase activity that decomposes starch and therefore increases soluble sugar contents (Ghasempour *et al.,* 1998; Vaezi, 2005). Farshadfar *et al.* (2008) evaluated the molecular indices of drought tolerance in twenty bread wheat (*Triticum aestivum* L.) genotypes and recorded more soluble sugar content during grain filling period than pre-anthesis stage under drought stress. They were of the view that post-anthesis stage and grain filling period are the best stage for the screening of drought tolerant cultivars (Hien *et al.,* 2003). Mostajeran and Rahimi-Eichi (2009) studied the accumulation of soluble sugars in sheath and blade of different age leaves of rice and found that young leaves accumulated more sugars than old ones under non-submerged conditions. The average amount of total soluble sugars in leaf sheath and blade was 219 and 212 mg g^{-1} respectively. The response of soluble sugars content in wheat leaves to different irrigation regimes of 100%, 75%, 50% and 25% field capacity (FC) at two growth stages was evaluated by Nazarli and Faraji (2011). They found that limited water supply increased production of total soluble sugars in leaves and irrigation at 25% FC resulted in maximum total soluble sugars content (49 mg $g^{-1}$of dry weight) in leaves. Non-significant difference was observed between different irrigation regimes and growth stages. Qayyum *et al.* (2011) stated that increase in osmotic stress progressively increases the production of total soluble sugars in wheat leaves. They observed that sugars contents increased from 1.49 mg g^{-1} in control to 2.65 mg g^{-1} under osmotic stress of -8 bars.

Chlorophyll content is an important index of photosynthetic activity in plants (Larcher, 2003) and thus plays an important role in precision agriculture. A broad range variation exists within leaf chlorophyll content (0.05 to 0.30% of fresh weight). The most widely reported ratio between chlorophylls a and b is 3:1. The variation among these values depend upon cultivars, plant growth and development and various environmental factors The highest chlorophyll content are observed at the onset of the flowering phase in plants (Simova *et al.,* 2001). About 4-5 mg of chlorophyll occurs per unit of leaf surface however it

may vary due to color of the leaves of certain species and cultivars (Bojović and Stojanovic, 2005). The decrease in Chl (a + b) contents under drought stress suggest that these can be used as indicators of crop stress (Tejada-Zarco *et al.,* 2004)

Mafakheri *et al.* (2010) studied the effect of drought stress on the chlorophyll contents of three chickpea cultivars and reported a significant decrease in chlorophyll a, chlorophyll b and total chlorophyll content of all cultivars. Similar results were stated by Fotovat *et al.* (2007) in wheat that severe drought stress significantly reduces leaf chlorophyll content however Schelmmer *et al.* (2005) observed no significant effect of drought stress on leaf chlorophyll content of maize plants. Kilic and Yagbasanlar (2010) evaluated yield and quality attributes of fourteen durum wheat (*Triticum turgidum* ssp. durum) cultivars under drought stress and found that the grain yield was positively associated with chlorophyll content, grain filling period, number of grains spike^{-1} and spikelets spike^{-1} under drought stress. The seed yield increases with increase in chlorophyll content and the variation exists among genotypes for chlorophyll contents under drought stress conditions (Alaei, 2010, Arjenaki *et al.,* 2012) which can be useful in screening of drought tolerant and susceptible cultivars. Almeselmani *et al.* (2012) evaluated physiological performance of nine tolerant, moderately tolerant and susceptible wheat genotypes under rainfed condition. They recorded non-significant difference in chlorophyll content of tolerant and susceptible genotypes at both vegetative and anthesis stage and found lowest chlorophyll content at anthesis stage.

Saeedipour (2011) examined the remobilization of carbon reserves in wheat under drought stress imposed at post anthesis stage. He recorded more significant loss in total soluble proteins at 50% FC imposed 14 days after anthesis to maturity than from anthesis to 14 days later. Farshadfar (2008) reported significant correlation between total proteins and grain yield of wheat under rainfed conditions and suggested that carbon reserves remobilization can be used as an indicator of drought tolerance in plants (Ghasempour *et al.,* 2001). The leaf soluble proteins have been reported to decrease under drought stress in sunflower (Rodriguez *et al.,* 2002) and cotton (Parida *et al.,* 2007). However, Ashraf and Mehmood (1990) were of the view that higher protein contents are the indicator of drought resistance in plants. Dehydrin proteins are produced by cell under drought stress (Ingram and

Barels, 1996) and act as compatible solutes through chaperone like properties to stabilize macromolecules in cell (Close, 1996; Hoekstra *et al.,* 2001).

Drought stress perturbs the balance between antioxidant defense and the amount of reactive oxygen species (Gill and Tuteja, 2010). Reactive oxygen species (ROS) play an important role in inter- and intracellular signaling to control plant growth and development (Breusegemetal., 2001) and are involved in the regulation of photosynthesis and programmed cell death (Turkan *et al.,* 2005, Foyer and Shigeoka, 2011). They serve as substrates and signals at low concentrations (Wang *et al.,* 2012) and trigger defense responses in plant cells during drought stress (Sofo *et al.,* 2005) but high concentrations of ROS such as singlet oxygen ($^1O_2$), superoxide radicals (O_2), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH) leads to damage at various levels of the organization (Asada, 1999). The accumulation of ROS must be kept in check through the action of scavenging enzymes and antioxidant molecules (Foyer and Shigeoka *et al.,* 2011; Wang *et al.,* 2012). Reactive oxygen species are produced from the action of photosynthetic apparatus, photorespiration pathways and mitochondrial respiration during drought periods (Mittler, 2002). They can directly attack membrane proteins and lipids, inactivate metabolic enzyme, damage nucleic acids and destroy cellular structures associated with photosynthesis (Apel and Hirt, 2004; Miller *et al.,* 2010).

In general, a well-known adaptive mechanism in plants in response to drought conditions is the production of antioxidant enzymes such as peroxidase (POD), catalase (CAT), Ascorbate peroxidase (APX), superoxide dismutase (SOD) and glutathione peroxidase (GPx) (Li *et al.,* 2009). Both enzymatic and non-enzymatic antioxidants such as glutathione (GSH), flavonoids, ascorbic acid (AsA), α-tocopherol, β-carotene and hydroquinones collaboratively serve as scavengers of ROS under stress (Chaves and Oliveria, 2004). Ascorbate peroxidase (APX) is one of the most important antioxidant enzyme involved in the scavenging of ROS to protect cells of higher plants (Gill and Tuteja, 2010). It is the constituent of water-water and ascorbate-glutathione (ASH-GSH) cycles that scavenge H_2O_2 in the chloroplasts of plant cells under stress (Asada, 1999; Gill and Tuteja, 2010). The antioxidant defense system is activated/modulated by plant water relations (Srivalli *et al.,* 2003; Selote and Khanna-Chopra, 2004) that results in the synthesis of APX

under drought stress. The non-availability of water significantly increased the activity of APX in three cultivars of P. asperata (Yang *et al.,* 2008) and P. vulgaris (Zlatev *et al.,* 2006). The APX activity increased in chloroplast of rice seedlings subjected to mild drought stress but higher levels of drought decreased its activity in stressed plants (Sharma and Dubey, 2005).

Nikolaeva *et al.,* (2010) stated that APX activity varies in different wheat cultivars and depends on duration of drought and stage of leaf development. They observed that mild drought stress enhanced APX activity in leaves but prolonged drought reduced its activity due to increase in malonic dialdehyde (MDA) content. In contrast, Nazarli and Faraji (2011) reported highest APX activity (42 μmol min^{-1} g^{-1} FW) in leaves of wheat plants at irrigation regime of 25% FC and were of the view that APX activity increases with increase in drought stress. Both antioxidant enzymes and concentrations increased in apple rootstocks and APX activity enhanced to a greater extent in response to drought stress to maintain cell structural integrity of the plants (Wang *et al.,* 2012).

The sequential and simultaneous action of various antioxidant enzymes like catalase (CAT) and peroxidase (POD) is a key factor for survival of plants in changing environments. Both CAT and POD are major scavengers of H_2O_2 and play an important role to convert toxic levels of accumulated H_2O_2 into water and oxygen (Apel and Hirt, 2004). Peroxidase uses several available reductants to reduce H_2O_2 into H_2O in plant cells under stress (Mittler, 2002). Wang *et al.* (2012) reported an increase in POD activity in drought stressed apple leaves and found more increase (141.97%) in POD activity in drought tolerant *Malus prunifolia* root stock than drought sensitive *Malus hupehensis* rootstock (84.19%). Similar results were reported by Turkan *et al.* (2005) in bean cultivars. Stoilova *et al.* (2010) examined CAT activity in wheat leaves under severe soil drought and were of the view that drought stress increases CAT activity especially in sensitive cultivars. Nazarli and Faraji (2011) reported no significant difference among different irrigation regimes on CAT activity in wheat leaves. In contrast, Sharma and Dubey *et al.* (2005) reported that water deficiency leads to a decrease in CAT activity in rice seedlings. CAT activity is enhanced in high light conditions under drought stress (Yang *et al.,* 2008). Pan *et al*. (2006) evaluated the combined

effect of drought and salt stress and reported a decrease in CAT activity in *Glycyrrhiza uralensis* seedlings.

The water availability improves crop growth as irrigated crops intercept more radiation than drought stressed crops that ultimately improves grain yield (Sharif, 1999). The wheat crop yield is equally sensitive to drought stress at both vegetative and reproductive stages (Qadir *et al.* 1999). Pirdashi *et al.* (2004) observed that water stress at flowering stage reduced grain yield by 50% in rice while the reduction (21%) was similar at both vegetative and reproductive stages. Nawaz *et al.* (2012) reported that late drought stress (LDS) imposed six weeks after emergence has more deleterious effect on water relations, nutrient uptake and yield of wheat than early drought stress (EDS) imposed three weeks after emergence. They recorded a decline of 60% in grain yield at LDS as against 24% reduction at EDS. The non-availability of water contributes to low solute potential and substrate availability within sink that reduces grain filling processes and synthetic capacity of the sink in wheat (*Triticum aestivum* L.) (Ahmadi and Baker, 2001). The pre-anthesis assimilates stored in stems contribute significantly in grain filling to prevent severe decrease in grain yield in wheat under water deficit conditions. The water deficiency increased remobilization of pre-anthesis assimilates by 20% during grain filling as compared to normal supply of water (Moayedi *et al.,* 2009).

The differential response of wheat cultivars to water stress indicates their ability of drought tolerance (Ahmad *et al.,* 2002). Researchers have used various physiological indices for the screening of wheat cultivars under drought stress. Labuschagne *et al.* (2003) suggested drought susceptibility index as a useful screening tool while Dhanda *et al.* (2004) reported seed vigor index as the most sensitive trait to evaluate performance of wheat cultivars under water stress conditions. Mushtaq *et al.* (2011) evaluated two indigenous wheat cultivars (Mairaj-2008 and Fareed-2006) under different irrigation regimes and found that both cultivars lack genetic potential to withstand drought stress. They reported that tillering and grain formation stages are most sensitive to drought stress. Akram *et al.* (2004) reported Barani-83 as drought tolerant and Inqlab-91 as drought sensitive wheat cultivar on the basis of osmotic adjustment and absolute grain yield under limited water conditions. The high yield stability in wheat under drought is correlated with the expression and induction of

senescence associated glutathione S-transferases (GSTs) in flag leaves during grain filling stage (Galle *et al.,* 2009).

2.3. Selenium

Selenium (Se) was discovered in 1817 by a Swedish chemist Jons Jacob Berzelius. It has the properties of both metals and non-metals and hence is classified as a metalloid. It is similar to sulphur (S) in many ways like electronegativity, atomic size and have similar major oxidation states (Johansson *et al.,* 2005). As an elemental Se or cadmium selenide, Se has wide range of applications in many industries. It is used in electronics and photography, semiconductors, medical imaging equipment, photocopiers and solar cells. It also finds its application in glass industry to give beautiful ruby red color to the glass (Dhillon and Dhillon, 2003).

Earlier Se was regarded as an undesirable and toxic element for higher organisms but in the second half of the 20th century, Schwarz and Foltz (1957) reported that low concentrations of Se are essential for dietary intake and interchangeable with vitamin E. Later in 1973, it was discovered that being an integral component of glutathione peroxidase (GPX) enzyme, Se protects cells against intracellular oxidative damage (Rotruck *et al.,* 1973). In 1979, the association of Keshan disease with Se deficiency in the Keshan region in China provided the direct evidence for Se requirement in human nutrition (Keshan Disease Research Group, 1979). Further studies revealed that selenoproteins are involved in various physiological processes in mammals and today more than 30 selenoproteins had been reported to have a significant role in human nutrition (Rayman, 2002; Brown and Arthur, 2007). Selenium toxicity causes distraction of nervous and digestive systems in humans and animals and results in hair and nail loss, poor reproduction rate and growth in animals (Hartikainen, 2005, Plant *et al.,* 2005). On the other hand, the inadequate dietary intake of Se results in many disorders and diseases related to free radical damage such as greater risk of tumor formation, immunity disorders and cardiovascular diseases like hypertension and atherosclerosis. A very narrow range between Se deficiency (< 40 μg d^{-1}) and toxicity (> 400 μg d^{-1}) has been reported (Plant *et al.,* 2005).

Organic forms of Se (selenomethionine, selenocysteine and Se-methylselenocysteine) are mostly found in foods while it rarely occurs in inorganic forms (selenite or selenate). In

most Se rich diets, selenomethionine is the major organic form however both organic and inorganic forms are involved in the formation of selenoproteins in the body (Shiobara *et al.,* 1998). The biological role of selenocompounds in humans has been well documented (Arthur, 1997). The first identified selenoprotein in mammals, glutathione peroxidase (GPX) protects cells from oxidative damage and is an important part of antioxidative defense system of the body. Thioredoxin reductase (TR) is recently discovered selenoprotein which is an integral part of the thioredoxin system, well known for its antioxidant and redox regulatory roles in cells (Arnér and Holmgren, 2000; Gromer *et al.,* 2004). Another important selenoenzyme, selenophosphate synthethase (SePsyn) is thought to be involved in the production of other selenoenzymes and also catalyzes the synthesis of selenophosphate which is essential for the production of selenocysteinylated tRNASec (Kim *et al.,* 1997). The protective role of Se against the toxicity of other heavy metals such as lead, silver and mercury has also been well reported (Frost, 1983; Cuvin-Aralar and Furness, 1991; Ellingsen, 1993). Selenium levels in fish are high enough to prevent Hg toxicity; however the exact mechanism of their interaction is not well known (Cuvin-Aralar and Furness, 1991).

The major sources of Se in most diets are meat, fish and cereals (Combs, 2001). The level of available Se in the soil used for growing food stuffs and dietary composition determines the Se intake by humans. Selenium usually occurs within the range of 0.10 – 0.60 mg kg^{-1} in fish, 0.05 – 0.60 in cereals, 0.05 – 0.30 in red meat, and 0.002 – 0.08 in fruit and vegetables (Combs, 2001). It depicts that cereals largely contribute to the total dietary intake of Se and nearly 70% dietary Se requirements are met with cereals and cereal products in Se deficient areas in China while they contribute about 40–54% to the Se dietary intake of low-income population in India (FAO, WHO, 2001). In developed countries like UK, 18-24% of the total Se intake comes from cereals and cereal products (Ministry of Agriculture Fisheries and Food, 1997). Among cereals, wheat is one of the most important Se sources for humans as it is the most efficient Se accumulator (Lyons *et al.,* 2003).

2.4. Selenium and plant Growth

Selenium due to its physiological and toxicological importance has become an element of interest to many plant scientists. There are reports in literature of selenium beneficial role in plants. It enhances growth of plants (Hartikainen and Xue, 1999), reduces

damage caused by UV-induced oxidative stress (Hartikainen and Xue, 1999; Hartikainen *et al.,* 2000; Valkama *et al.,* 2003), enhances chlorophyll contents under light stress (Seppanen *et al.,* 2003), stimulates senescing plants to produce antioxidants, and improves plant tolerance to drought stress by regulating water status (Kuznetsov *et al.,* 2003; Djanaguiraman *et al.,* 2005; Ekelund and Danilov, 2001). Selenium is known to increase superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities thus activating the protective mechanisms against oxidative stress. It is also reported to exert an antioxidant effect directed towards a decreased concentration of intracellular active oxygen species by inducing the biosynthesis of proline and peroxidase production (Kuznetsov *et al.,* 2003).

In plants, selenium uptake and translocation takes place through sulphate transporters (STs) and phosphate transporters (PiTs). Once absorbed by the plants, the inorganic Se is converted into more bioavailable organic forms like selenomethionine (SeMet). It is presumed that STs located in root cell membranes are involved in the uptake of selenate while PiTs are generally accepted to be involved in selenite uptake (Hopper and Parker, 1999). A large number of enzymes such as APS reductase (APSR), ATP sulphurylase (ATPS), sulphite reductase (SiR) etc. take part in sulphate assimilatory pathway to incorporate selenate or selenite into selenocysteine (SeCys) and selenomethionine (SeMet) (Rotte and Leustek, 2000). Selenium uptake may be influenced by genetic factors and cultivars may differ in their Se content (Eurola *et al.,* 2004). Rate and method of Se application are reported to affect grain Se levels while other factors like the yield of the crop may also affect grain Se concentration through dilution effects, i.e., a low yield crop of dry areas may have higher grain Se than a high yielding crop grown on an irrigated site (Curtin *et al.,* 2006).

The soil and/or foliar application of Se fertilizers (agronomic biofortification) are often used to improve the Se concentration of food. Moreover, plants act as an effective buffer that can protect humans from toxic Se intakes that may take place with direct Se supplementation (Hartikainen, 2005). The application of selenate fertilizers (soil and/or foliar application) has proved to be more effective to increase plant Se concentrations than selenite fertilization (Gissel-Nielsen *et al.,* 1984; Singh, 1991) and hence selenate is the predominant form of Se in Se fertilization of plants (Broadley *et al.,* 2007). The chemical form of Se, soil characteristics, time of foliar and method of basal application affects the relative

effectiveness of soil or foliar applied Se fertilizers (Lyons *et al.,* 2003). Stephen *et al.* (1989) reported an increase of 32% in Se concentrations of wheat plants by sodium selenate fertilization on silt loam soil. They supplied sodium selenate at varying rates of 5, 10, 15 and 20 g Se ha^{-1} as prills drilled with the wheat seed, Se seed coating or a foliar spray at mid-tillering and/or ear emergence and found foliar application at ear emergence stage to be more effective than other methods of Se application. In Finland, Se is added at a rate of 10 mg kg^{-1} as a soil amendment in NPK fertilizer (Eurola and Hietaniemi, 2000) and has proved to be a safe, easy, effective and cost efficient approach to increase Se levels in a human population.

Cartes *et al.,* (2005) found that treatment of soil with selenite and selenate (0-10 mg kg^{-1}) increased the Se concentration in ryegrass seedlings and recorded a significant positive correlation between shoot Se concentration and glutathione peroxidase (GSH-Px) activity. The higher shoot Se concentration in selenate treated plants suggested that activity of this enzyme was related with the chemical form of applied Se rather than with the concentration of Se in plant tissues. More recently, Ducsay *et al.* (2009) reported that soil Se application significantly increased the Se content in the dry matter of roots, straw and grains of wheat. The application of 0.2 mg Se kg^{-1} of soil gave highest Se content in grain (0.732 mg kg^{-1}), straw (0.227 mg kg^{-1}) and roots (1.375 mg kg^{-1}) dry matter whereas the lowest dose of Se (0.05 mg Se kg^{-1} of soil) gave 0.155 mg Se kg^{-1} in grain. The results of the study showed that Se concentration was highest in grain and lowest in the straw dry matter.

The foliar application of Se on wheat significantly increased the levels of plasma Se (53% increase after 6 weeks) in Serbia. It caused an increase in the activity of glutathione peroxidase in blood and decreased oxidative stress parameters (Djujic *et al.* 2000a). An increase in the levels of copper, iron and zinc by Se-enriched wheat was observed in erythrocytes, compared to consumption of low-Se wheat (Djujic *et al.* 2000b). Germ *et al.* (2007) found that foliar application of 1 mg sodium selenate per liter doubled the concentration of Se (43-46 ng Se g^{-1} DM) compared to the control (21-24 ng Se g^{-1} DM) and it also increased the respiratory potential in young plants without any visible toxic effects. Xu and Hu (2004) reported in their study on Se-enriched rice that Se concentration was significantly enhanced dose dependent in rice. They reported an increase in the antioxidant activity of Se enriched rice by Se foliar application.

The low rate of selenate (10 g ha^{-1}) was found to be equally effective in both basal and foliar application while foliar gave better results at 50 g ha^{-1} and no significant difference was observed at the high rate of 500 g ha^{-1} (Ylaranta, 1983b). In the follow-up trials, foliar applied selenate at the 3-4 leaf stage was found to be more effective than basal application in variety of soils. The wheat grain Se level increased from 16 to 168 μg kg^{-1} by the foliar application of selenate at the rate of 10 g ha^{-1} while basal application of 9g raised it to just 77 μg kg^{-1}. Thus it was concluded that foliar application was the more effective method with the exception from low rainfall areas (Ylaranta, 1984).

The variation among cereal crop cultivars for nutrients like zinc and iron (Graham *et al.,* 2001) suggest the possibility of such high variation for Se. In an experiment conducted in controlled field by Lyons *et al.* (2005), it was observed that significant genetic variation does not exist among modern wheat genotypes however, larger variation and higher grain Se concentration was observed in diploid wheat (*Aegilops taushii*) and rye. The soil physical and chemical properties significantly affect variation in Se accumulation in wheat and even within few meters of field; large variation in grain Se concentration might occur (Lyons *et al.,* 2005). Therefore the homogeneity of the field site for available soil Se is very important for the assessment of genotypic variation in grain Se concentration and content.

The hydroponic conditions provide a better medium to study the uptake efficiency for Se. It not only removes the limitations of heterogonous soil conditions but also provide more reliable information about Se accumulation by different genotypes (Cary and Allaway, 1969). The experiments carried out in water cultures on lettuce revealed that sodium selenite ($Na_2SeO_3.5H_2O$) applied at the rate of 5 μM in nickel (Ni) contaminated medium prevented the toxic effects of Ni and stimulated plant growth by increasing the concentration of assimilation pigments (Hawrylak *et al.,* 2007).

2.5 Selenium and Plant Drought Tolerance

The physiological and antioxidant properties of Se have increased the curiosity of many biologists in recent past. Although it does not take part in various vital metabolic processes in plants but may help to reduce the damage under physiological stresses (Hanson *et al.,* 2003; Seppanen *et al.,* 2003). Several studies have confirmed the positive role of Se against various environmental stresses such as cold (Chu *et al.,* 2010), high temperature

(Djanaguiraman *et al.,* 2010), salt (Hasanuzzaman *et al.,* 2011), heavy metals (Kumar *et al.,* 2012), senescence (Hartikainen *et al.,* 2000), UV-B (Yao *et al.,* 2010 a, b), excess water (Wang, 2011) and desiccation (Pukacka *et al.,* 2011). However, reports on the role of Se in plants under water stress conditions are scanty. It may regulate water status (Kuznetsov *et al.,* 2003) and increases biomass production (Nawaz *et al.,* 2013) by the activation of antioxidant apparatus of water stressed plants (Yao *et al.,* 2009; Hasanuzzaman and Fujita, 2011).

The antioxidative effect of Se is closely associated with the increased activity of glutathione peroxidase (GPX) (Xue *et al.,* 2001). The use of Se on a spring wheat cultivar showed that it exerts an antioxidant effect directed towards a decreased concentration of intracellular active oxygen species by inducing the biosynthesis of proline and peroxidase production (Kuznetsov, 2003). The addition of Se up to 1.0 mg kg^{-1} significantly increased GPX activity in rye grass (*Lolium perenne*) that led to a simultaneous decrease in lipid peroxidation. The Se induced GPX activity suggests the presence of Se-dependent GPX and indicate positive relationship between the Se concentration and GPX activity (Hartikainen *et al.,* 2000). Ramos *et al.* (2010) observed an increase in lipid peroxidation at higher Se concentrations that decreased the yield of lettuce plants. They suggested low doses of Se optimal for increasing antioxidants activity and were of the view that Se application as selenate is more favorable for increasing Se translocation and Se levels in the shoot biomass than selenite. This high selenate uptake and translocation is due to high affinity of sulphate transporters for selenate (Zhang *et al.,* 2003). In contrast, Cartes *et al.* (2005) reported that selenite was more efficient than selenate in increasing enzymatic activity.

Numerous strategies viz. seed dressing/coating (Bittman *et al.,* 2000), seed soaking (Nawaz *et al.,* 2013), soil application (Temmerman *et al.,* 2014) and foliar spray (Boldrin *et al.,* 2013) have been used to supply Se in plants. However, the simplicity and practicability of soil and foliar application make them widely accepted among these methods. Several studies confirmed the positive role of soil Se fertilization in various crops/plants such as rice (Wang *et al.,* 2013; Boldrin *et al.,* 2013), maize (Chilimba *et al.,* 2012), wild barley (Yan *et al.,* 2011) and soybean (Yang *et al.,* 2003). The foliar Se application has been reported to significantly promote growth in vegetables such as onion bulbs and leaves (Kápolna *et al.,*

2012), carrot roots and leaves (Kápolna *et al.,* 2009), radish flowers and leaves (Hladun *et al.,* 2013) as well as garlic bulbs (Põldma *et al.,* 2011) and in cereals like wheat (Curtin *et al.,* 2006).

Selenium application method and rates affect its uptake and concentration in plants. Curtin *et al.,* (2006) evaluated different methods and rates of Se application in wheat. They applied Se at rates ranging from 5-20 g ha^{-1}. They found foliar application @ 20 g ha^{-1} as the most effective method for increasing grain Se contents in wheat as it increased grain Se concentration to 0.4-0.5 mg kg^{-1} as compared to control treatment (0.03 mg/kg). It was also observed that application of Se fertilizer at sowing gave low grain Se levels as compared to application of Se fertilizer at later growth stages of wheat. In another study, Ducsay *et al.* (2009) evaluated the response of wheat to different Se doses ranging from 0.05 mg to 0.20 mg kg^{-1}. They reported that application of Se @ 0.20 mg kg^{-1} of soil produced the maximum Se in grains (0.732 mg kg-1), in straw (0.227 mg kg-1) and in roots dry matter (1.375 mg kg^{-1}) whereas application of Se @ 0.05 mg kg^{-1} resulted in 0.155 mg kg^{-1} grain Se contents. Sajedi *et al.* (2011) evaluated the interactive effects between Se and micronutrients in corn plants exposed to drought stress. They found that single but not the combined use of Se was more effective in alleviating the adverse effects of drought stress on corn yield by affecting plant metabolism including antioxidant activity.

The uptake and accumulation of Se within a narrow range is beneficial for plants (Terry, 2000) and is determined by the plants ability to absorb and metabolize Se. It is well documented that increase in acidity, iron oxides/hydroxides, organic matter and high clay content of soil decreases the bioavailability of Se to plants (Mikkelsen *et al.,* 1989; Kabata-Pendias, 2001). The soil moisture also affects the availability of Se to plants as it is more available under low precipitation conditions (Zhao *et al.,* 2007). Moreover, actively growing tissues usually contain large amounts of Se (Kahakachchi *et al.,* 2004) and accumulation is higher in shoot and leaf than in root tissues (Zayed *et al.,* 1998). Therefore, Se fertigation and foliar spray are much more viable and effective approaches than soil application to increase Se translocation within plants.

Priming of plant seeds is an easy, low cost, low risk and effective approach to improve plant tolerance under stressful environments (Ashraf and Foolad, 2005; Wahid and

Shabbir, 2005). Seed priming is a controlled hydration process that promotes metabolic activities before radical protrusion (Sivritepe *et al.,* 2005). A number of osmotica have been reported to enhance germination, emergence, growth, and/or grain yield of wheat like potassium chloride (Misra and Dwibedi, 1980), polyethylene glycol and potassium hydro phosphate (KH_2PO_4) monobasic solutions (Dell'Aquila and Taranto, 1986). Frias *et al.* (2009) reported that the treatment of lupin seeds with different inorganic Se solutions (sodium selenate and selenite) significantly increased essential amino acids, Se content and antioxidant activity in Se-enriched sprouts. They observed higher germination rate in seeds treated with selenate solutions as compared to selenite solutions and suggested that selenate concentration of 8 mg L^{-1} is optimal for the production of Se-enriched sprouts. The increase in germinability and antioxidant activity of bitter gourd seedlings by Se seed priming under sub-optimal temperature has been reported by Chen and Sung (2001). They observed that free radical and peroxide-scavenging activities were enhanced by Se treatment of seeds and suggested that GPX is a Se inducible enzyme because it showed positive response with increasing Se level up to 10 mg L^{-1}.

Selenium increases the tolerance of plants to drought stress by regulating their water status. The foliar application of Se @ 20 g ha^{-1} in maize under drought stress increased yield and water use efficiency as compared to no Se application under water deficit conditions (Sajedi *et al.,* 2009). In a similar study, Valadabadi *et al.* (2010) evaluated the role of Se in improving drought tolerance of rapeseed cultivars and found that Se application significantly mitigated the adverse effects of drought on total dry weight, leaf area index (LAI), relative growth rate (RGR) and crop growth rate (CGR) of the cultivars. The combined treatment of re-watering and Se helped to improve biomass in wheat seedlings as compared to drought treatment and re-watering alone and promoted the recovery of malondialdehyde (MDA) content, superoxide radical ($O_2^{\cdot-}$) production, soluble protein content and catalase (CAT) activity to the control values (Yao *et al.,* 2011). Zahedi *et al.* (2012) reported foliar application of Se helpful in reducing electrolyte leakage in canola cultivars subjected to drought stress. Proietti *et al.* (2013) observed that foliar spray of Se significantly improved photosynthesis, fruit yield and leaf water content of olive trees under water deficit conditions. An increase in GPX, CAT and APX activity was noted that suggests the protective role of Se against oxidative damage. However, Germ (2008) observed an evident

decrease in respiratory potential and mass of Se treated potato tubers exposed to water deficit conditions, whereas non-significant effect of Se treatment was recorded on number and size of leaf stomata. Selenium also promotes the growth of ageing seedlings (Germ *et al.,* 2007). Djanaguiraman *et al.,* 2005 reported that foliar Se application to soybean promoted plant growth during senescence.

Khattab (2004) reported an increase in total free amino acids in the tops of rocket plants exposed to 5 μM or 100 μM selenate while Hu *et al.* (2001) reported similar results in selenite-treated green tea. The Se causes disorder in amino acid metabolism (Gowily *et al.,* 1996; Wu, 1998) which may increase total free amino acids under drought stress. It interacts with sulphydryl containing enzymes to regulate the biosynthetic pathway of chlorophyll. Hawrylak *et al.,* (2007) observed that Chl and carotenoids (CAR) contents are Se dose dependent and reported that addition of 5 μM Se in nutrient solution enhanced chlorophyll a and b content of lettuce seedlings by 34% and 21% respectively, while same dose increased CAR by 74%. A higher dose of 20 μM did not significantly decrease Chl while it caused 50% reduction in CAR.

Wang (2011) noted a significant increase in fresh weight, Chl and enzymatic activity of Se treated *Trifolium repens* exposed to PEG induced water deficit conditions and were of the view that Se enhanced tolerance by alleviation of lipid peroxidation and activation of antioxidant enzymes such as APX, SOD and GR. An increase in biomass and reduction in membrane lipid peroxidation and free radicals production in wheat seedlings by Se supply has also been reported by Chu *et al.* (2010). The application of Se was observed to significantly improve plant height, Chl and proline accumulation in barley seedlings (Akbulut and Cakir, 2010). Selenium is also reported to enhance starch accumulation in chloroplasts to promote plant growth (Pennanen *et al.,* 2002) and plays a positive role in carbohydrate accumulation in potato (Turakainen *et al.,* 2004). The highest Se addition (0.3 mg Se kg^{-1}) resulted in maximum accumulation of soluble sugar concentration (75cca-90cca mg g^{-1} DW) in upper leaves four weeks after planting while the roots and stolons accumulated maximum soluble sugars at maturity (Seppänen *et al.* 2003).

Selenium supply favors increase in plant biomass by increasing root and antioxidant activity. Yao *et al.,* (2009) in a study observed increased biomass in wheat seedlings under

well watered conditions. Tadina *et al.* (2007) reported Se foliar application of 1g to be favorable for well watered buckwheat, however, no accumulation in biomass was observed by Se application under water deficit conditions. The application of 0.01 mg kg^{-1} of Se increased the dry weight of plants by 3.5% and improved the stress recovery of maize but caution should be taken with regard to timing and concentration of Se application because of its potential growth restricting role (Qiang-yun *et al.,* 2008).

CHAPTER-3 MATERIALS AND METHODS

The present study was conducted with the objectives to evaluate the role of selenium (Se) in enhancing the drought tolerance potential and productivity of wheat (*Triticum aestivum* L.) genotypes through laboratory, wire-house, lysimeter and field experiments. Laboratory and wire house experiments were conducted during 2010-11 to evaluate 15 wheat genotypes for drought tolerance using physiological indices as screening tool. The lysimeter and field experiments were conducted during the years 2011-12 and 2012-13 (spring wheat growing season in Pakistan), to identify an appropriate rate, time and method of Se application helpful in improving growth and yield of wheat under drought stress. Materials and methods used during the course of above mentioned studies are given below:

3.1 Experimental Site and Conditions

The research activities were carried out in the Department of Crop Physiology, University of Agriculture, Faisalabad and in the stress physiology laboratory at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. A series of laboratory, wire-house, lysimeter and field experiments were conducted for this study. The laboratory experiments were conducted in petri dishes whereas the wire house experiments were conducted in plastic pots (8 cm diameter × 15 cm length) containing 430 g of sterilized, washed, fine river sand. Laboratory, wire house and lysimeter experiments were laid out in completely randomized design (CRD) with three replications whereas for field experiments split-plot design with three replications was used. The textural class and physiochemical characteristics (Electrical conductivity, pH and ion contents) of the soil used in this study are presented in Table 3.1. The soil texture was and physiochemical properties were using methods of Dewis and Freitas (1970) and Jackson (1962). The weather data of the experimental site in respect of minimum and maximum temperature (°C), relative humidity (%) and rainfall (mm of the experimental site for the year 2011-2012 and 2012-2013 are given in the figure (Fig. 3.1).

Table 3.1 Physiochemical characteristics of the soil used for field experiments

Soil Characteristics	**Values**
Physical	
Soil texture	Sandy clay loam
Chemical	
Saturation percentage (%)	37.4
EC_e (dSm^{-1})	0.72-0.92
Soil pH_s	7.5-7.8
Organic matter (%)	0.4-0.6
Ca+Mg (meq L^{-1})	3.75-5.76
CO_3 (meq L^{-1})	Nil
HCO_3 (meq L^{-1})	3.5-4.0
NO_3-N (mg kg^{-1})	10.7-14.7
Available K (mg kg^{-1})	108-200
Available P (mg kg^{-1})	8.2-10.8
Total Se content	0.049 mg kg^{-1}

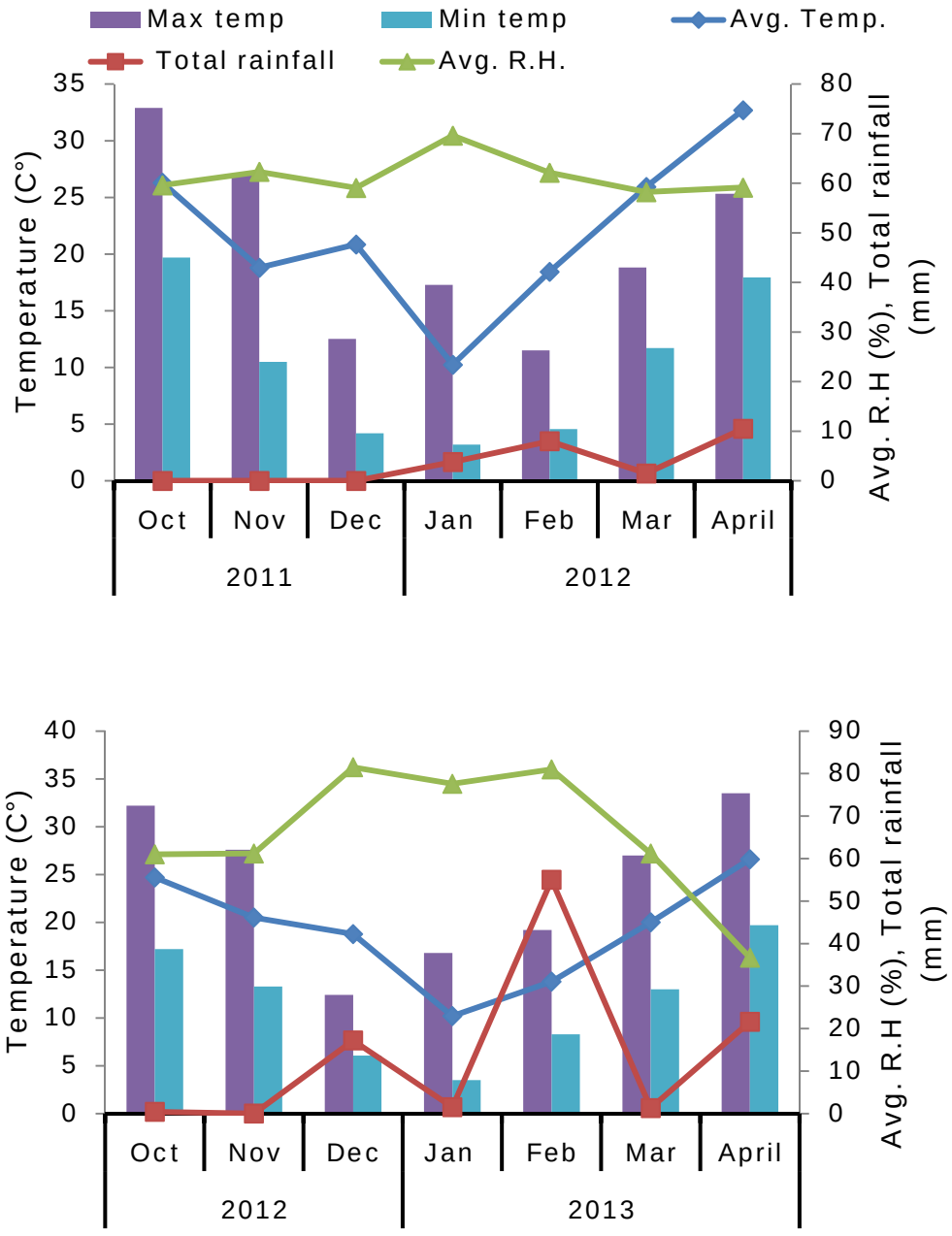

Fig. 3.1 Meteorological data of the experimental site for field experiment for the growing season 2011-12 and 2012-13.

3.2 Wheat Seed

Fifteen local wheat genotypes/lines (Chakwal-86, Pak-81, Manthar-03, Kohistan-97, Chakwal-50, Fsd-08, Sehar-06, Inqlab-91, Pasban-90, Shafaq-06, V_0-5082, V_0-5066, V_0-4178, Lasani-08 and Ufaq-06) were used for this study. The seed of these genotypes was obtained from Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan.

3.3 Laboratory Experiments

Fifteen wheat genotypes were evaluated for their drought tolerance potential at germination and seedling stages in two laboratory experiments. The first experiment was carried out in Petri-dishes to assess germination percentage (GP), promptness index (PI), germination stress tolerance index (GSI), emergence index (EI) and mean emergence time (MET). The solution of PEG-6000 with -0.5 MPa osmotic potential was prepared according to the method of Michel and Kaufmann (1973). Osmometer (Wescor, Model 5520) was used for the determination of osmotic potential of PEG. Sterilized Petri-dishes of 9 cm diameter, each containing twenty seeds (sterilized with 5% sodium hypochlorite solution for five minutes) placed on top of filter paper moistened with distilled water (control) and PEG-6000 solution of osmotic potential -0.5 MPa, were used for this experiment. The Petri-dishes were kept in an incubator (Sanyo-Gallenkamp, UK) for eight days at 25±2°C (Ghodsi, 2004). The data were recorded daily till eight days. The number of seeds germinated was counted to estimate GP, EI, PI and GSI and MET. The seeds that gained approximately 2 mm of root length were considered to be germinated (Afzal *et al.* 2004). Five seedlings were randomly selected from each Petri-dish for the measurement of root length, shoot length and plant fresh weight (FW).

The second laboratory experiment was conducted in the growth chamber (Sanyo-Gallenkamp, UK) having controlled temperature at 25°C; 16 h day length, 200 µmol m^{-2} s^{-1} photosynthetic active radiations (PAR) and 75-80% relative humidity. Ten seeds of each genotype were sown at 100 % field capacity (FC) in plastic pots (8 cm diameter × 15 cm length) containing 430 g of fine, washed and sterilized river sand. One set of pots (control)

was watered regularly while to other set water stress was imposed by withholding water application after seedling emergence. Amount of water evaporated was calculated daily and control plants were re-watered accordingly. At appearance of the wilting symptoms in stressed plants (after three weeks), five plants were harvested randomly from each pot and the root length, shoot length and seedling biomass were recorded. For seedlings biomass, seedlings were placed in an oven at 65°C for 72 hours and then dry weight was recorded. From these data the physiological indices were calculated using the formulae as described below. Both the experiments were repeated thrice and the data presented is the mean of values obtained in three experiments.

3.4 Wire House Experiments

Three pot experiments were conducted in wire house to determine appropriate rates of Se application as seed priming, fertigation and foliar spray, effective in improving the drought tolerance and biomass in wheat plants subjected to water stress at seedling stage. One each drought tolerant (Kohistan-97) and sensitive wheat genotype (Pasban-90) selected from the screening experiments were used for these experiments. Ten healthy sterilized seeds (as described earlier) were sown in plastic pots (8 cm diameter × 15 cm length) containing sand as growth medium. After emergence only five seedlings were maintained in each pot. For Se seed priming, the Se solutions of 25 (1.7 mg L^{-1}), 50 (3.400mg L^{-1}), 75 (5.1 mg L^{-1}) and 100 (6.8 mg L^{-1}) µM were prepared by dissolving Na_2SeO_4 in distilled water. The seeds were soaked in distilled water and the Se solutions and for 0.5 h and one 1 h at 25 °C and later re-dried to their original moisture level. For Se foliar application, the Se solutions of 1.76 (0.12 mg L^{-1}), 3.53 (0.24 mg L^{-1}), 5.29 (0.36 mg L^{-1}) and 7.06 µM (0.48 mg L^{-1}) were developed by dissolving Na_2SeO_4 in distilled water. For fertigation experiment, 3.68 (0.25mg L^{-1}), 7.35 (0.50mg L^{-1}), 11.03 (0.75mg L^{-1}) and 14.70 µM (1.00mg L^{-1}) were developed by dissolving Na_2SeO_4in distilled water. Drought stress in these three experiments was imposed after one week of seed emergence by withholding of water to stressed plants. At appearance of the wilting symptoms in stressed plants (after three weeks), seedlings were harvested, recorded biomass and different physiological indices were calculated. Selenium levels were developed by dissolving sodium selenate (Sigma-Aldrich USA) in distilled water.

Appropriate rate and duration of Se for seed priming, foliar application and fertigation were selected on the basis of maintenance of the highest vales for physiological indices.

3.5 Calculations of Physiological Indices

The number of seeds germinated after eight days were counted for each treatment and replication. The GP, PI, EI, MET and GSI were calculated as:

$$PI = nd_2(1.00) + nd_4(0.75) + nd_6(0.50) + nd_8(0.25)$$ (Sapra *et al.,* 1991)

Where nd_2, nd_4, nd_6 and nd_8 represent the number of seeds germinated on 2^{nd}, 4^{th}, $6^{th}$and 8^{th} day respectively.

EI = (number of seeds germinated/days of first count) +.....+ (number of seeds germinated/days of final count) (AOSA 1983)

$$MET = (\Sigma Dn/\Sigma n)$$ (Moradi-Dezfuli *et al.,* 2008)

Where n represents the number of seeds emerged on day D and D represents the number of days from the onset of seed germination.

GSI = [PI of stressed seeds/ PI of control seeds] × 100 (Bouslama and Schapaugh, 1984)

The plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI) and fresh and dry matter stress tolerance indices (FMSI, DMSI) using the formulae described by Ashraf *et al.* (2008).

PHSI = [Plant height of stressed plant/Plant height of control plant] × 100

DMSI = [Dry matter of stressed plant/Dry matter of control plant] × 100

RLSI = [Root length of stressed plant/Root length of control plant] × 100

SFSI = [Shoot fresh weights of stressed plants / Shoot fresh weights of control plants] x 100

RFSI = [Root fresh weights of stressed plants / Root fresh weights of control plants] x 100

The genotypes were ranked on the basis of their performance using physiological indices as screening tool. Least significant difference (LSD) test (P<0.05) was used to evaluate the significant differences among genotypes and MSTAT-C software package was used for the statistical analysis of the data.

3.6 Lysimeter Experiment

Lysimeters consisting of four cemented tanks each measuring (9 m^2 (3 x 3 m) and 1 m in depth were used to optimize the methods and time of foliar application (vegetative or reproductive growth stage) of Se helpful in improving drought tolerance potential and productivity of wheat plants. The same wheat genotypes, viz. Kohistan-97 and Pasban-90 as used in wire house experiments and two water stress levels i.e., control (100% FC) and water stress (60% FC) were used for this experiment. Individual tanks were separated by 15 cm thick cemented wall on each side of the tank which acted as buffer zone to prevent seepage. Prior to planting, lysimeters were precisely levelled to ensure even distribution of water. A basic dose of urea (110 kg N ha^{-1}) and diammonium phosphate (70 kg $P_20_5ha^{-1}$) was broadcasted and mixed with the surface layer (0 to 15 cm) immediately prior to sowing. The pre-planting irrigation (75 mm) was applied. Seeds were hand drilled when the soil was at field capacity condition. Each genotype was allotted three rows of 0.80 m length and row to row distance of 0.30 m. The control plants were irrigated with 75 mm water (100% field capacity), whereas drought stress was imposed by the application of 45 mm irrigation water (60% field capacity) after sowing. The amount of irrigation water applied to the lysimeter tanks at regular intervals was measured using a water meter. All lysimeters were protected from rain by manually operated shelter equipped with movable sheet of transparent flexible plastic. The lysimeters were manually weeded and hoed whenever found necessary.

Plants were grown up to maturity and data on various physiological, biochemical, plant nutrients analyses and yield and yield components were recorded following methods described in section 3.8.

3.7 Field Experiments

Two field experiments were conducted during two consecutive years (2011-12 and 20112-13) to optimize the methods and time of foliar application (vegetative or reproductive growth stage) of Se helpful in improving drought tolerance potential and productivity of wheat plants. The experiments were laid out in a split-split plot design and replicated thrice. The wheat genotypes, viz. Kohistan-9 and Pasban-90 as used in pot and lysimeter experiments and two water stress levels i.e., no stress (normal conditions) and water stress imposed at tillering and anthesis stages were used for these experiments. The stress was imposed by withholding the irrigation. During 2011-12, the no stress treatment received 397 mm water (375 mm irrigation + 22 mm rainfall) during the whole wheat growing season, whereas water- stressed plants received 322 mm water (300 mm irrigation + 22 mm rainfall). Similarly in the next year (2012-13), the normal plants received 473 mm water (375 mm irrigation + 98 mm rainfall), whereas the plants exposed to drought stress received 398 mm water (300 mm irrigation + 98 mm) during the whole crop growth period. Recommended dose of N, P and K as urea (110 kg N ha^{-1}), diammonium phosphate (70 kg P_2O_5 ha^{-1}) and potassium sulphate (50 kg SOP ha^{-1}) was applied. All P and K were applied at the time of sowing but N was applied in three split doses. Plants were grown up to maturity and data regarding yield and yield components was recorded.

3.8 Data Collection

3.8.1 Physiological Parameters

3.8.1.1 Leaf Water Potential (-MPa)

The second leaf from top (fully expanded youngest leaf) of plants from each treatment was used to determine leaf water potential (Ψ_w). The measurements were made between 8.00 to 10.00 a.m. with Scholander type pressure chamber (ARIMAD-2, ELE-International).

3.8.1.2 Leaf Osmotic Potential (-MPa)

For leaf osmotic potential (Ψ_s) measurements, the same leaves as used for water potential measurements, were frozen at -20^{o}C. The frozen leaf material was then thawed and

cell sap was extracted while crushing the leaves with a glass rod. The sap so extracted was directly used for the determination of Ψ_s using an Osmometer (Wescor 5520).

3.8.1.3 Turgor Potential (MPa)

Turgor potential (Ψ_p) was calculated as the difference between Ψ_w and Ψ_s values.

$$(\Psi_p) = (\Psi_w) - (\Psi_s)$$

3.8.1.4 Relative Water Contents (%)

For relative water content's (RWR) measurements, three leaves (flag leaf) from each treatment were taken. Fresh weight (FW) of each sample was recorded using a digital electrical balance (Chyo, MK-500C) and leaves were dipped in test tube containing distilled water for 24 hours. Then leaves were taken out, wiped with the tissue paper and the turgid weight (TW) was recorded. The samples were dried at 65°C for 72 h and dry weight (DW) of each sample was recorded. Relative water contents were calculated using the formula given by Cornic (1994).

$$RWC = [(FW-DW) / (TW-DW)] \times 100$$

3.8.1.5 Gas Exchange Characteristics

A fully expanded youngest leaf of each plant (the second leaf from top) was used to measure the instantaneous net photosynthetic rate (*Pn*), transpiration rate (*E*) and stomatal conductance (*gs*) by using photosynthesis measuring-system CI-340 portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England). These measurements were taken between 9.00 to 11.00 a.m. with the following adjustments: molar flow of air per unit leaf area 403.3 mmol m^{-2} S^{-1}, atmospheric pressure 99.9kPa, water vapor pressure into chamber ranged from 6.0 to 8.9 m bar, PAR at leaf surface was maximum up to 1711 $molm^{-2}$ s^{-1}, temperature of leaf ranged from 28.4 to 32.4ºC, ambient temperature ranged from 22.4-27.9ºC and ambient CO_2 concentration was 352 mol mol^{-1}.

3.8.1.6 Pigments

Chlorophyll (Chl) and carotenoid (Car) contents were determined using the methods of Arnon (1949) and Davies (1976). Fresh leaves of (0.5 g) were chopped into segments of 0.5 cm and extracted with 5 mL acetone (80%) at 10^0C over-night. Centrifuged the material at 14000 x g for 5 minutes and measured the absorbance of the supernatant at 645, 652, 663 and 480 nm on spectrophotometer (Hitachi, U-2800). Calculated Chl a, Chl b, total Chl and Car contents as described below.

Chl_a (mg g^{-1} FW) = [12.7 (OD 663) -2.69 (OD 645)] x V/1000 x W

Chl_b (mg g^{-1} FW) = [22.9 (OD 645) -4.68 (OD 663)] x V/1000 x W

Total Chl_{a+b} (mg g^{-1} FW) = [20.2 (OD 645) + 8.02 (OD 663)] x V/100 x W

Carotenoids (µg g^{-1} FW) = A^{car}/E_{mx}^{100}

Where V is the volume of sample extract and W is the weight of the sample and

Acar = (OD480) + 0.114 (OD663)-0.638 (OD645); E_{max}^{100} cm =2500

3.8.2 Biochemical parameters

3.8.2.1 Total Soluble Proteins (mg g^{-1} FW)

Total soluble proteins (TSP) were determined using the method of Lowry *et al.* (1951), detail is given below:

Reagents

Phosphate buffer (0.2 M): Following chemicals were used to prepare the phosphate buffer.

1. One-molar solution of $NaH_2PO_4.2H_2O$ (156.01 g L^{-1}) was prepared as the stock.
2. One-molar solution of Di-sodium hydrogen phosphate ($Na_2HPO_4.2H_2O$, 177.99 g L^{-1}) was prepared as the stock.

Copper Reagents

Solution A

Na_2CO_3	=	2.0 g
NaOH	=	0.2 g
Sodium potassium tartarate	=	1.0 g

All the three chemicals were dissolved in distilled water and the volume was made to 100 mL.

Solution B

$CuSO_4.5H_2O$ solution: 0.5g $CuSO_4.5H_2O$ was dissolved in 100 mL distilled water

Solution C

Fifty mL of solution A and 1.0 mL of solution B were mixed to prepare alkaline solution. This solution was always prepared fresh.

Folin Phenol Reagent

One hundred g of sodium tungstate and 25 g of sodium molybdate were dissolved in 700 mL of distilled water. Fifty mL of 85% orthophosphoric acid and 100 mL of HCl were added and the mixture was refluxed for 10 h. Then 150 g of lithium sulfate was added along with 50 mL of distilled water. A few drops of Br_2 were also added. The mixture was boiled without condenser for 15 min to remove extra Br_2. The mixture was then cooled and diluted to 1000 mL.

Standard Bovine Serum Albumin (BSA) solution (1 µg mL^{-1})

Ten mg of Bovine serum albumin (BSA) was dissolved in 10.0 mL of distilled water.

Extraction

Fresh leaf material (0.5 g) was chopped in 10 mL of phosphate buffer (0.2 M) of pH 7.0 and was ground. The ground leaf material was centrifuged at 5000 x g for 5 min. The supernatant was used for protein determination.

Procedure

One mL of the leaf extract from each treatment was taken in a test tube. The blank contained 1 mL of phosphate buffer (pH 7.0). One mL of solution C was added to each test tube. The reagents in the test tube were thoroughly mixed and allowed to stand for 10 min at room temperature. Then 0.5 mL of Folin-Phenol reagent (1:1 diluted) was added, mixed well and incubated for 30 min. at room temperature. The optical density (OD) was read at 620 nm on a spectrophotometer (Hitachi, U-2800). The protein concentration was calculated by using standard curve developed by different concentration of Bovine serum albumin (BSA).

3.8.2.2 Total Free Amino Acids (µmol g^{-1} FW)

Total free amino acids (TFA) were determined according to Hamilton and Van Slyke (1973). Fresh plant leaves (0.5 g) were chopped and extracted with phosphate buffer (0.2 M) having pH 7.0. Took 1 mL of the extract in 50 mL volumetric flask, added 1 mL of pyridine (10 %) and 1mL of ninhydrin (2%) solutions in flask. Ninhydrin solution was freshly prepared by dissolving 2 g ninhydrin in 100 mL distilled water. The flasks with sample mixture, heated in boiling water bath for about 30 min. Volume of each flask was made up to 50 mL with distilled water. Read the optical density of the colored solution at 570 nm using spectrophotometer (Hitatchi, U-2800). Developed a standard curve with Leucine and calculated free amino acids using the formulae given below:

Total free amino acids (µmol g^{-1} FW) = (Graph reading of sample) X (Dilution factor)

Dilution Factor = (Volume of the sample/weight of the sample)

3.8.2.3 Total Soluble Sugars (mg g^{-1} FW)

Total soluble sugars (TSS) were determined according to the method of Yemm and Willis (1954).

Extraction

Dried plant material was ground well in a micro mill and the material was sieved through 1 mm sieve of micro mill. Plant material (0.5 g) was extracted 5 mL of 80% ethanol solution for 6 h at 60^{o}C. This extract was used for the estimation of total soluble sugars.

Reagents

Anthrone reagent was prepared by dissolving 150 mg of anthrone in 72% H_2SO_4 solution. This reagent was freshly prepared whenever needed.

Procedure

Plant extract 0.1 mL was taken in 25 mL test tubes and 6 mL anthrone reagent was added to each tube, heated in boiling water bath for 10 min. The test tubes were ice-cooled

for 10 min. and incubated for 20 min. at room temperature (25ºC). Optical density was read at 625 nm on a spectrophotometer (Hitatchi, U-2800). The concentration of soluble sugars was calculated from the standard curve developed by using different concentration of glucose according to the above procedure.

3.8.2.4 Proline Determination

The proline was determined according to the Bates *et al.* (1973) method. Fresh leaf material of 0.5 g was ground in 10 mL of 3% sulfo-salicylic acid. The sample material was filtered by using Whatman No. 40 filter paper. Two mL of the filtrate was taken in a 25 mL test tube and reacted with 2 mL acid ninhydrin solution (Acid ninhydrin solution was prepared by dissolving 1.25 g ninhydrin in 30 mL of glacial acetic acid and 20 mL of 6 M orthophosphoric acid) and 2 mL of glacial acetic acid and test tubes were heated for 1 h at 100ºC. Reaction was terminated in an ice bath, the reaction mixture was extracted with 10 mL toluene which form a chromophore. Continuous air stream was passed vigorously for 1-2 minutes in the reaction mixture to separate aqueous phase from the chromophore containing toluene. Isolated colored phase was allowed to stand for 2-3 minutes room temperature and its absorbance was noted at 520 nm using above mentioned model of spectrophotometer. Toluene was used as a blank. The proline concentration was calculated by using a standard curve developed by Analar grade proline and calculated on FW basis as follows:-

mmole proline g^{-1} FW = [{(mg of proline mL^{-1}) x (mL of toluene)}/ (wt. of sample/5)]/115

3.8.3 Antioxidant Enzymes

The activities of peroxidase (POX), catalase (CAT), and ascorbate peroxidase (APX) were determined spectrophotometrically. Leaves were homogenized in a medium composed of 50 m*M* phosphate buffer with 7.0 pH and 1 m*M* dithiothreitol (DTT) as described by Dixit *et al*., (2001).

3.8.3.1 Catalase (Units min^{-1} g^{-1} FW)

Catalase activity (CAT) was assayed by measuring the conversion rate of hydrogen peroxide to water and oxygen molecules, following the method described by Chance and Maehly (1955). The activity was assayed in 3 mL reaction solution comprising of 50 m*M*

phosphate buffer with 7.0 pH, containing 5.9 m*M* of H_2O_2 and 0.1 mL enzyme extract. The catalase activity was determined by decline in absorbance at 240 nm after every 20 sec due to consumption of H_2O_2. Absorbance change of 0.01 unit min^{-1} was defined as one unit catalase activity.

3.8.3.2 Peroxidase (Units min^{-1} g^{-1} FW)

The activity of peroxidase (POX) was determined by measuring peroxidation of H_2O2 with guaiacol as an electron donor (Chance and Maehly, 1955). The reaction solution for POD consists of 50 m*M* phosphate buffer with pH 5, 20 m*M* of guaiacol, 40 m*M* of H_2O_2 and 0.1 mL enzyme extract. The increase in the absorbance due to the formation of tetraguaiacol at 470 nm was assayed after every 20 sec. One unit of the enzyme was considered as the amount of the enzyme that was responsible for the increase in OD value of 0.01 in 1 min. The enzyme activity was determined and expressed as unit min^{-1} g^{-1}FW basis.

3.6.3.3 Ascorbate Peroxidase (ABA digested g^{-1} FW h^{-1})

Ascorbate peroxidase (APX) activity was measured by monitoring the decrease in absorbance of ascorbic acid at 290 nm (extinction coefficient 2.8 mM cm-1) in a 1 mL reaction mixture containing 50 m*M* phosphate buffer (pH 7.6), 0.1 m*M* Na-EDTA, 12 m*M* H_2O_2, 0.25 mM ascorbic acid and the sample extract as described by Cakmak (1994).

3.8.4 Nutrients Analyses

Digestion

Dried ground material (0.5 g) was taken in digestion tubes and 5 mL of concentrated H_2SO_4 were added to each tube (Wolf, 1982). All the tubes were incubated overnight at room temperature. Then 0.5 mL of H_2O_2 (35%) poured down the sides of the digestion tube, ported the tubes in a digestion block and heated at 350^oC until fumes were produced. They were continued to heat for another 30 min. The digestion tubes were removed from the block and cooled and 0.5 mL of H_2O_2 was slowly added and the tubes were placed back into the digestion block. The above step was repeated till the cooled digested material was colorless. The volume of the extract was maintained up to 50 mL in volumetric flasks. The extract was

filtered and used for determining selenium (Se), phosphorous (P), potassium (K), iron (Fe), zinc (Zn), magnesium (Mg) and calcium (Ca).

3.8.4.1 Estimation of P

Phosphorus (P) was determined by a spectrophotometer (Jackson, 1962). The extracted material (2 mL) was dissolved in 2 mL of Barton's reagent and total volume was made 50 mL.

The Barton's reagent was prepared as described below:

Barton's Reagent

Solution A:

Ammonium molybdate 25 g was dissolved in 400 mL of distilled water.

Solution B:

Ammonium metavandate 1.25 g was dissolved in 300 mL of boiling water, cooled, and 250 mL of conc. HNO_3 were added. The solution was again cooled at room temperature.

The solution A and B were mixed and the volume was made to 1 L. It was stored at room temperature. The samples were kept for half an hour before analyzing P. The values of P were calculated by using standard curve.

3.8.4.2 Estimation of K

Potassium (K) was determined using flame photometer (Jenway PFP 7).

3.8.4.3 Estimation of Se, Fe, Zn, Mg and Ca

Digested samples were tested for Se, Ca, Mg, Fe and Zn by ICP-OES (Optima 2100 DV Perkin-Elmer).

3.8.5 Yield and Yield Components

3.8.5.1 Number of Tillers m^{-2}

A steel quadrant of one meter square was used to record number of tillers m^{-2}. Total number of tillers of randomly selected quadrant in each plot was counted at the final harvest and then average number of tillers of each plot was recorded.

3.8.5.2 Number of Productive Tillers m^{-2}

Productive tillers of randomly selected quadrates in plot were counted by subtracting the non-productive tillers from total number of tillers.

3.8.5.3 Spike Length (cm)

To record spike length, five plants were selected from each quadrate and the spike length of these were measured separately and then average spike length was calculated.

3.8.5.4 Number of Spikelets $Spike^{-1}$

Five spikes were removed from randomly selected plant in each quadrate. Numbers of spikelets in each spike were counted and average was calculated.

3.8.5.5 Number of Grains $spike^{-1}$

Five spikes were taken randomly from each quadrate after threshing manually, their grains were counted and average was worked out.

3.8.5.6 Thousand-grains Weight (g)

The thousand grains from each quadrate were randomly selected/counted and their weight was recorded with the help of an electric balance to get 1000-grain weight.

3.8.5.7 Biological Yield (t ha^{-1})

Biological yield per meter square was calculated by weighing plants on an electric balance and was converted in to tons ha^{-1}.

3.8.5.8 Grains Yield (t ha^{-1})

The grain yield per meter square was calculated after threshing plants manually and weighing grains on an electric balance and was converted in to tons ha^{-1}.

3.8.5.9 Harvest Index (%)

It was recorded for each quadrate by using the formula:

HI = [(Economic yield* / Biological yield**] × 100

* Economic yield = grain yield

**Biological yield = grain + straw

3.9 Statistical Analyses

The data recorded in different experiments were analyzed statistically using analysis of variance technique and the MSTAT-C Computer Program was used for this purpose. Least Significant Difference (LSD) test at 5% probability level was used to compare the significant means (Steel *et al.,* 1997).

CHAPTER-4 RESULTS

4.1. Screening of Wheat Genotypes/Lines for Drought Tolerance

Screening of 15 wheat genotypes was carried out in lab/greenhouse experiments using physiological indices as selection tools for drought tolerance. The results are given below:

4.1.1. Promptness index (PI)

A significant decrease ($P \leq 0.01$) in seed PI was recorded due to osmotic stress created by PEG-6000 as compared to control treatment (Table 4.1). Osmotic stress decreased the PI of plants by 39%. The highest value for PI (47.8) was noted under normal supply of water while the lowest value (29.2) was recorded in genotypes exposed to osmotic stress of -0.5 MPa.

The different wheat genotypes also differed significantly for this variable. Among genotypes, the maximum value (53.5) was observed in Kohistan-97 sown in distilled water (control) which was statistically at par with PI of Manthar-03 (50.7) while its minimum value (27.0) was observed in Pasban-90 (Fig. 4.1). The genotypes Chakwal-86, Chakwal-50, V_0-5082 and Lasani-08 had PI values between 40 and 45 and can be ranked as moderately tolerant to drought stress. The low values of PI for Inqlab-91 (31.3), FSD-08 (31.3), Pak-81 (31.8) and Pasban-90 (27.0) marked them as the most susceptible genotypes to drought stress. The interaction between genotypes and drought stress was also significant. The highest value (60.3) was obtained in Sehar-06 which was statistically at par with PI value of Chakwal-50 (56.0) while the lowest (17.7) was in Manthar-03 under osmotic stress of -0.5 MPa.

4.1.2. Germination stress tolerance index (GSI)

The promptness index of stressed and controlled seeds of different wheat genotypes was used to calculate GSI. A significant reduction in GSI was observed under PEG-6000 induced osmotic stress of -0.5 MPa. Data regarding GSI showed that maximum value (80%) was noted in Manthar-03 which was found to be statistically at par with Kohistan-97 (78%) and Chakwal-86 (71%).Wheat genotypes Pak-81, Chakwal-50,

Table 4.1: Analysis of variance for promptness index (PI), emergence index (EI) and mean emergence time (MET) in 15 wheat genotypes exposed to PEG-6000 induced osmotic stress

SOV[a]	Promptness Index (PI)	Emergence Index (EI)	Mean Emergence Time (MET)
Genotypes (G)	***	***	NS
Water stress level (W)	***	***	NS
G×W	***	NS	NS
CV[b]	6.78	15.84	4.67

NS= non-significant, *significant at $P< 0.05$, ** significant at $P< 0.01$,

*** significant at $P< 0.001$

[a] Source of variation, [b] Co-efficient of variation

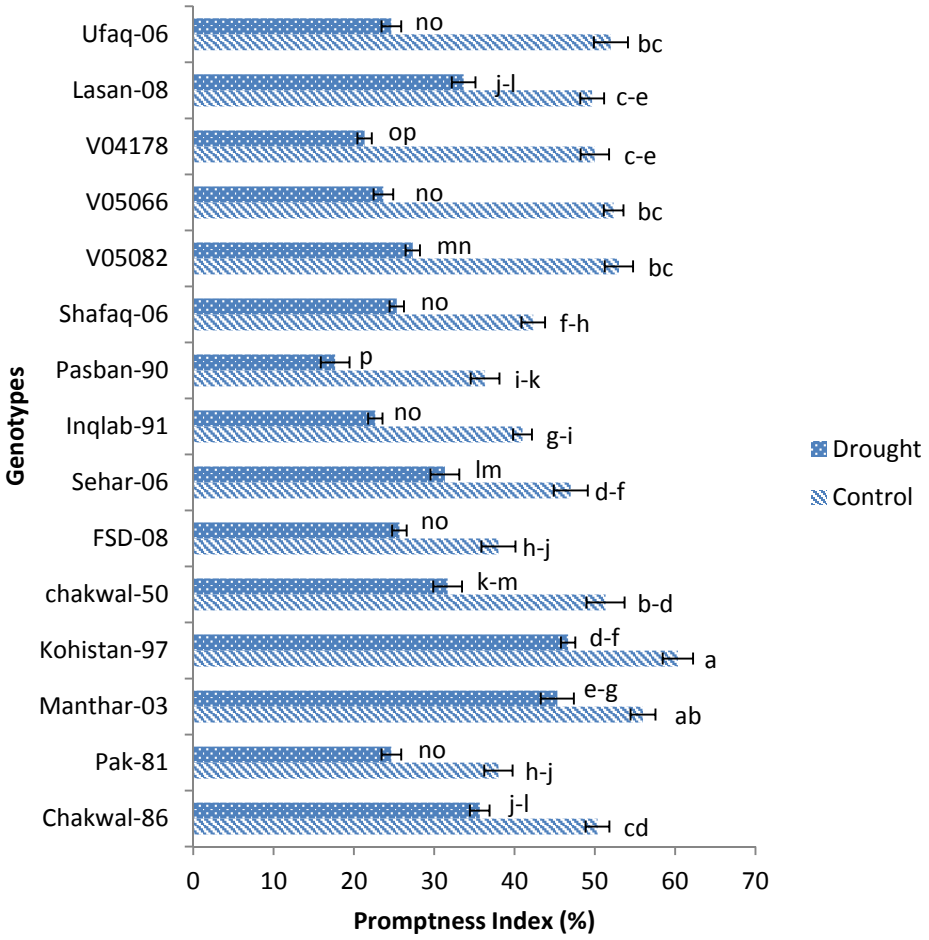

Figure 4.1: Promptness index (PI) of 15 wheat *(Triticum aestivum* L.) genotypes subjected to PEG-6000 induced osmotic stress (-0.5 MPa). Values are the means of three experiments±SE and represent data significantly different at $P < 0.05$.

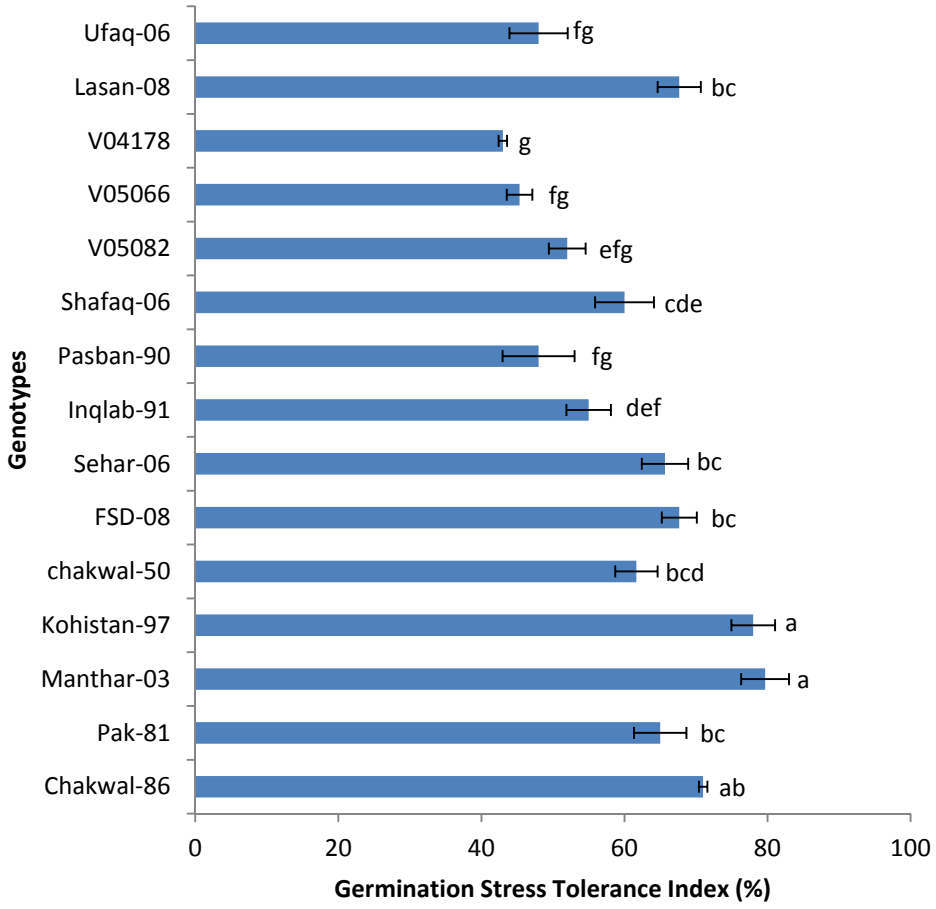

Figure 4.2: Germination stress tolerance index (GSI) of 15 wheat *(Triticum aestivum* L.) genotypes subjected to PEG-6000 induced osmotic stress (-0.5 MPa). Values are the means of three experiments±SE and represent data significantly different at $P < 0.05$.

FSD-08, Sehar-06 and Lasani-08 had GSI values ranged between 60-70% and were categorized as medium tolerant to drought stress. The minimum value (43%) was recorded in V_0-4178 which was statistically at par with GSI values estimated for V_0-5066 (45%), Pasban-90 (48%), Ufaq-06 (48%) and V_0-5082 (52%) (Fig. 4.2).

4.1.3. Emergence index (EI)

The number of seeds germinated on each alternate day till day of final count was used to calculate emergence index (EI) of different wheat genotypes. The PEG-6000 induced osmotic stress decreased EI by 41% as compared to that recorded for normal supply of water. The maximum value for EI (19.7) was observed in seed exposed to distilled water while genotypes subjected to osmotic stress (-0.5 MPa) had the minimum value (11.6) for this variable.

Analysis of variance showed highly significant ($P \leq 0.001$) difference among genotypes for EI (Table 4.1). The maximum E.I (22.0) was observed in Kohistan-97, which was statistically at par with Manthar-03 (20.8) while Pasban-90 attained the minimum value for E.I (11.0) statistically at par with EI value of Inqlab-91 (12.7), Pak-81 (13.3), FSD-08 (13.3), and Shafaq-06 (13.5) (Fig. 4.2). The second highest value for EI was recorded in Chakwal-86 (17.3) which non-significantly differed to EI of V_0-5082 (16.5), Lasani-08 (16.5) Chakwal-50 (16.2), Sehar-06 (16.00), Ufaq-06 (15.7), V_0-5066 (15.5), V_0-4178 (14.5), and (Fig 4.3). The interaction between genotypes and water stress treatment was non-significant for EI (Table 4.1).

4.1.4. Mean emergence time (MET)

The average between number of seeds emerged and the number of days from the onset of seed germination was used to calculate mean emergence time (MET). Analysis of variance showed non-significant difference between water stress level and normal water supply (Table 4.1). Similarly non-significant difference was observed between genotypes (G) and water stress level (W) for this variable which indicates that drought stress does not affect the MET of wheat genotypes (Fig. 4.4).

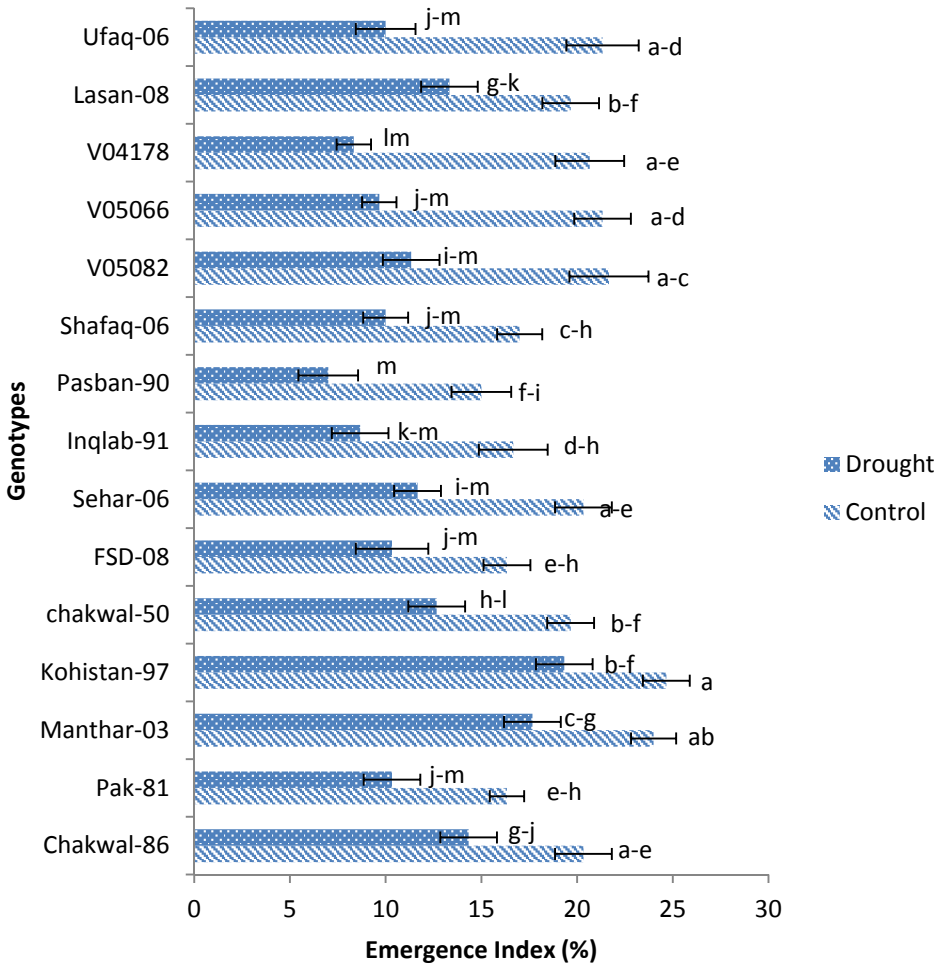

Figure 4.3: Emergence index (EI) of 15 wheat *(Triticum aestivum* L.) genotypes subjected to PEG-6000 induced osmotic stress (-0.5 MPa). Values are the means of three experiments±SE and represent data significantly different at $P < 0.05$.

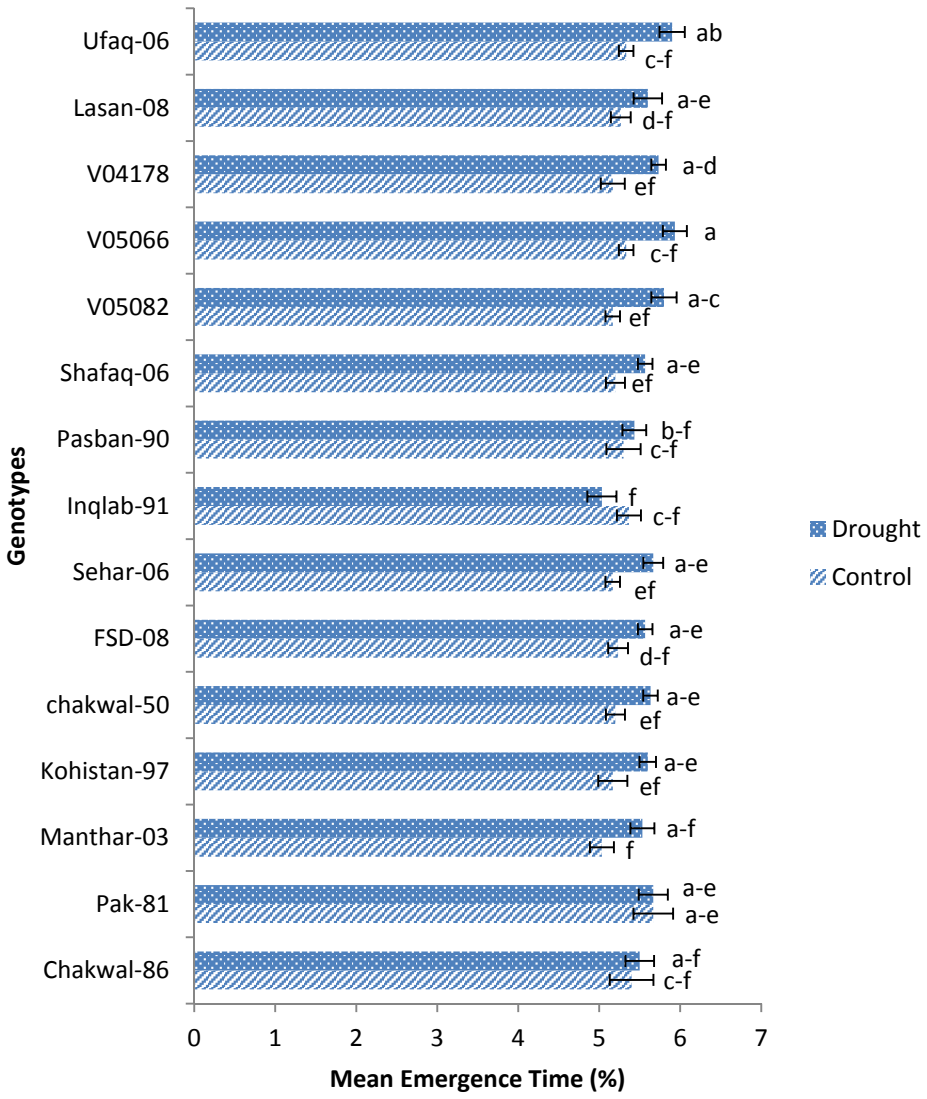

Figure 4.4: Mean emergence time (MET) of 15 wheat *(Triticum aestivum* L.) genotypes subjected to PEG-6000 induced osmotic stress (-0.5 MPa). Values are the means of three experiments±SEand represent data significantly different at $P < 0.05$.

4.2. Screening of Wheat Genotypes/Lines for Drought Tolerance

A second experiment was conducted in plastic pots (8×12 cm) containing 430 g of sterilized sand maintained at 100% field capacity (FC). Ten seeds in each pot were sown and kept in growth room running at 28±2ºC with 10h photoperiod. During the course of experiment, one set of pots (control) was irrigated regularly to maintain 100%FC, while water stress was imposed in the other set of pots by withholding water. The seedlings were allowed to grow for 3 weeks, then seedlings were harvested and different physiological indices were estimated. Results regarding these indices are given below:

4.2.1. Plant height stress tolerance index (PHSI)

The shoot length of controlled and water stressed seedlings was used to calculate plant height stress tolerance index (PHSI) in 15 different wheat genotypes. A significant reduction in plant height was noted in the seedlings, exposed to PEG-6000 induced osmotic stress, of all genotypes. Maximum value for PHSI was recorded in Chakwal-50 (89%) which did not differ significantly to that obtained for Kohistan-97 (87%) and Pak-81 (86%). Wheat genotype Pasban-90 possessed the minimum value (45%) for PHSI (Fig. 4.5). The comparison among wheat genotypes indicated that V_0-5082, Lasani-08, Chakwal-86, V_0-4178 and V_0-5066 had statistically similar values for PHSI (81%, 81%, 79%, 79% and 77% respectively).

4.2.2. Root length stress tolerance index (RLSI)

The root length of seedlings under normal water supply and water stress conditions was used to calculate root length stress tolerance index (RLSI). An increase in RLSI due to limited water supply was noted in almost all the genotypes. The maximum value (151.7) for RLSI was obtained in Pak-81 which was statistically similar to that of Chakwal-86 (151%), FSD-08 (150.0) and Chakwal-50 (141%) while minimum value was recorded in Manthar-03 (108%) and was statistically at par withV_0-4178 (109%), Shafaq-06 (110%), Kohistan-97 (113%), Sehar-06 (117%) and Pasban-90 (119%) (Fig. 4.6).

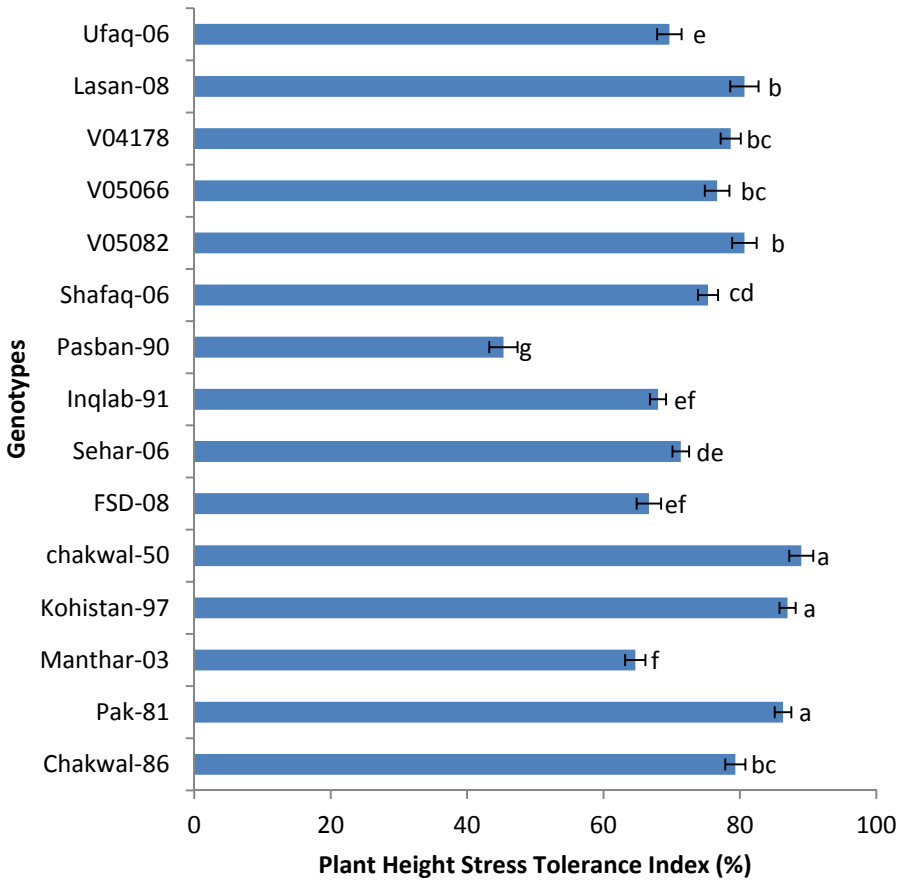

Figure 4.5: Plant height stress tolerance index (PHSI) of 15 wheat *(Triticum aestivum* L.) genotypes subjected to drought stress. Values are the means of three experiments±SE and represent data significantly different at $P < 0.05$.

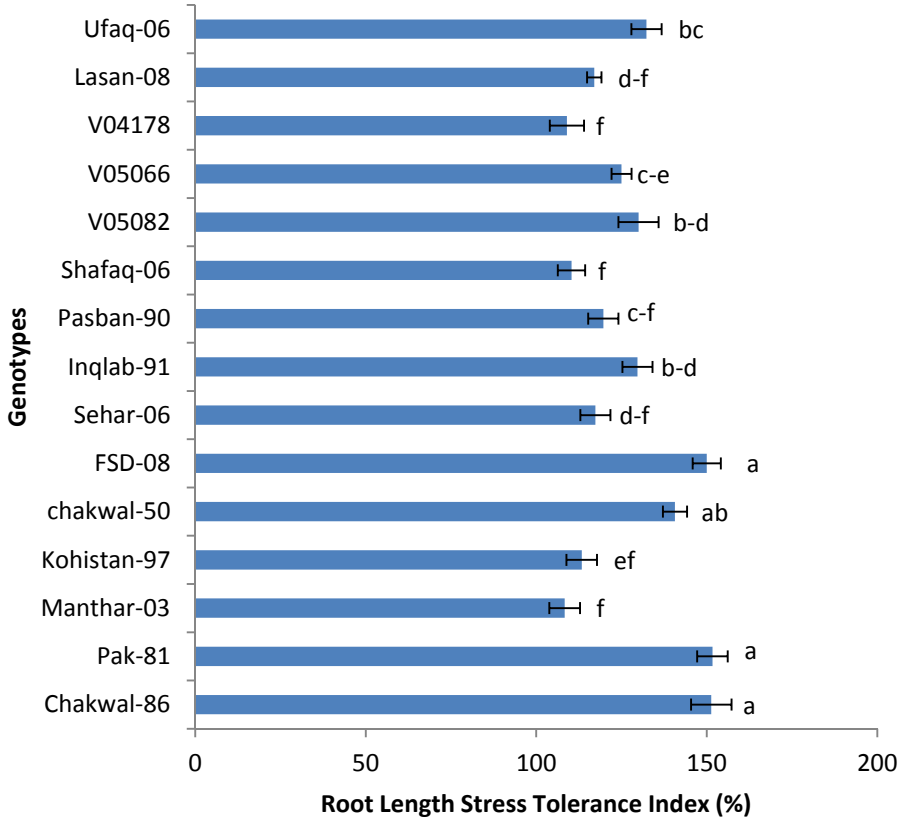

Figure 4.6: Root length stress tolerance index (RLSI) of 15 wheat *(Triticum aestivum* L.) genotypes subjected to drought stress. Values are the means of three experiments±SE and represent data significantly different at $P < 0.05$.

4.2.3. Dry matter stress tolerance index (DMSI)

The dry weight of shoot and root of seedlings under both normal and water stress conditions was used to calculate dry matter stress tolerance index (DMSI). The dry weight of all genotypes decreased significantly under drought stress. The highest value (50%) for DMSI was recorded in V_0-5082 and V_0-5066 which was statistically at par with Kohistan-97 (46%) while the minimum value (17%) was noted in FSD-08 (Fig. 4.8). Non-significant variations for DMSI were estimated in Shafaq-06 (38%), Sehar-06 and Ufaq-06 (36%), Chakwal-86 and (35%), Manthar-03 and Pasban-90 (33%) (Fig. 4.7).

Ranking of wheat genotypes on the basis of performance under drought stress

The ranking of genotypes on the basis of their performance under drought stress was done on the basis of score allotted to each index (Table 4.2). The maximum score was attained by Kohistan-97 and was ranked at first position; Manthar-03, Chakwal-86, Pak-81 and Chakwal-50 were the next and the last position was occupied by Pasban-90.

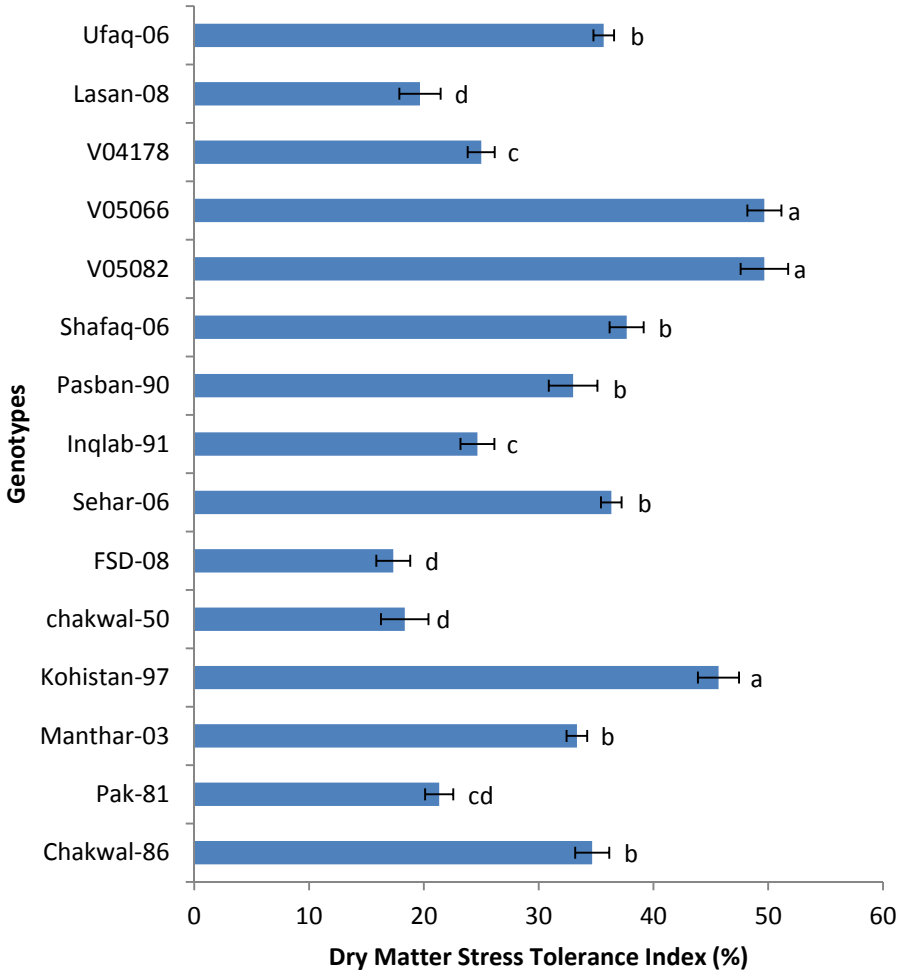

Figure 4.7: Dry matter stress tolerance index (DMSI) of 15 wheat *(Triticum aestivum* L.) genotypes subjected to drought stress. Values are the means of three experiments±SE and represent data significantly different at $P < 0.05$.

Table 4.2: Ranking of wheat genotypes on the basis of performance under drought stress

Sr #	Genotypes/ Lines	P.I (10)	E.I (10)	MET (10)	GSI (10)	PHSI (20)	DMSI (20)	RLSI (20)	Total (100)	Ranking
1	Chakwal-86	7.6	7.6	9.4	8.9	17.8	14.0	20.0	85.3	2
2	Pak-81	5.2	5.3	9.7	8.2	19.4	8.6	20.0	76.4	8
3	Manthar-03	9.4	9.5	9.3	10.0	14.5	13.4	14.3	80.4	4
4	**Kohistan-97**	**10.0**	**10.0**	**9.5**	**9.8**	**19.6**	**18.4**	**14.9**	**92.2**	**1**
5	Chakwal-50	6.7	6.9	9.5	7.7	20.0	7.4	18.5	76.7	7
6	FSD-08	5.5	5.6	9.5	8.5	15.0	7.0	19.8	70.9	12
7	Sehar-06	6.5	6.5	9.7	8.2	16.0	14.6	15.5	77	6
8	Inqlab-91	4.8	5.0	9.4	6.9	15.3	9.9	17.1	68.4	13
9	**Pasban-90**	**3.7**	**3.8**	**9.3**	**6.0**	**10.2**	**13.3**	**15.8**	**62.1**	**15**
10	Shafaq-06	5.2	5.3	9.4	7.6	16.9	15.2	14.5	74.1	10
11	V_0-5082	5.8	5.9	9.8	6.5	18.1	20.0	17.2	83.3	3
12	V_0-5066	4.9	5.1	9.9	5.7	17.2	20.0	16.5	79.3	5
13	V_0-4178	4.5	4.5	9.7	5.4	17.7	10.1	14.4	66.3	14
14	Lasani-08	7.1	7.2	9.6	8.5	18.1	7.9	15.4	73.8	11
15	Ufaq-06	5.3	5.6	10.0	6.0	15.7	14.4	17.4	74.4	9

4.3. Optimizing Selenium Level and Duration for Seed Priming

To select optimal Se level and duration optimum for seed priming, two wheat genotypes, one drought tolerant (Kohistan-97) and the other sensitive (Pasban-90) one, were used. Effective Se level and duration of seed priming was estimated on the basis of physiological indices.

4.3.1. Plant height stress tolerance index (PHSI)

The analysis of variance showed that seed priming with Se solutions significantly increased ($P< 0.001$) PHSI of wheat seedlings (Table 4.3).The plants maintained the highest PHSI (79%) by Se seed treatment of 100 µM which was statistically related to the value (78%) obtained with 75 µM. A non-significant ($P> 0.05$) difference was recorded between 100 µM (74%) and 25 µM (72%) for this variable whereas hydro-priming gave the lowest value (66%) for PHSI (Fig. 4.8 a, b).

A highly significant difference ($P<0.001$) was also observed for duration of seed priming. One hour priming (1 h) with Se solutions gave significantly higher PHSI (75%) than half hour (0.5 h) priming (72%). Similarly, drought tolerant wheat genotype (Kohistan-97) exhibited higher value than sensitive genotype (Pasban-90) for this variable (Fig. 4.8 b).

The interaction among genotypes (G), Se treatments (S) and seed priming duration (T) was also significant ($P<0.05$). The maximum value (81%) was recorded in Kohistan-97 whose seeds were primed with Se solution of 100 µM for 1 h. The minimum value for PHSI (60%) was observed in Pasban-90 seedlings raised from the distilled water primed seeds for 0.5 h (Fig. 4.8 a, b).

4.3.2. Root length stress tolerance index (RLSI)

The different Se seed priming solutions exhibited highly significant variation ($P<0.001$) for RLSI. The maximum value was observed in seedlings grown from seeds primed with Se solution of 100 µM closely followed by Se seed treatment of 75 µM whereas Se seed priming @ 25 µM gave the minimum value for RLSI (114%) which was statistically at par with priming of seeds with distilled water (117%) (Fig. 4.9 a, b).

Table 4.3: Analysis of variance for plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI), shoots fresh weights stress tolerance index (SFSI), roots fresh weights stress tolerance index (RFSI) and dry matter stress tolerance index (DMSI) in two wheat genotypes primed with different selenium solutions for half hour (0.5 h) and one hour (1 h) under drought stress.

SOV[a]	PHSI (%)	RLSI (%)	SFSI (%)	RFSI (%)	DMSI (%)
Genotypes (G)	***	NS	***	***	***
Selenium treatments (S)	***	***	***	***	***
Priming duration (T)	***	***	***	***	***
G×S	NS	NS	***	NS	*
G×T	***	***	*	NS	NS
S×T	NS	**	***	NS	***
G×S×T	*	*	*	*	NS
CV[b]	4.17	2.50	3.48	8.29	3.79

NS= non-significant, *significant at $P< 0.05$, ** significant at $P< 0.01$,

*** significant at $P< 0.001$

[a] Source of variation, [b] Co-efficient of variation

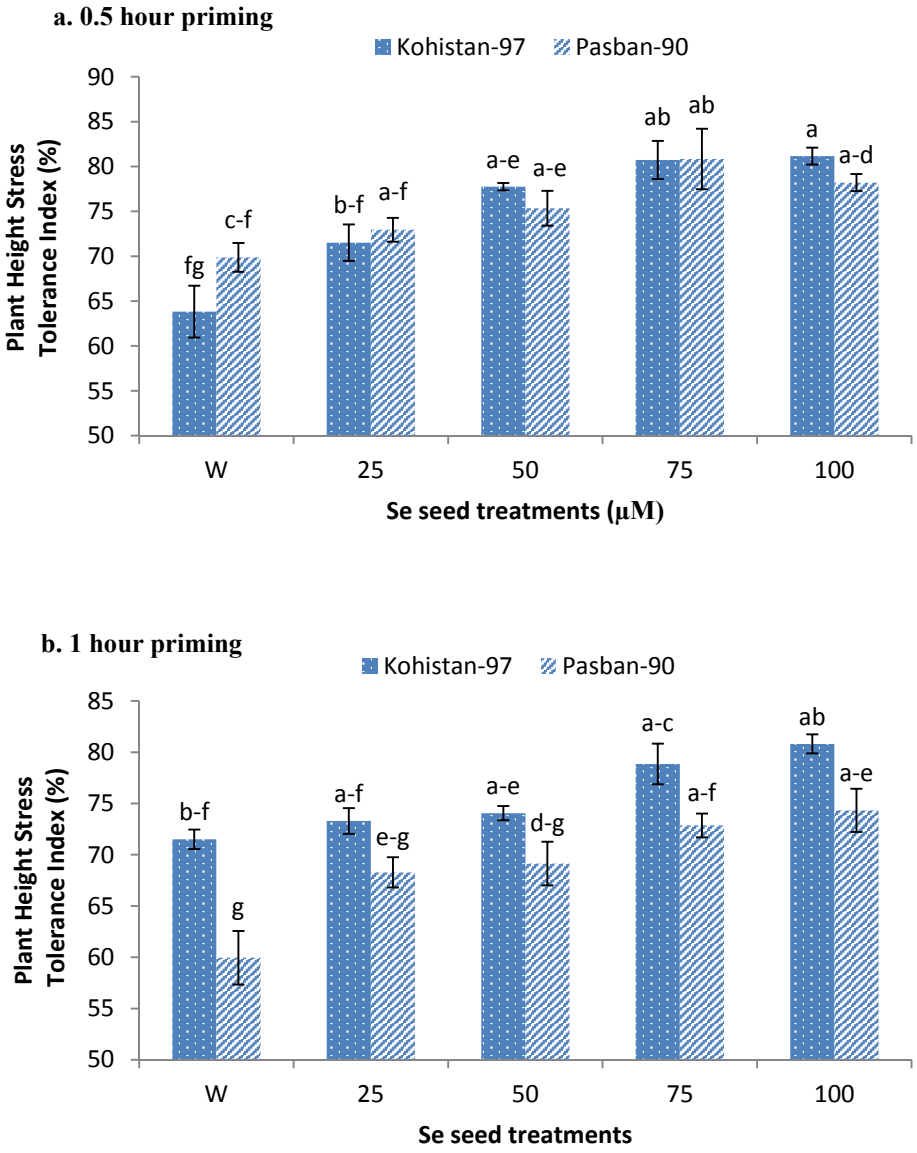

Figure 4.8 a, b: Effect of seed priming with Selenium (Se) on plant height stress tolerance index (PHSI) in wheat. Priming treatments include priming with distilled water (W) and priming with 25 µM, 50 µM, 75 µM and 100 µM Se. Values are mean±standard error.

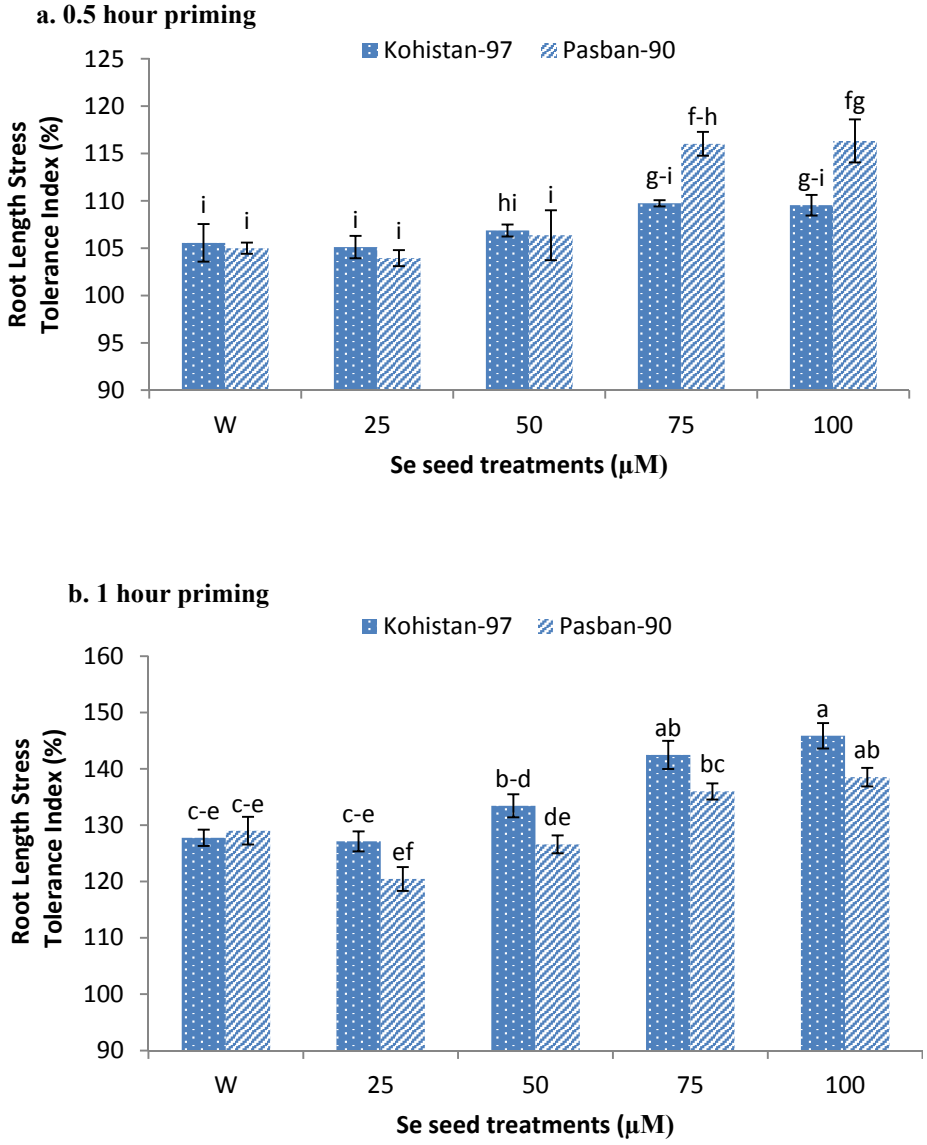

Figure 4.9 a, b: Effect of seed priming with Selenium (Se) on root length stress tolerance index (RLSI) in wheat. Priming treatments include priming with distilled water (W) and priming with 25 µM, 50 µM, 75 µM and 100 µM Se. Values are mean±standard error.

The duration of Se seed priming was found highly significant ($P< 0.001$) for RLSI. The highest value (133%) for this index was obtained by priming seeds with Se for 1 h (Fig. 4.9 b). Wheat genotypes differed non-significantly ($P>0.05$) for this variable (Table 4.3).

The significant interaction G×S×T revealed that soaking of seeds in Se solutions for 0.5 h had no significant effect on RLSI of both wheat genotypes, however, 1 h priming significantly increased the RLSI values which was maximum in Kohistan-97 (146%), where Se Seed priming was carried out with 100 µM which was statistically similar to that of seed priming @ 75 µM (142%) (Fig. 4.9 a, b).

4.3.3. Shoots fresh weights stress tolerance index (SFSI)

Highly significant differences ($P<0.001$) were observed among various Se seed treatments for SFSI. The seedlings exhibited maximum SFSI by Se seed priming @ 100 µM. A significant increase in SFSI was also observed by priming of seeds with Se solutions of 75 µM (50%) and 50 µM (43%). However, Se level of 25 µM gave the minimum value (38%) and differed non-significantly from hydro-priming (39%) for SFSI (Fig. 4.10 a, b).

The priming of seeds with Se solutions for 1 h resulted in significantly higher SFSI (47%) than 0.5 h priming (42%). The seedlings of genotype Kohistan-97 gave higher value (46%) for this index than Pasban-90 (43%).

The interaction among different factors (G×S×T) was significant. Drought tolerant genotype (Kohistan-97) maintained highest SFSI by Se seed priming with 100 µM for 1 h which was statistically at par with seed treatment @ 75 µM for the same duration (Fig. 4.10 a, b). All other interactions were non-significant for this variable.

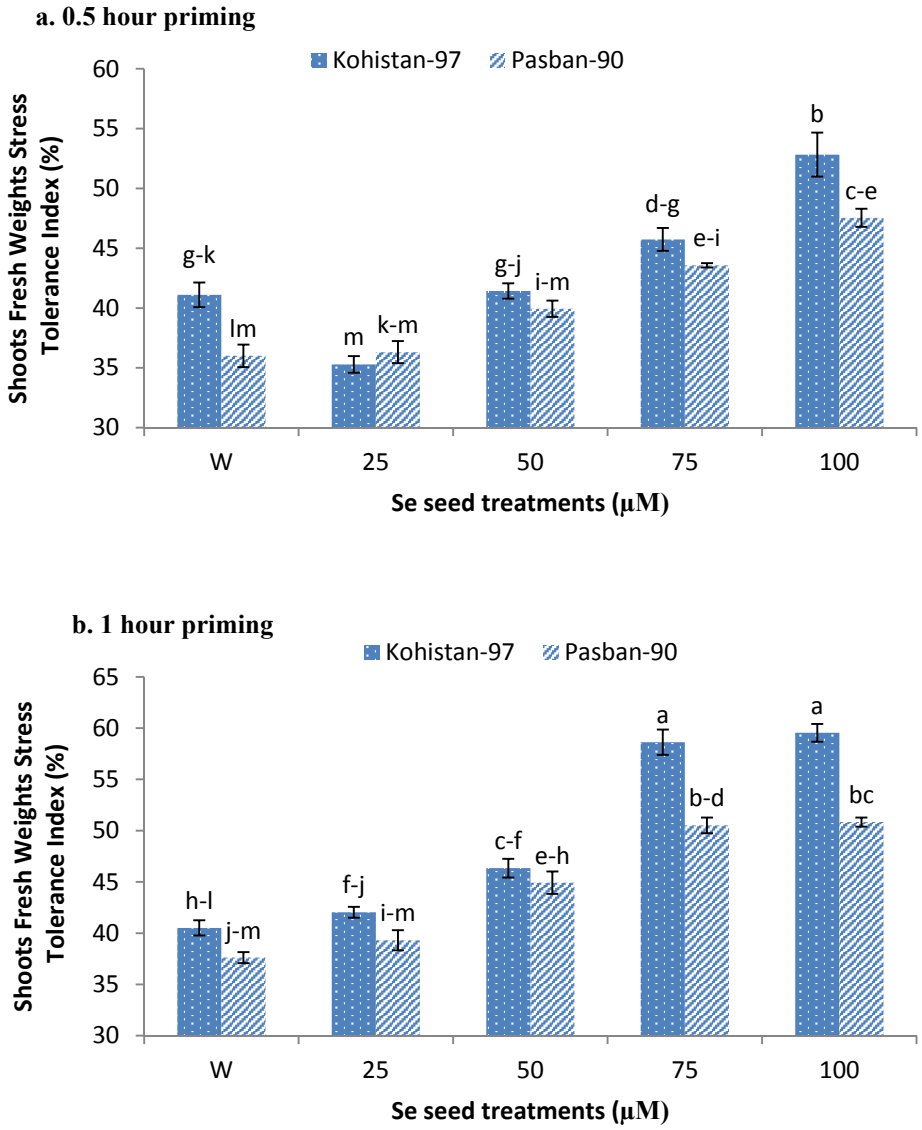

Figure 4.10 a, b: Effect of seed priming with Selenium (Se) on shoots fresh weights stress tolerance index (SFSI) in wheat. Priming treatments include priming with distilled water (W) and priming with 25 µM, 50 µM, 75 µM and 100 µM Se. Values are mean±standard error.

4.3.4. Roots fresh weights stress tolerance index (RFSI)

The data regarding RFSI showed highly significant variation (P<0.001) among different Se seed priming treatments (Table 4.3). The maximum RFSI (79%) was observed by Se seed priming @ 75 µM which was statistically at par with the value (76%) obtained by highest level (100 µM) for Se seed treatment (Fig. 4.11 a, b). All other treatments (hydro-priming, 25 µM and 50 µM) differed non-significantly for this variable (Table 4.3).

The seedlings raised from seeds primed with Se solutions for 1 h maintained significantly higher RFSI (74%) than 0.5 h priming (68%). Similarly, genotype Kohistan-97 exhibited significantly higher value (77%) than Pasban-90 (65%) for RFSI (Fig. 4.11 a, b).

The interaction G×S×T was also significant (P<0.05). The maximum value (87%) was recorded in Kohistan-97 by priming of seeds with Se solution of 100 µM for 1 h whereas Se seed priming @ 25 µM for 0.5 h resulted in minimum value (53%) for RFSI in Pasban-90 (Fig. 4.11 a, b).

4.3.5. Dry matter stress tolerance index (DMSI)

A highly significant effect (P< 0.001) of duration and rate of Se seed priming was noted for DMSI. It was maximum (83%) in seedlings raised from seeds treated with Se solution of 100 µM which was statistically related to the value (81%) obtained by 75 µM. The seedlings also maintained high DMSI by Se seed treatments of 50 µM (72%) and 25 µM (74%) whereas hydro-priming of seeds gave minimum value (60%) for this variable (Fig. 4.12 a, b).

The priming of seeds with 1 h resulted in significantly higher DMSI (75%) than 0.5 h priming (69%). Wheat genotype Kohistan-97 exhibited significantly greater value (74%) than Pasban-90 (70%) for DMSI (Fig. 4.12 a, b). The interaction among different factors (G×S×T) was non-significant (Table 4.3).

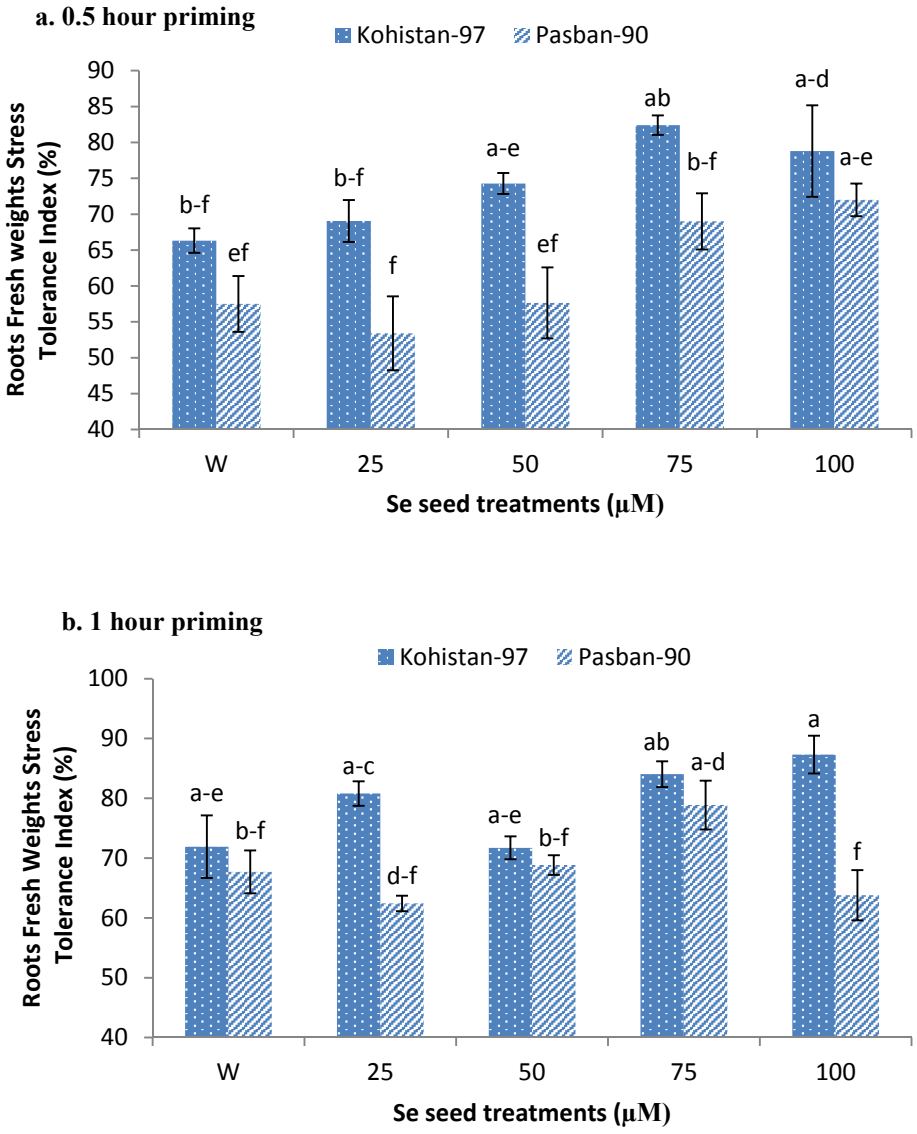

Figure 4.11 a, b: Effect of seed priming with Se on roots fresh weights stress tolerance index (RFSI) in wheat. Priming treatments include priming with distilled water (W) and priming with 25 µM, 50 µM, 75 µM and 100 µM selenium. Values are mean±standard error.

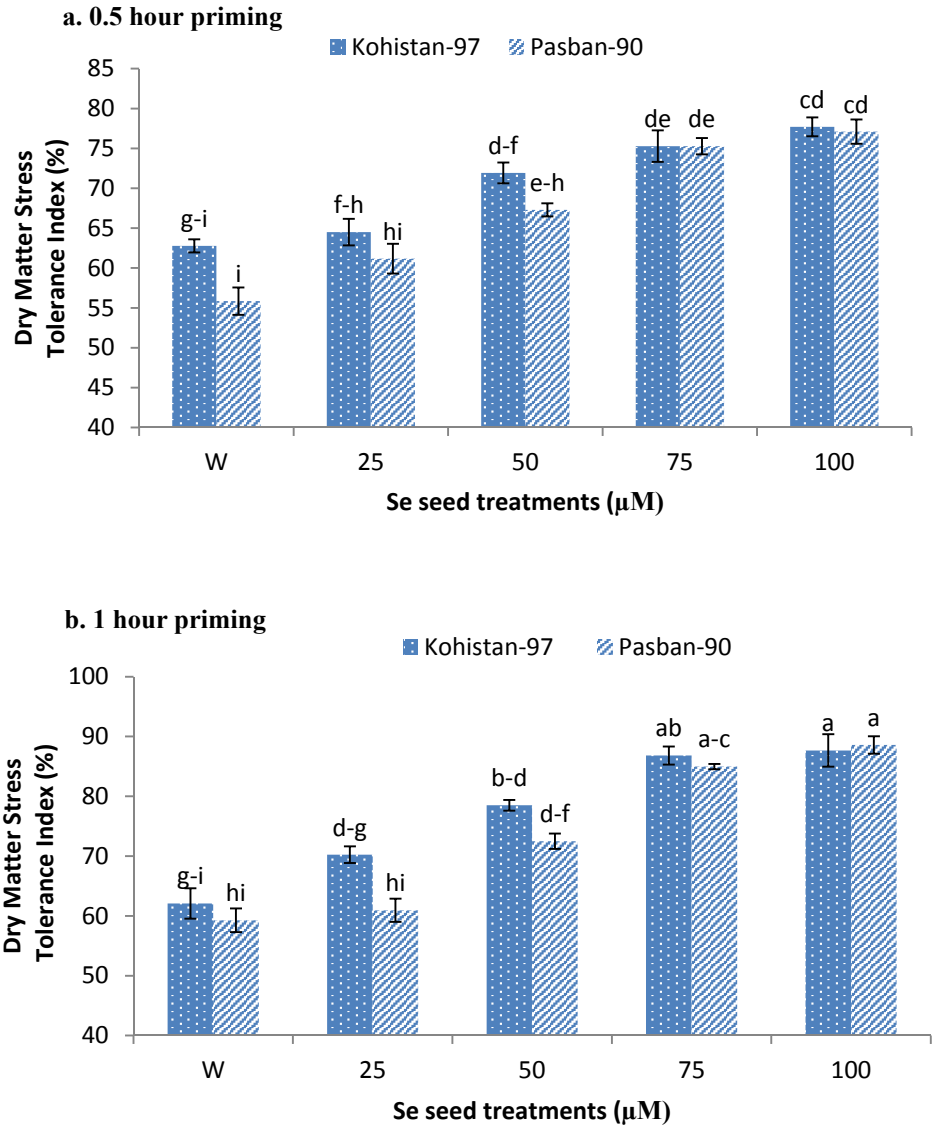

Figure 4.12 a, b: Effect of seed priming with Se on dry matter stress tolerance index (DMSI) in wheat. Priming treatments include priming with distilled water (W) and priming with 25 µM, 50 µM, 75 µM and 100 µM selenium. Values are mean±standard error.

4.4. Optimizing Selenium Level for Fertigation

To identify the optimum level of Se fertigation, an experiment with two screened out wheat genotypes (one drought tolerant and the other sensitive one) was conducted in green/wire-house under natural conditions. The Se level was selected on the basis of improvement in physiological indices.

4.4.1. Plant height stress tolerance index (PHSI)

Analysis of variance showed highly significant difference (P<0.01) between different Se fertigation levels for PHSI (Table 4.4). The maximum PHSI value (86%) was noted in seedlings grown with 7.35 µM (0.5 mg L^{-1}) Se fertigation, while a decrease in PHSI was observed at higher or lower levels of Se fertigation. The seedlings fertigated with 11.03 µM (0.75 mg Se L^{-1}) exhibited the second highest value (70%) which was statistically at par with Se fertigation @ 3.68 µM (0.25 mg L^{-1}) and 14.70 µM (1.00 mg L^{-1}) with the values of 69% and 68%, respectively (Fig. 4.13). Differences for PHSI between genotypes were non-significant. The interaction between Se treatments (S) and genotypes (G) was significant. The highest PHSI value (88%) was obtained in Pasban-90, fertigated with 7.35 µM (0.5 mg L^{-1}) while the lowest (67%) was in Kohistan-97 where Se @ 14.70 µM (1.00 mg L^{-1}) was applied (Fig. 4.13).

4.4.2. Root length stress tolerance index (RLSI)

The RLSI of both wheat genotypes and different Se treatments was highly significant (P<0.01) (Table 4.4). Minimum value for RLSI (141%) was recorded in seedlings fertigated with 3.68 µM (0.25 mg L^{-1}) while maximum value (159%) was recorded in plants supplied with 7.35 µM Se (0.5 mg L^{-1}). The non-significant difference (P>0.05) was observed between higher Se treatments i.e., 11.03 µM (0.75 mg L^{-1}) and 14.70 µM (1.00 mg L^{-1}), which had RLSI values of 150% and 152 % respectively and ranked as second best for fertigation (Fig. 4.14). The interaction (G×S) was significant and genotype Kohistan-97 exhibited higher RLSI value (160%) at fertigation with 7.35 µM (0.5 mg L^{-1}) level of Se than Pasban-90 at same level. The lowest RLSI value (138%) was recorded in Kohistan-97, fertigated by 3.68 µM (0.25 mg Se L^{-1}) (Fig. 4.14).

Table 4.4: Analysis of variance for plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI) shoots fresh weights stress tolerance index (SFSI), roots fresh weights stress tolerance index (RFSI) and dry matter stress tolerance index (DMSI) in two wheat genotypes fertigated with different selenium (Se) solutions under drought stress.

SOV[a]	PHSI (%)	RLSI (%)	SFSI (%)	RFSI (%)	DMSI (%)
Genotypes (G)	NS	***	NS	NS	***
Selenium treatments (S)	***	**	***	***	*
G×S	NS	*	NS	*	*
CV[b]	5.71	1.69	4.92	6.26	4.86

NS= non-significant, *significant at $P< 0.05$, ** significant at $P< 0.01$,

*** significant at $P< 0.001$

[a] Source of variation, [b] Co-efficient of variation

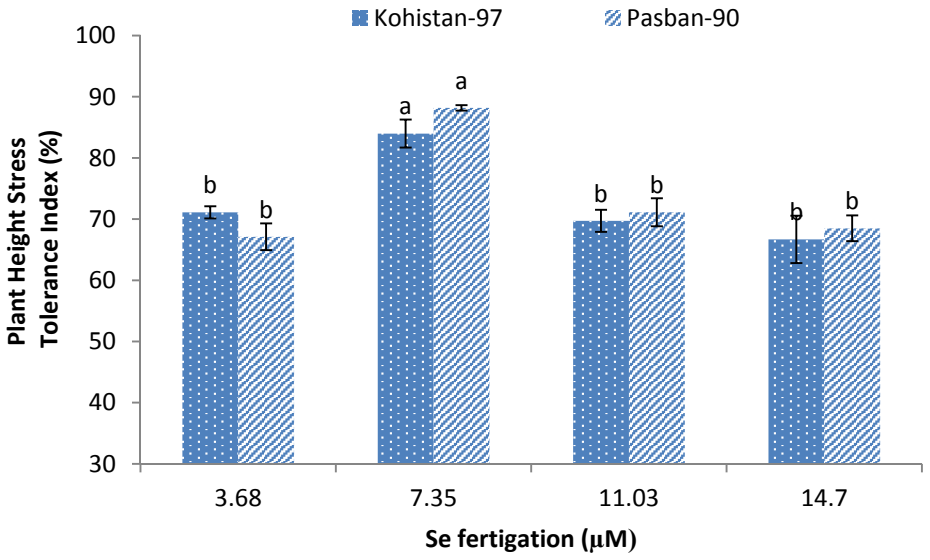

Figure 4.13: Effect of Se fertigation on plant height stress tolerance index (PHSI) of wheat seedlings. Fertigation treatments include fertigation with 3.68 (0.25 mg L^{-1}), 7.35 (0.50 mg L^{-1}), 11.03 (0.75 mg L^{-1}) and 14.70 µM (1.00 mg L^{-1}). Values are mean±standard error.

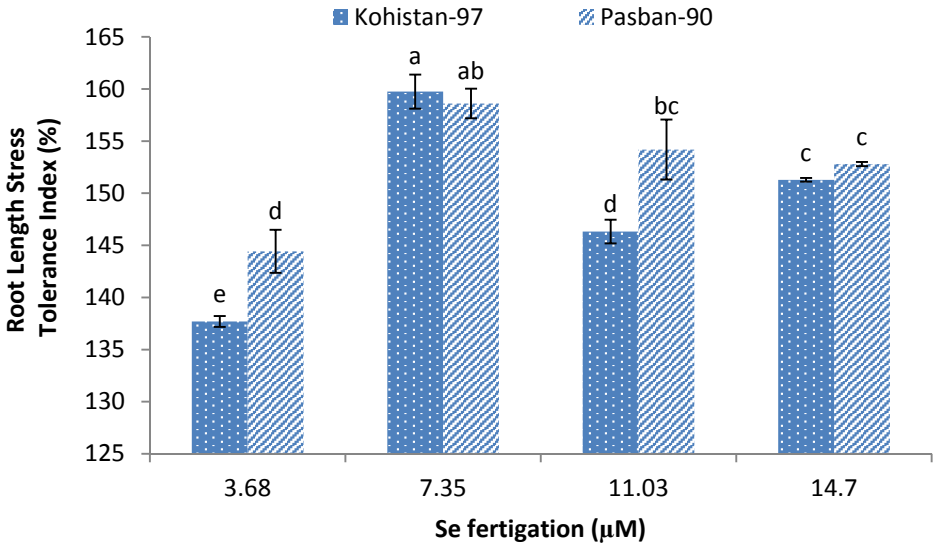

Figure 4.14: Effect of Se fertigation on root length stress tolerance index (RLSI) of wheat seedlings. Fertigation treatments include fertigation with 3.68 (0.25 mg L^{-1}), 7.35 (0.50 mg L^{-1}), 11.03 (0.75 mg L^{-1}) and 14.70 µM (1.00 mg L^{-1}). Values are mean±standard error.

4.4.3. Shoots fresh weights stress tolerance index (SFSI)

The exogenous Se supply as fertigation treatment had highly significant effect (P<0.001) on SFSI of wheat seedlings (Table 4.4). The plants fertigated with Se treatment of 7.35 µM (0.5 mg L^{-1}) maintained the highest SFSI (53%). The fertigation levels of 11.03 µM (0.75 mg L^{-1}) and 14.70 µM (1.00 mg L^{-1}) also gave high values (49% and 48% respectively) for SFSI whereas Se fertigation @ 3.68 µM (0.25 mg L^{-1}) resulted in the lowest value (44%) for this index (Fig. 4.15).

Wheat genotypes (Kohistan-97 and Pasban-90) exhibited non-significant differences for SFSI. Similarly, the interaction G×S was also non-significant (Table 4.4).

4.4.4. Roots fresh weights stress tolerance index (RFSI)

Wheat seedlings varied significantly (P<0.001) in response to different Se fertigation treatments for RFSI (Table 4.4). The maximum value (80%) was observed with Se fertigation dose of 7.35 µM (0.5 mg L^{-1}). A non-significant difference was recorded between seedlings fertigated with Se treatments of 11.03 µM (69%) and 14.70 µM (65%). The lowest fertigation level (3.68 µM) gave the minimum value (63%) for RFSI (Fig. 4.16).

Wheat genotypes differed non-significantly (P>0.05) for this index. However, the interaction between genotypes and Se treatments (G×S) was significant (Table 4.4). The fertigation treatment of 7.35 µM gave the highest RFSI (84%) in Kohistan-97. The lowest value (60%) was also recorded in same genotype by Se fertigation @ 11.03 µM statistically at par with 3.68 µM (Fig. 4.16).

4.4.5. Dry matter stress tolerance index (DMSI)

. The highly significant differences (P< 0.01) were observed among different Se treatments for DMSI. The fertigation of seedlings with 7.35 µM (0.5 mg Se L^{-1}) and 11.03 µM (0.75 mg L^{-1}) gave the maximum value (72%) for this index while, at fertigation level of 3.68 µM (0.25 mg Se L^{-1}), minimum value (67%) was recorded. The genotypes also differed significantly for this index. The interaction between Se levels and genotypes was significant.

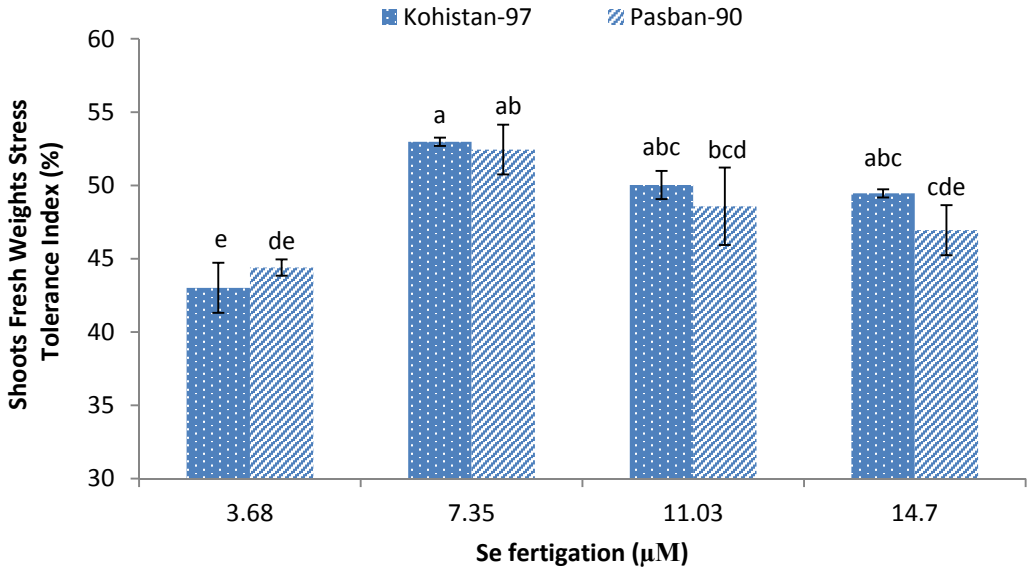

Figure 4.15: Effect of Se fertigation on shoots fresh weights stress tolerance index (SFSI) of wheat seedlings. Fertigation treatments include fertigation with 3.68 (0.25 mg L^{-1}), 7.35 (0.50 mg L^{-1}), 11.03 (0.75 mg L^{-1}) and 14.70 µM (1.00 mg L^{-1}). Values are mean±standard error.

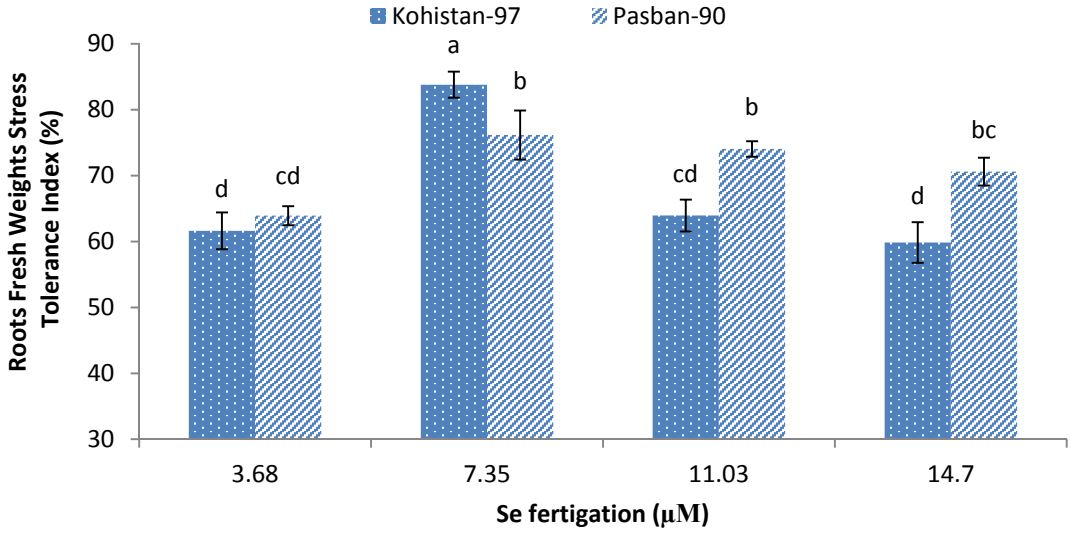

Figure 4.16: Effect of Se fertigation on roots fresh weights stress tolerance index (RFSI) of wheat seedlings. Fertigation treatments include fertigation with 3.68 (0.25 mg L^{-1}), 7.35 (0.50 mg L^{-1}), 11.03 (0.75 mg L^{-1}) and 14.70 µM (1.00 mg L^{-1}). Values are mean±standard error.

Genotype Kohistan-97 had greater value (78%) with Se fertigation @ 7.35 µM (0.5 mg L^{-1}) while treatment of seedlings with 14.70 µM (1.00 mg Se L^{-1}) gave the lowest value (64%) in Pasban-90 (Fig. 4.17).

Results of this experiment showed that only 7.35 µM (0.5 mg Se L^{-1}) level was better than lower and higher rates of Se in improving the above mentioned physiological indices, so, this level was selected for further lysimetric and field studies.

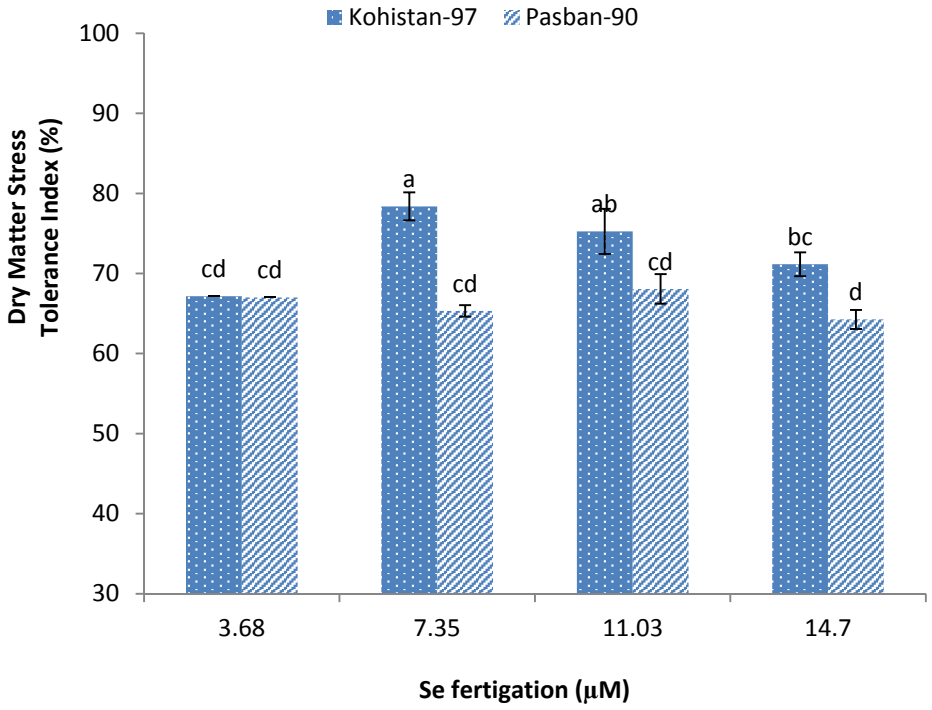

Figure 4.17: Effect of Se fertigation on dry matter stress tolerance index (DMSI) of wheat seedlings. Fertigation treatments include fertigation with 3.68 (0.25 mg L^{-1}), 7.35 (0.50 mg L^{-1}), 11.03 (0.75 mg L^{-1}) and 14.70 µM (1.00 mg L^{-1}). Values are mean±standard error.

4.5. Optimizing Selenium Level for Foliar Application

4.5.1. Plant height stress tolerance index (PHSI)

Analysis of variance showed highly significant difference (P<0.01) among different foliarly applied Se treatments for PHSI (Table 4.5). A gradual increase in PHSI was observed by increasing Se levels. The application of Se @ 7.06 µM (0.48 mg L^{-1}) gave the maximum value (88%) for this index, while, minimum value (63%) was recorded in seedlings sprayed with water which was statistically at par with value (64%) obtained for the lowest Se treatment of 1.76 µM (0.12 mg L^{-1}). The Se treatments of 3.53 µM (0.24 mg L^{-1}) and 5.29 µM (0.36 mg L^{-1}) increased PHSI by 18% and 24%, respectively as compared to the values obtained for seedlings sprayed with water (Fig. 4.18).

Non-significant difference was observed between genotypes Kohistan-97 and Pasban-90. The interaction of Se treatments and genotypes was also non-significant for PHSI (Table 4.5).

4.5.2. Root length stress tolerance index (RLSI)

The different Se treatments exhibited highly significant variations (P<0.01) for RLSI, which increased gradually with increase in Se levels (Table 4.5). The foliar application of Se @ 7.06 µM (0.48 mg L^{-1}) resulted in the maximum value (121%) for RLSI, which was 15% higher as compared to water sprayed seedlings which maintained the minimum value (103%) for this index. The foliar application of Se @ 3.53 µM (0.24 mg L^{-1}) and 5.29 µM (0.36 mg L^{-1}) increased RLSI by 8% and 12%, respectively as compared to water sprayed seedlings (Fig. 4.19).

Variations between genotypes were significant (P<0.05) for RLSI. Genotype Kohistan-97 had significantly higher value (113%) than Pasban-90 (110%). However, the interaction between genotypes and different Se treatments (G×S) was non-significant (Table 4.5).

Table 4.5: Analysis of variance for plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI), shoots fresh weights stress tolerance index (SFSI), roots fresh weights stress tolerance index (RFSI) and dry matter stress tolerance index (DMSI) in two wheat genotypes foliarly sprayed with selenium (Se) under drought stress

SOV[a]	PHSI (%)	RLSI (%)	SFSI (%)	RFSI (%)	DMSI (%)
Genotypes (G)	NS	***	***	NS	***
Selenium treatments (S)	***	*	***	***	***
G×S	NS	NS	NS	NS	NS
CV[b]	3.57	2.82	2.87	4.89	3.11

NS= non-significant, *significant at $P< 0.05$, ** significant at $P< 0.01$,

*** significant at $P< 0.001$

[a] Source of variation, [b] Co-efficient of variation

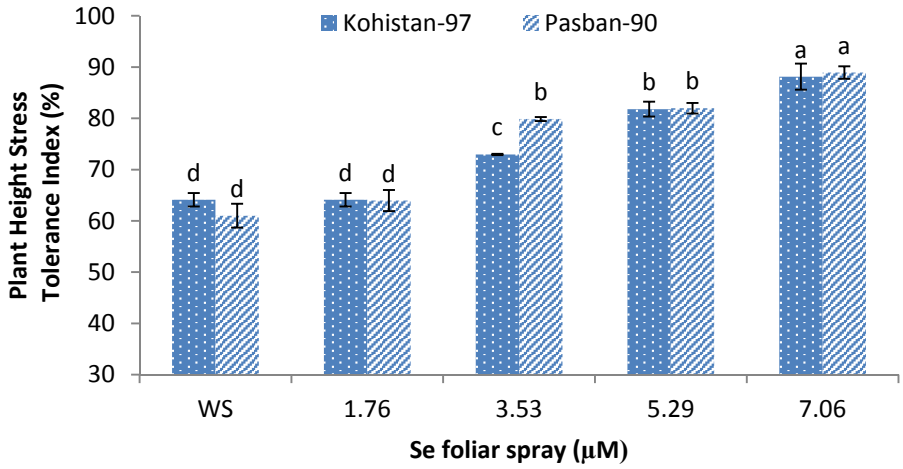

Figure 4.18: Effect of Se foliar application on plant height stress tolerance index (PHSI) of wheat seedlings. Foliar treatments include foliar spray with water (WS) and Se foliar spray with 1.76 (0.12 mg L^{-1}), 3.53 (0.24 mg L^{-1}), 5.29 (0.36 mg L^{-1}) and 7.06 µM (0.48 mg L^{-1}). Values are mean±standard error.

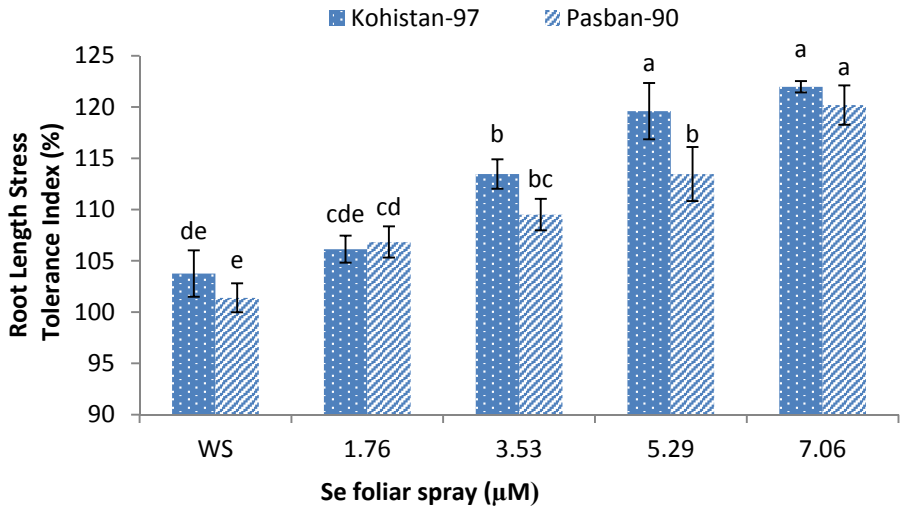

Figure 4.19: Effect of Se foliar application on root length stress tolerance index (RLSI) of wheat seedlings. Foliar treatments include foliar spray with water (WS) and Se foliar spray with 1.76 (0.12 mg L^{-1}), 3.53 (0.24 mg L^{-1}), 5.29 (0.36 mg L^{-1}) and 7.06 µM (0.48 mg L^{-1}). Values are mean ± standard error.

4.5.3. Shoots fresh weights stress tolerance index (SFSI)

The seedlings foliarly sprayed with different Se treatments exhibited highly significant (P<0.001) differences for SFSI (Table 4.5). The application of Se @ 7.06 µM (0.48 mg L^{-1}) gave the maximum value (85%) for SFSI. The seedlings foliarly sprayed with Se @ 5.29 µM (0.36 mg L^{-1}) and 3.53 µM (0.24 mg L^{-1}) also maintained higher values (75% and 66% respectively) than water sprayed (51%) seedlings (Fig. 4.20).

Wheat genotype Pasban-90 exhibited higher SFSI (71%) than Kohistan-97 (66%). The interaction G×S was non-significant for SFSI (Table 4.5).

4.5.4. Roots fresh weights stress tolerance index (RFSI)

The analysis of variance for the data showed highly significant (P<0.001) differences among different Se foliar treatments for RFSI. The seedlings foliarly sprayed with Se treatment of 7.06 µM (0.48 mg L^{-1}) maintained the highest value for RFSI. The foliar Se treatment of 5.29 µM (0.36 mg L^{-1}) also improved this index whereas non-significant difference (P>0.05) was recorded among other treatments (water spray, 1.76 µM and 3.53 µM Se) for this index (Fig. 4.21).

Wheat genotypes differed non-significantly for RFSI. The interaction G×S was also non-significant (Table 4.5).

4.5.5. Dry matter stress tolerance index (DMSI)

Highly significant effect (P<0.01) of different Se treatments for DMSI of wheat seedlings was observed (Table 4.5). The seedlings applied with foliar Se treatment of 7.06 µM (0.48 mg L^{-1}) showed an increase of 46% and had maximum value (89%) as compared to plants sprayed with water exhibiting minimum value (48%) for DMSI (Fig. 4.22). Similarly, an increase of 41% and 36% was recorded in seedlings exposed to foliar spray of Se @ 3.53 µM (0.24 mg L^{-1}) and 5.29 µM (0.36 mg L^{-1}) respectively.

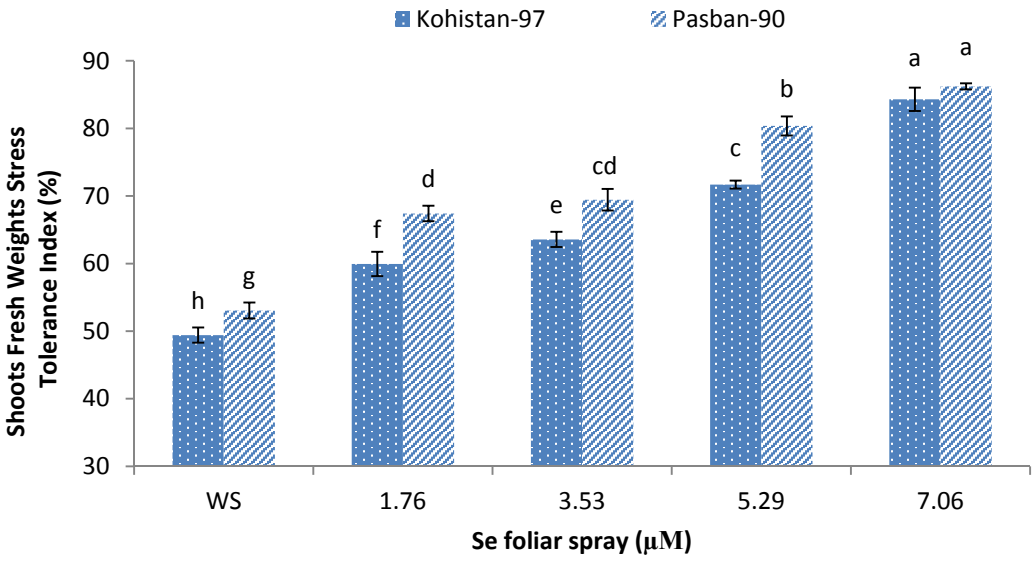

Figure 4.20: Effect of Se foliar application on shoots fresh weights stress tolerance index (SFSI) of wheat seedlings. Foliar treatments include foliar spray with water (WS) and Se foliar spray with 1.76 (0.12 mg L^{-1}), 3.53 (0.24 mg L^{-1}), 5.29 (0.36 mg L^{-1}) and 7.06 µM (0.48 mg L^{-1}). Values are mean ± standard error.

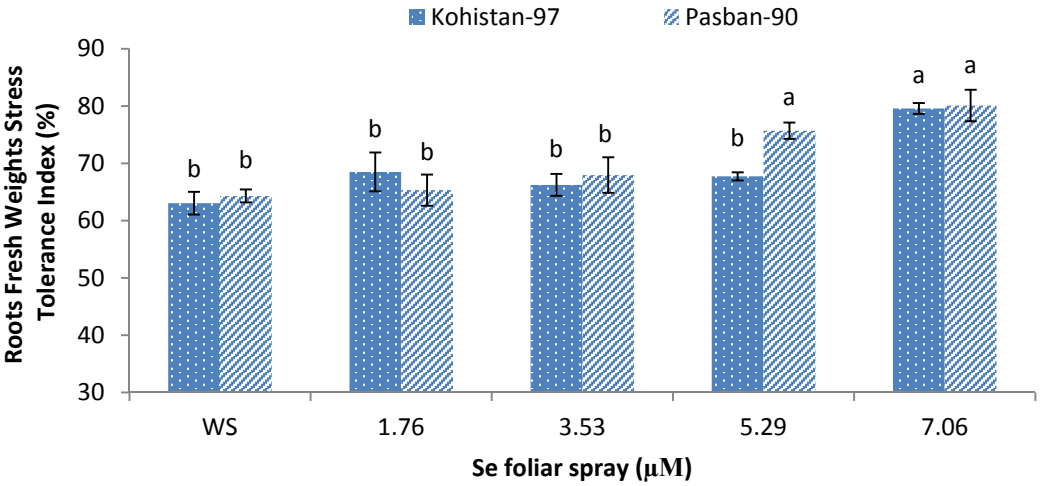

Figure 4.21: Effect of Se foliar application on roots fresh weights stress tolerance index (RFSI) of wheat seedlings. Foliar treatments include foliar spray with water (WS) and Se foliar spray with 1.76 (0.12 mg L^{-1}), 3.53 (0.24 mg L^{-1}), 5.29 (0.36 mg L^{-1}) and 7.06 µM (0.48 mg L^{-1}). Values are mean ± standard error.

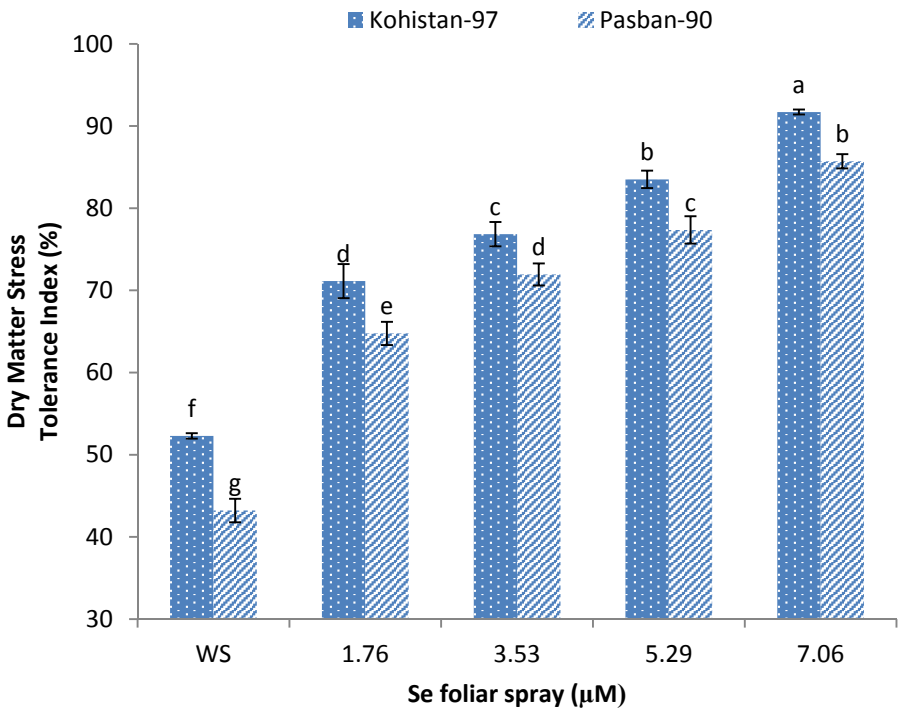

Figure 4.22: Effect of Se foliar application on dry matter stress tolerance index (DMSI) of wheat seedlings. Foliar treatments include foliar spray with water (WS) and Se foliar spray with 1.76 (0.12 mg L^{-1}), 3.53 (0.24 mg L^{-1}), 5.29 (0.36 mg L^{-1}) and 7.06 µM (0.48 mg L^{-1}). Values are mean ± standard error.

Dry matter stress tolerance index (DMSI) also differed significantly between genotypes. Wheat genotype Kohistan-97 maintained significantly greater value (75%) for DMSI than that of Pasban-90 (69%). The interaction between treatments and genotypes was non-significant for this index (Table 4.5).

The results showed that seedlings sprayed with Se @ 7.06 µM (0.48 mg L^{-1}) gave the highest values for PHSI, RLSI and DMSI, thus it is the optimum Se level for foliar application to improve the stress tolerance potential of wheat genotypes. Keeping in view the above results, Se level of 5.29 µM (0.36 mg Se L^{-1}) can be ranked as second among other Se treatments.

4.6. Optimization of Method and Time of Selenium Application

An experiment was conducted in lysimeters covered with plastic sheets to protect from rainfall. The above selected optimum Se levels for priming, fertigation and foliar spray with same wheat genotypes were utilized to select the best suited method and time of Se application. During the course of study necessary physiological, biochemical, growth and yield parameters were recorded, the results of those are given below:

4.6.1. Leaf water potential (ψ_w)

Analysis of variance regarding ψ_w revealed that it was significantly reduced (P<0.01) in water stressed wheat plants than of non-stressed plants (Table 4.6). Drought stress caused a 30% reduction in ψ_w as compared to normal irrigated plants of both wheat genotypes i.e. Kohistan-97 and Pasban-90 (Table 4.6). A more pronounced decrease was recorded at anthesis (-1.02MPa) as compared to tillering stage (-0.61 MPa).

The different selenium (Se) treatment differed significantly (P<0.01) for ψ_w. The Se applied through foliar (-0.75MPa) and fertigation (-0.74 MPa) methods at tillering stage exhibited higher values for ψ_w as compared to other Se application methods. The lowest ψ_w (-1.00 MPa) was recorded in plants which were grown without Se application. However, differences in foliar at anthesis (-0.814 MPa) and seed priming (-0.79MPa) methods were non-significant for ψ_w (Fig. 4.23). Variation for $\psi_w$betweenKohistan-97 and Pasban-90 was statistically similar.

The interaction among growth stages (P), water stress levels (W), selenium treatments (S) and genotypes (G) were also significant for ψ_w. The maximum value (-0.41 MPa) was recorded in Pasban-90 plants where Se was foliarly applied at tillering stage under normal supply of water. The minimum value for ψ_w (-1.46 MPa) was recorded in Pasban-90 grown under drought stress without Se supply at anthesis stage (Fig. 4.23).

Table: 4.6. Analysis of variance of water potential (-MPa), osmotic potential (-MPa), turgor potential (MPa) and relative water contents (%) in two wheat genotypes exposed to exogenous selenium supply under drought stress.

SOV[a]	Water Potential (-MPa)	Osmotic Potential (-MPa)	Turgor Potential (MPa)	Relative Water Contents (%)
Growth Stages (P)	***	***	***	***
Water stress levels (W)	***	***	***	***
Selenium treatments (S)	***	***	NS	***
Genotypes (G)	NS	NS	NS	NS
P×W	***	***	NS	*
P×S	***	***	NS	NS
P×G	***	*	NS	NS
W×S	NS	NS	NS	NS
W×G	**	NS	NS	NS
S×G	NS	NS	NS	NS
P×W×S	NS	NS	NS	NS
P×W×G	***	**	NS	NS
P×S×G	NS	NS	NS	NS
W×S×G	*	NS	NS	NS
W×S×G×P	**	NS	NS	NS
CV[b]	6.25	7.10	23.39	8.19

NS= non-significant, *significant at $P< 0.05$, ** significant at $P< 0.01$,

*** significant at $P< 0.001$,

[a] Source of variation, [b] Co-efficient of variation

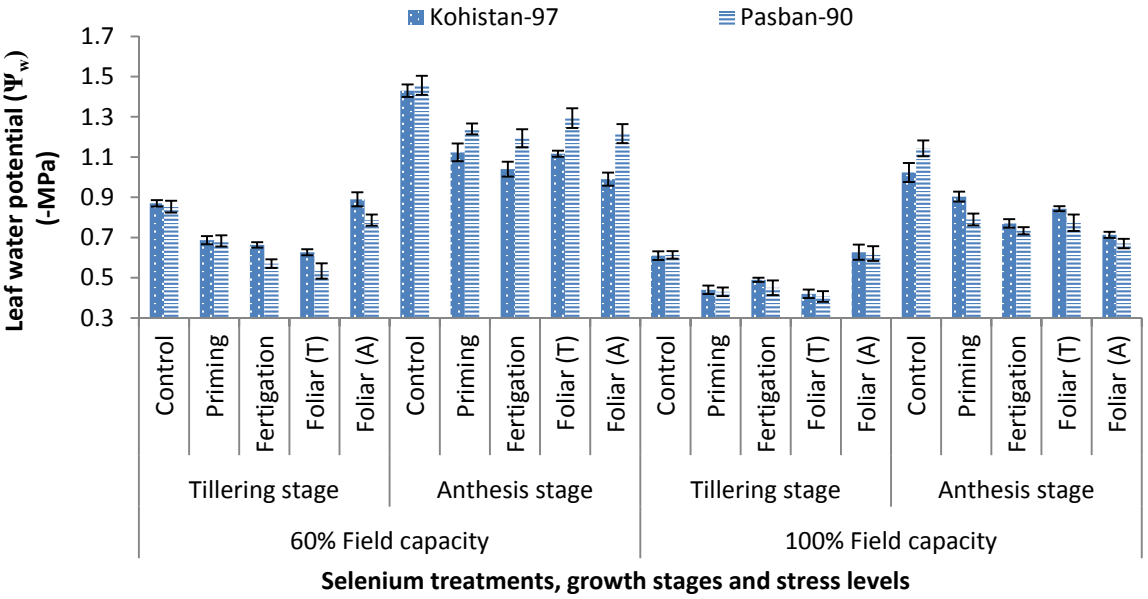

Figure 4.23: Effect of exogenous Se supply on water potential (-MPa) of two wheat (*Triticum aestivum* L.) genotypes at tillering and anthesis stage under normal (100% FC) and drought stress (60% FC) conditions. Values are mean±standard error.

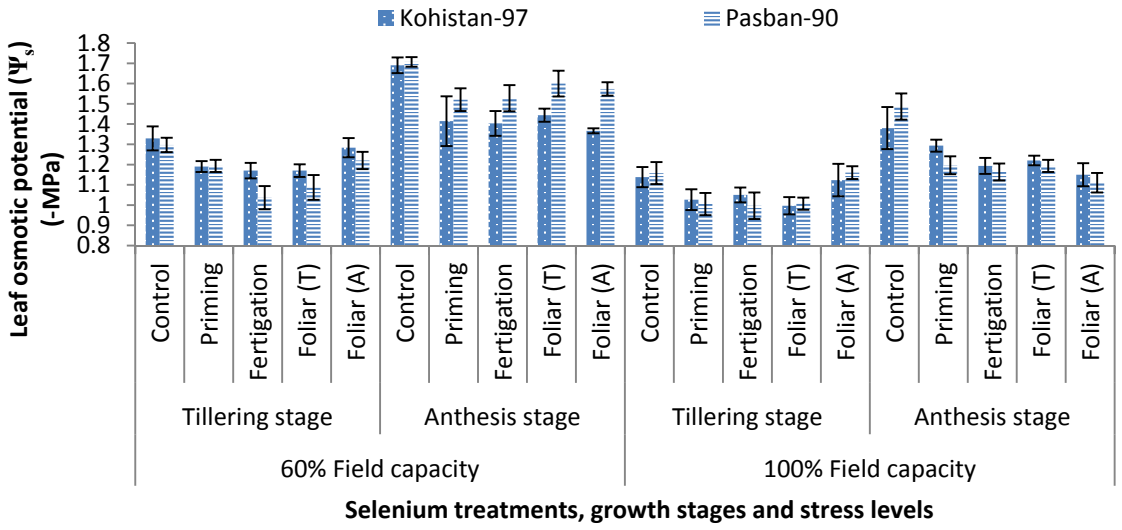

Figure 4.24a: Effect of exogenous Se supply on osmotic potential (-MPa) of two wheat (*Triticum aestivum* L.) genotypes at tillering and anthesis stage under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

4.6.2. Leaf osmotic potential (ψ_s)

Highly significant effect ($P<0.01$) of drought stress was found on ψ_s (Table 4.6). Drought stress decreased ψ_s by 18% as compared to normal supply of water. The comparison between stages indicated that Se application at anthesis stage (-1.38MPa) affected ψ_s more adversely than Se supply at tillering stage (-1.13MPa) (Fig. 4.24c). No significant difference was observed between genotypes Kohistan-97 and Pasban-90 for this variable (Table 4.6).

Exogenous Se supply methods significantly ($P<0.01$) influenced the ψ_s of both wheat genotypes. Application of Se through fertigation at tillering stage gave the highest value (-1.19 MPa) closely followed by foliar application of Se at tillering (-1.22MPa) and Se seed priming method (-1.23MPa). The lowest ψ_s was recorded in plants with no Se supply (-1.40MPa) (Fig. 4.24b).

The interactions among WxSxPxG were non-significant (Table 4.6; Fig. 4.24a).

4.6.3. Turgor potential (ψ_p)

Water stress had highly significant ($P>0.01$) effect on ψ_p of both wheat genotypes (Table 4.6). A marked reduction of 17% was recorded in ψ_p of the plants grown under limited water conditions as compared to control. The decrease in ψ_p was more pronounced (31%) for anthesis than tillering stage. Non-significant difference for ψ_p was also observed for wheat genotypes i.e., Kohistan-97 and Pasban-90.

Comparison among Se application methods showed that all the Se treatments affected ψ_p significantly as compared to control. However, all Se application methods did not differ significantly for ψ_p. The highest value (0.46 MPa) was obtained by Se foliar application at tillering stage while minimum value (0.40 MPa) was recorded in plants subjected to no Se supply (Fig. 4.25). All the interactions among growth stages, water stress levels, Se application methods and genotypes were non-significant for ψ_p (Table. 4.6).

4.6.4. Relative water contents (RWC)

Significant (P<0.05) differences between plants grown under normal and drought stress conditions were recorded for RWC. It was observed that plants growing under limited water

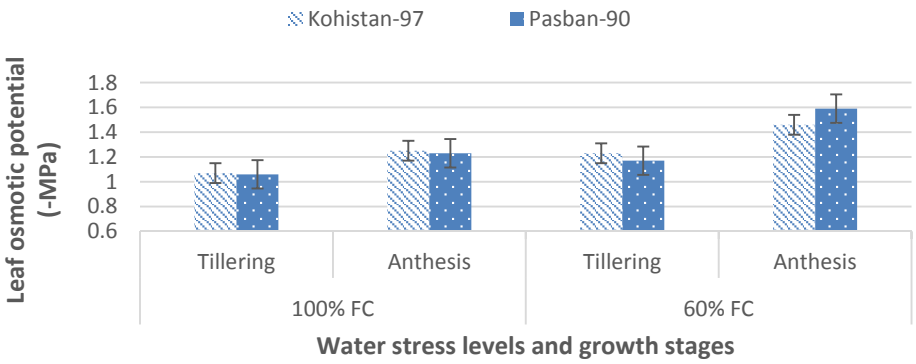

Figure 4.24b: Interaction effect between growth stages (P), water stress levels (W) and genotypes (G) on leaf osmotic potential (-MPa). Values are mean ± standard error.

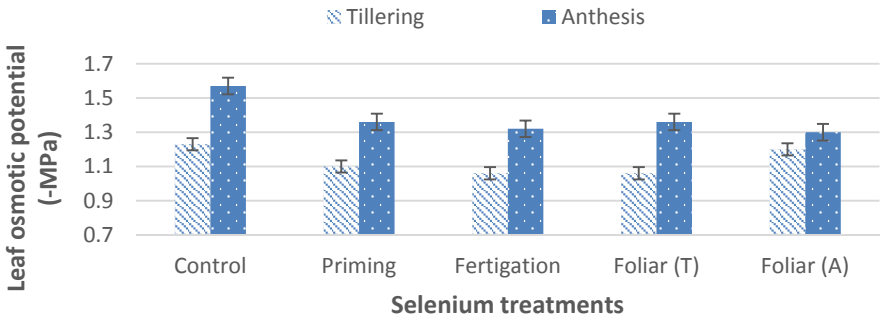

Figure 4.24c: Interaction effect between growth stages (P) and selenium treatments (S) on leaf osmotic potential (-MPa). Values are mean ± standard error.

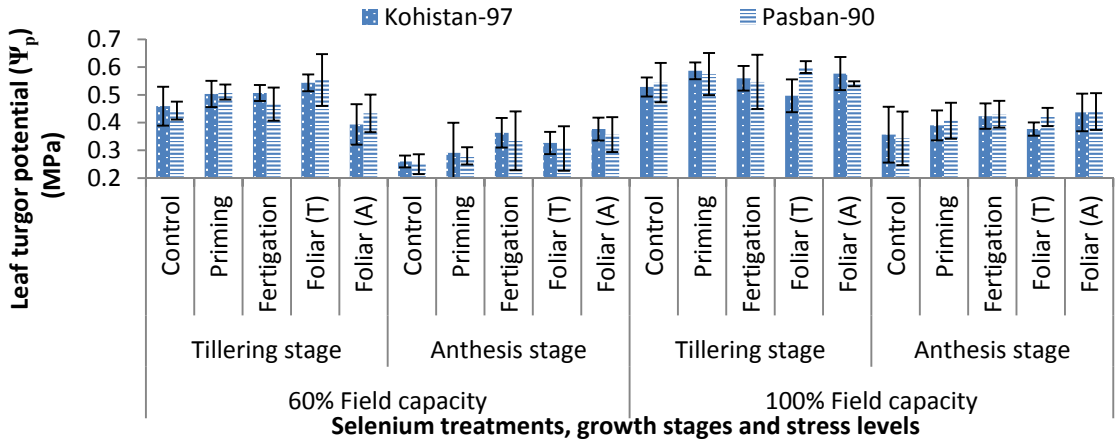

Figure 4.25: Effect of exogenous Se supply on turgor potential (MPa) of two wheat (*Triticum aestivum* L.) genotypes at tillering and anthesis stage under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

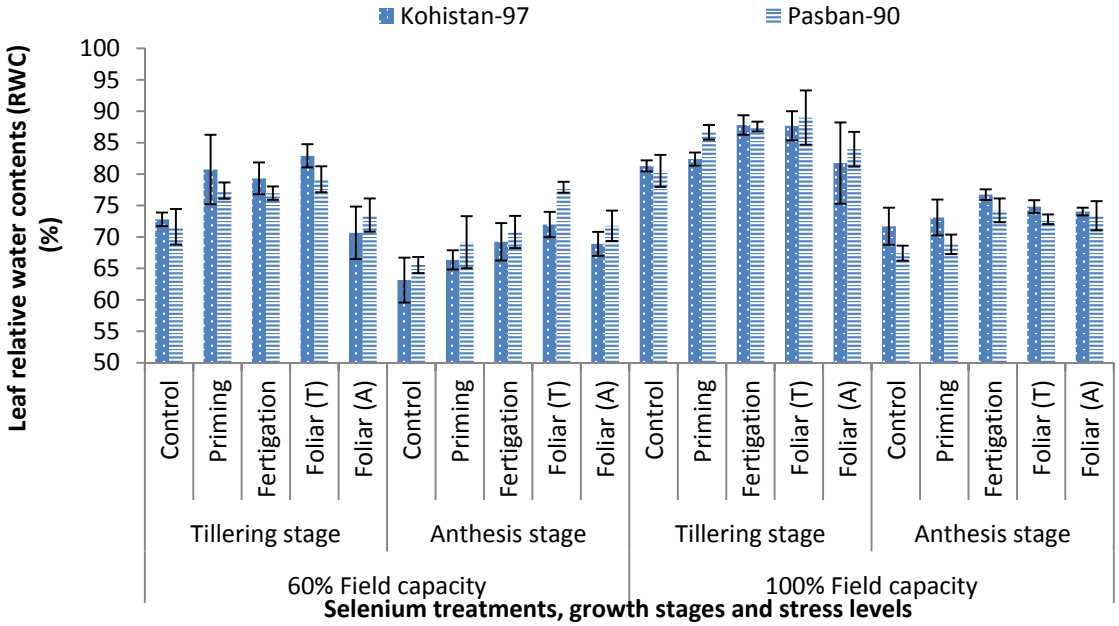

Figure 4.26a: Effect of exogenous Se supply on relative water contents (%) of two wheat (*Triticum aestivum* L.) genotypes at tillering and anthesis stage under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

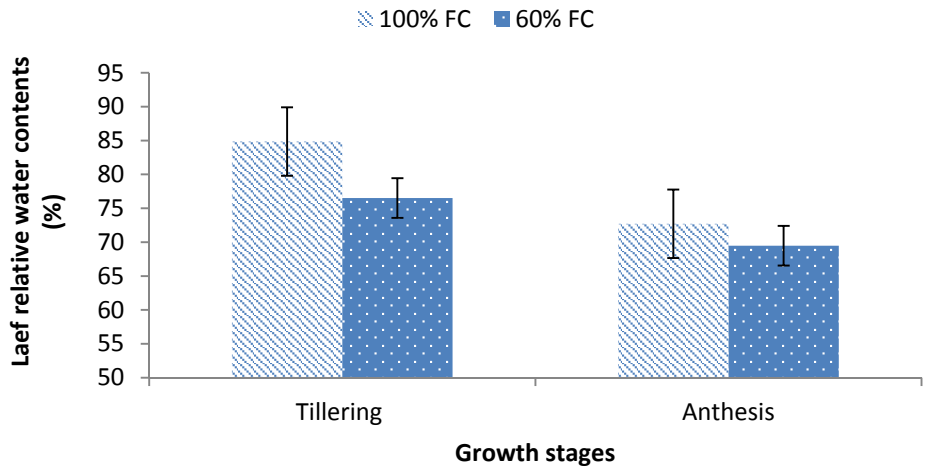

Figure 4.26b: Interaction effect between growth stages (P) and water stress levels (W) on leaf relative water contents (RWC). Values are mean ± standard error.

conditions maintained lower RWC (73%) than that of normal plants (79%). Application of Se at tillering stage (81%) was effective in maintaining higher RWC than at anthesis stage (71%) (Fig. 4.24b).

Relative water contents were significantly (P<0.01) influenced by Se application. The foliar application of Se at tillering stage was successful in maintaining the highest RWC (80%) closely followed by Se supply by fertigation (78%).The Se seed priming gave the value of (76%) for RWC which was statistically at par with foliar application of Se at anthesis stage (75%). The minimum value (72%) was estimated in plants where no Se was applied (Fig. 4.26a).

The interaction WxSxPxG was non-significant for RWC.

4.6.5. Leaf chlorophyll contents

Analysis of variance for the data regarding leaf chlorophyll contents showed highly significant difference (P<0.001) between water stress (60% FC) and normal irrigation (100% FC) (Table 4.7). Exogenous Se supply was found to be effective in increasing the leaf chlorophyll contents.

Highly significant effect (P<0.001) of water deficit was recorded on leaf chlorophyll a (Chl_a) contents (Table 4.7). The plants exposed to drought stress exhibited a reduction of 4% in Chl_a contents as compared to normal plants. The decrease in Chl_a contents was higher (8%) at tillering (0.47mg g^{-1} FW) than anthesis stage (0.51 mg g^{-1} FW).Wheat genotypes also differed significantly (P<0.01) for leaf Chl_a contents. Exogenous Se supply increased Chl_ainKohistan-97 by 15% than Pasban-90 (Fig. 4.27).

The different Se application methods also varied significantly (P<0.001) for this variable. The Se fertigation and seed primed plants had maximum Chl_a contents (0.54 mg g^{-1} FW). The plants where Se was applied foliarly at tillering stage also exhibited significantly higher Chl_a (0.48 mg g^{-1} FW) than those which were not treated with Se (0.44 mg g^{-1} FW) and plants sprayed with Se at anthesis stage (0.46 mg g^{-1} FW) (Fig. 4.27).

The interaction between water stress levels (W) and growth stages (P) was also highly significant (P<0.01). The highest Chl_a contents (0.53 mg g^{-1} FW) were maintained by the

Table: 4.7. Analysis of variance of chlorophyll a (mg g^{-1}), chlorophyll b (mg g^{-1}), total chlorophyll (mg g^{-1}) and total carotenoids contents (µg g^{-1}) in two wheat genotypes exposed to exogenous selenium (Se) supply under drought stress.

SOV[a]	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total Chlorophyll (mg g^{-1})	Total Carotenoids (µg g^{-1})
Growth Stages (P)	***	NS	***	NS
Water stress levels (W)	**	***	***	***
Selenium treatments (S)	***	***	***	***
Genotypes (G)	***	*	***	**
P×W	**	*	NS	NS
P×S	***	***	***	***
P×G	***	***	***	***

W×S	***	***	***	***
W×G	**	NS	***	NS
S×G	***	***	**	*
P×W×S	***	***	***	NS
P×W×G	***	**	***	*
P×S×G	***	***	***	*
W×S×G	***	*	*	NS
W×S×G×P	***	NS	***	NS
CV[b]	6.20	15.71	5.57	8.86

NS= non-significant, *significant at $P< 0.05$, ** significant at $P< 0.01$,

*** significant at $P< 0.001$

[a] Source of variation, [b] Co-efficient of variation

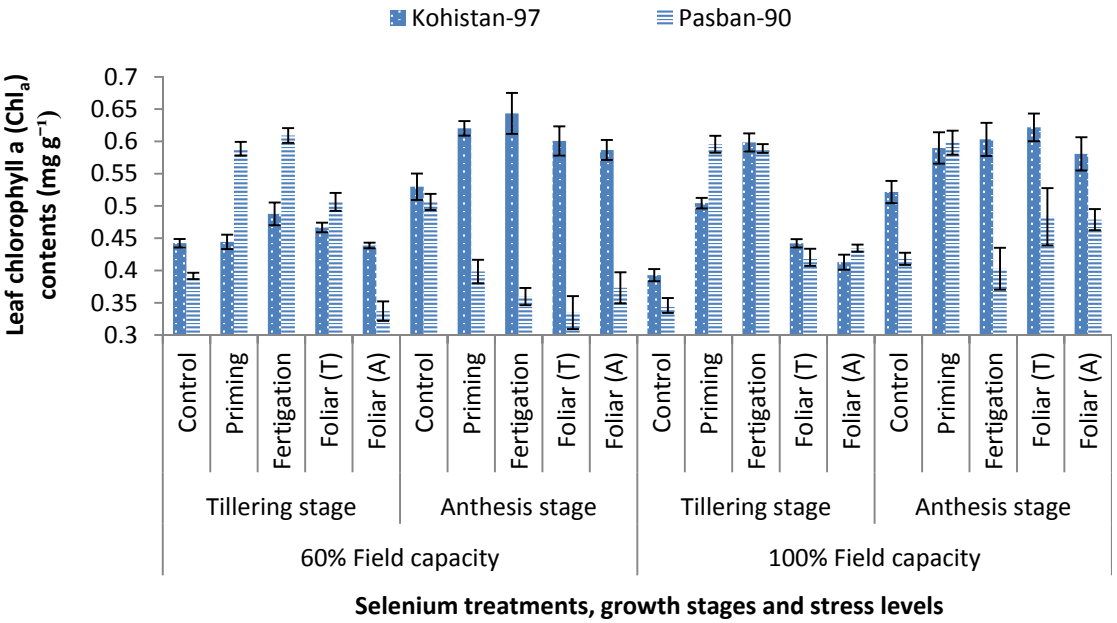

Figure 4.27: Effect of exogenous Se supply on chlorophyll a (Chl_a) contents (mg g^{-1} FW) of two wheat (*Triticum aestivum* L.) genotypes at tillering and anthesis stage under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

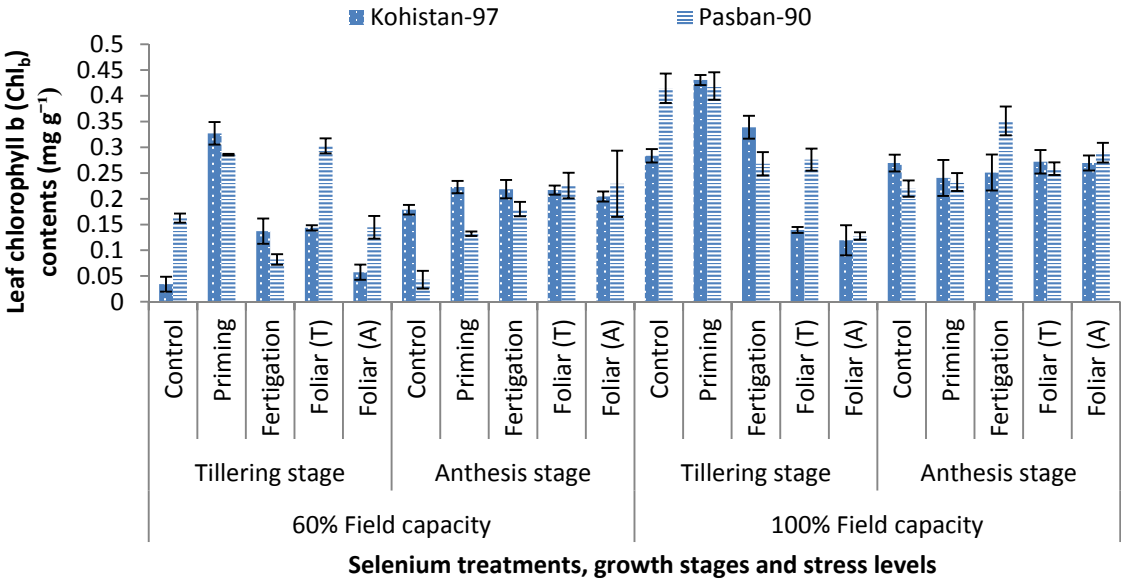

Figure 4.28: Effect of exogenous Se supply on Chlorophyll b (Chl_b) contents (mg g^{-1} FW) of two wheat (*Triticum aestivum* L.) genotypes at tillering and anthesis stage under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

normal plants (100% FC) at anthesis stage whereas the lowest value (0.47 mg g^{-1} FW) for Chl_a was noted in plants growing under limited water supply (60% FC) at tillering stage which did not differ significantly (0.47 mg g^{-1} FW) to the plant grown in normal water supply at tillering stage (Fig. 4.27). The significant interaction was recorded between water stress levels (W) and Se application methods (S). The data clearly indicated that Se seed priming method in normal plants gave the highest value (0.57 mg g^{-1}) for Chl_a contents statistically at par with value (0.55 mg g^{-1}) obtained in plant where Se was applied through fertigation at 100% FC. Under drought stress, Se fertigation gave the maximum value (0.53 mg g^{-1} FW) for Chl_a contents which was statistically related (P>0.05) to Se seed priming (Fig. 4.27). Interaction WxSxPxG was also significant for Chl_a contents (Table 4.7).

Imposition of drought stress had highly significant effect (P<0.001) on Chlorophyll b (Chl_b) contents of wheat leaves (Table 4.7).Under drought stress, a significant decrease of 36% was recorded in Chl_b contents with respect to normal supply of water. However, no significant difference (P>0.05) in Chl_b contents was observed between tillering and anthesis stages (Fig. 4.28). Wheat genotype Pasban-90 maintained significantly higher (P<0.05) Chl_b contents (0.23 mg g^{-1} FW) than Kohistan-97 (Table 4.7).

Exogenous Se application methods showed highly significant differences (P<0.001) for Chl_b contents. The plants grown with Se seed priming exhibited the highest value (0.29 mg g^{-1} FW) for Chl_b contents. Non-significant differences (P>0.05) were recorded for Se fertigation and foliar application at tillering stage (0.24 mg g^{-1} FW)whereas plants foliarly sprayed with Se at anthesis stage gave the lowest value (0.18 mg g^{-1} FW) for this variable (Fig. 4.28).

The interaction P×W×S was also significant for leaf Chl_b contents (Table 4.7). The highest value (0.42 mg g^{-1} FW) was noted in plants raised from seeds primed with Se at tillering stage under normal water supply while the lowest value (0.10 mg g^{-1} FW) was recorded in plants grown without the supply of Se under drought stress (60% FC) at tillering stage. The Se fertigation gave the maximum value (0.25 mg g^{-1} FW) under limited water conditions (60%FC) statistically at par with Se foliar application at tillering and anthesis stage (0.24 and 0.25 mg g^{-1} FW, respectively) (Fig. 4.28). The interaction among WxSxPxG was statistically non-significant (Table 4.7).

The leaf total chlorophyll (Chl_t =Chl_a+ Chl_b) contents significantly (P<0.01) reduced due to limited supply of water to plants (Table 4.7). The reduction in Chl_t was up to 15% in drought stressed plants as compared to normal ones. The plants treated at tillering stage maintained significantly higher (0.74 mg g^{-1} FW) Chl_t contents than that of anthesis stage (0.70 mg g^{-1} FW). Wheat genotypes also differed significantly (P<0.01) for this variable (Table 4.7) as Kohistan-97 exhibited 8% higher leaf Chl_t than Pasban-90 (Fig. 4.29).

The exogenous Se application methods also varied significantly (P<0.001) for this variable. The highest Chl_t contents were recorded by Se seed priming (0.83 mg g^{-1} FW). The other Se application methods i.e. Se fertigation (0.76 mg g^{-1} FW) and Se foliar application at tillering stage (0.71 mg g^{-1} FW) also significantly increased leaf Chl_t (Fig. 4.29). However, no Se application and Se application at anthesis stage resulted in minimum value for Chl_t (0.64mg g^{-1} FW).

The interaction between stages (P) and water stress levels (W) was also significant. The maximum value (0.8 mg g^{-1} FW) for Chl_t was recorded in normal plants (100% FC) at anthesis stage while minimum value (0.64 mg g^{-1} FW) was recorded at tillering stage under

water deficit conditions (60% FC) which showed that plants exhibited 6% higher Chl_t contents at anthesis stage than tillering stage (Fig. 4.29). A highly significant (P<0.01) interaction W×S showed that the most effective Se application method was Se seed priming which gave the maximum value (0.90 mg g^{-1}) under normal water supply with respect to no Se supply (0.57 mg g^{-1} FW) under drought stress (Fig. 4.29). Similarly, Se seed priming also gave the highest value for Chl_t (0.75 mg g^{-1} FW) under drought stress. A significant interaction among P×W×S revealed that Se fertigation and Se foliar application at tillering stage significantly increased Chl_t by 16% and 19%, respectively under water deficit conditions (Fig. 4.29). However, foliar supply of Se at anthesis stage under limited water conditions did not significantly improve the leaf Chl_t contents. The interaction among different factors (WxSxPxG) was also significant (Table 4.7).

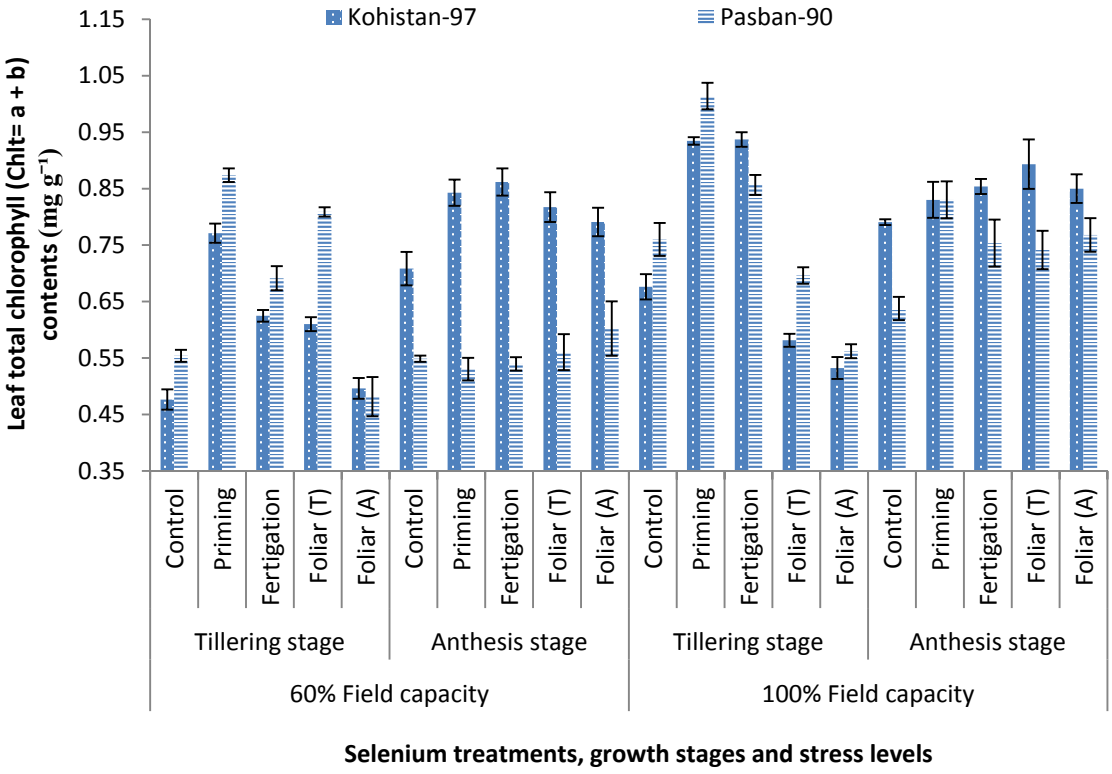

Figure 4.29: Effect of exogenous Se supply on total Chlorophyll (Chl_t = a+b) contents (mg g^{-1} FW) of two wheat (*Triticum aestivum* L.) genotypes at tillering and anthesis stage under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

4.6.6. Leaf carotenoid contents (Car)

A marked effect ($P<0.01$) of drought stress on leaf carotenoid contents (Car) was recorded in both wheat genotypes (Table 4.7). The limited water conditions (60% FC) reduced it by 10% as compared to normal supply of water (100% FC). Genotype Kohistan-97 maintained significantly higher Car contents (1.89 μg g^{-1}) than Pasban-90 (1.81 μg g^{-1}). However, non-significant difference ($P>0.05$) was observed between tillering and anthesis stage for this variable (Table 4.7).

A significant increase ($P<0.01$) in Car contents was observed by exogenous Se supply. Among different Se application methods, priming of seeds with Se was the most effective method that gave the maximum value for leaf Car contents (2.15 μg g^{-1} FW) and increased it by 30% as compared to those with no Se supply (1.65μg g^{-1} FW). The fertigation and Se foliar application at tillering also caused a significant increase in leaf Car contents by 19% and 7%, respectively as compared to control (Fig. 4.30b).

The interaction W×S was also significant for leaf Car contents (Table 4.7). The plants grown from Se primed seeds exhibited higher Car contents under both normal (2.37 μg g^{-1}FW) and water stress (1.92 μg g^{-1} FW) conditions. The lowest Car contents in normal plants (1.71 μg g^{-1} FW) were recorded by Se foliar application at anthesis stage while no Se application resulted in minimum Car contents (1.49 μg g^{-1} FW) under drought stress (Fig. 4.30a). The interaction WxSxPxG was non-significant (Table 4.7).

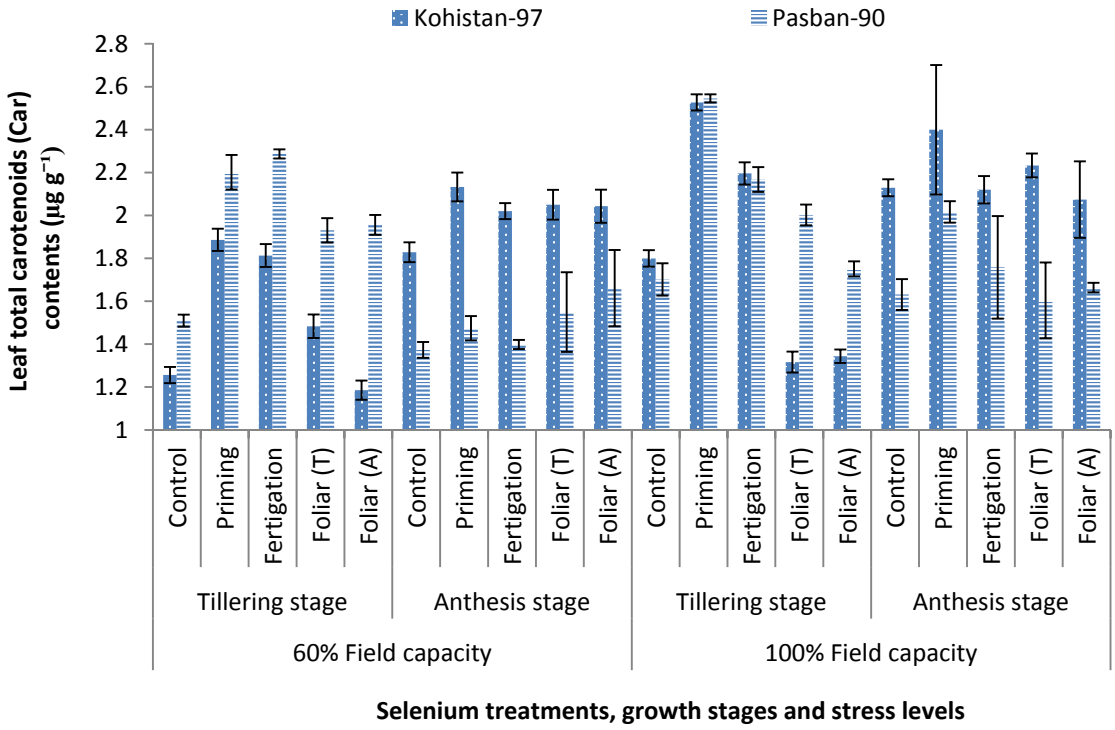

Figure 4.30a: Effect of exogenous Se supply on total Carotenoid (Car) contents (µg g^{-1} FW) of two wheat (*Triticum aestivum* L.) genotypes at tillering and anthesis stage under normal (100% FC) and drought stress (60% FC) conditions. Values are mean±standard error.

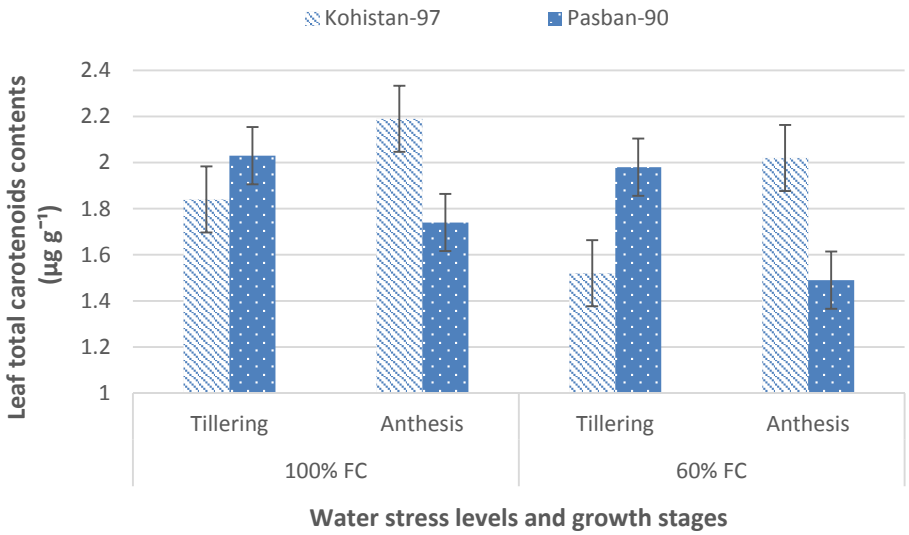

Figure 4.30b: Interaction effect between growth stages (P), water stress levels (W) and genotypes (G) on leaf carotenoid contents (Car). Values are mean ± standard error.

Table: 4.8. Analysis of variance of net CO_2 assimilation rate (µmol CO_2 m^{-2} S^{-1}), transpiration rate (mmol H_2O m^{-1} s^{-1}), and stomatal conductance (mmol H_2O m^{-1} s^{-1}) in two wheat genotypes exposed to exogenous selenium supply under drought stress.

SOV[a]	Net CO_2 assimilation rate (µmol CO_2 m^{-2} s^{-1})	Transpiration rate (mmol H_2O m^{-1} s^{-1})	Stomatal conductance (mmol H_2O m^{-1} s^{-1})
Growth Stages (P)	***	***	***
Water stress levels (W)	***	***	***
Selenium treatments (S)	***	***	***
Genotypes (G)	NS	***	***
P×W	***	***	***
P×S	***	***	***
P×G	**	NS	NS
W×S	***	***	**
W×G	**	NS	NS
S×G	NS	NS	NS
P×W×S	NS	NS	NS
P×W×G	NS	NS	NS
P×S×G	NS	NS	NS
W×S×G	NS	NS	NS
W×S×G×P	NS	NS	NS
CV[b]	4.65	6.17	6.50

NS= non-significant, *significant at $P< 0.05$, ** significant at $P< 0.01$,

*** significant at $P< 0.001$

[a] Source of variation, [b] Co-efficient of variation

4.6.7. Net CO_2 assimilation rate (P_n)

Highly significant effect ($P<0.01$) of drought stress was recorded on P_n of wheat leaves (Table 4.8). Imposition of water stress reduced it by 26% with respect to the plants grown under normal supply of water. The plants maintained significantly higher P_n ($P<0.01$) at tillering (9.65 µmol CO_2 m^{-2} s^{-1}) than anthesis stage (8.20 µmol CO_2 m^{-2} s^{-1}) (Fig. 4.31). Non-significant difference ($P>0.05$) was observed between wheat genotypes Kohistan-97 and Pasban-90 for this variable (Table 4.8).

Different Se application methods also varied significantly ($P<0.01$) for P_n (Table 4.8). The plants foliarly applied with Se at tillering stage maintained significantly higher P_n (9.60 µmol CO_2 m^{-2} s^{-1}) as compared to those supplemented with Se by other methods. The Se fertigation of plants also significantly increased P_n (9.35 µmol CO_2 m^{-2} s^{-1}) closely followed by Se seed priming (9.17 µmol CO_2 m^{-2} s^{-1}). The lowest value (7.89 µmol CO_2 m^{-2} s^{-1}) was recorded in control plants (without Se) (Fig. 4.31).

The highly significant interaction W×S revealed that under normal conditions, foliar application of Se at tillering stage gave the highest value (10.86 µmol CO_2 m^{-2} s^{-1}) for this variable statistically at par with the value (10.67 µmol CO_2 m^{-2} S^{-1}) obtained for Se seed priming. Similarly, in water stressed plants, the foliarly applied Se at tillering stage gave the maximum value (8.35 µmol CO_2 m^{-2} s^{-1}) by increasing P_n by 34% with respect to plants without Se supply that gave the minimum value (6.25 µmol CO_2 m^{-2} S^{-1}) for this variable (Fig. 4.31).The interaction among different factors such as growth stages (P), water stress levels (W), Se treatments (S) and genotypes (G) was non-significant (Table 4.8).

4.6.8. Transpiration rate (*E*)

Analysis of variance for the data regarding *E* revealed that drought stress significantly ($P<0.01$) reduced it by 26% in wheat (Table 4.8). The reduction in *E* was much higher at anthesis (3.21 mmol H_2O m^{-1} s^{-1}) than tillering stage (3.75 mmol H_2O m^{-1} s^{-1}). Wheat genotype Kohistan-97 exhibited significantly higher ($P<0.01$) *E* (3.56 mmol H_2O m^{-1} s^{-1}) than Pasban-90 (3.39 mmol H_2O m^{-1} s^{-1}) (Fig. 4.32).

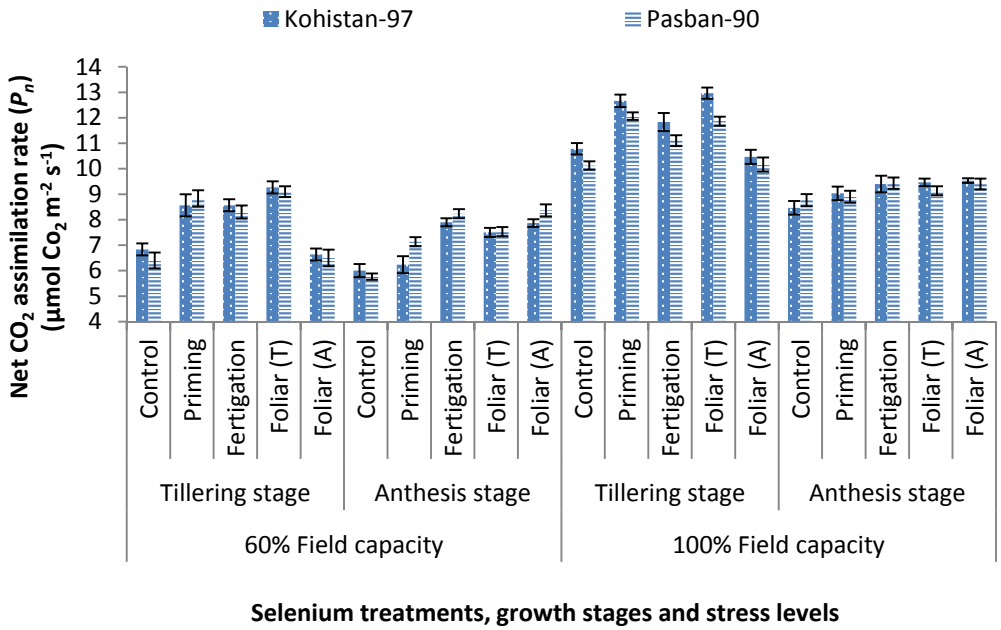

Figure 4.31: Effect of exogenous Se supply on net CO_2 assimilation rate (µmol CO_2 m^{-2} s^{-1}) of two wheat (*Triticum aestivum* L.) genotypes at tillering and anthesis stage under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

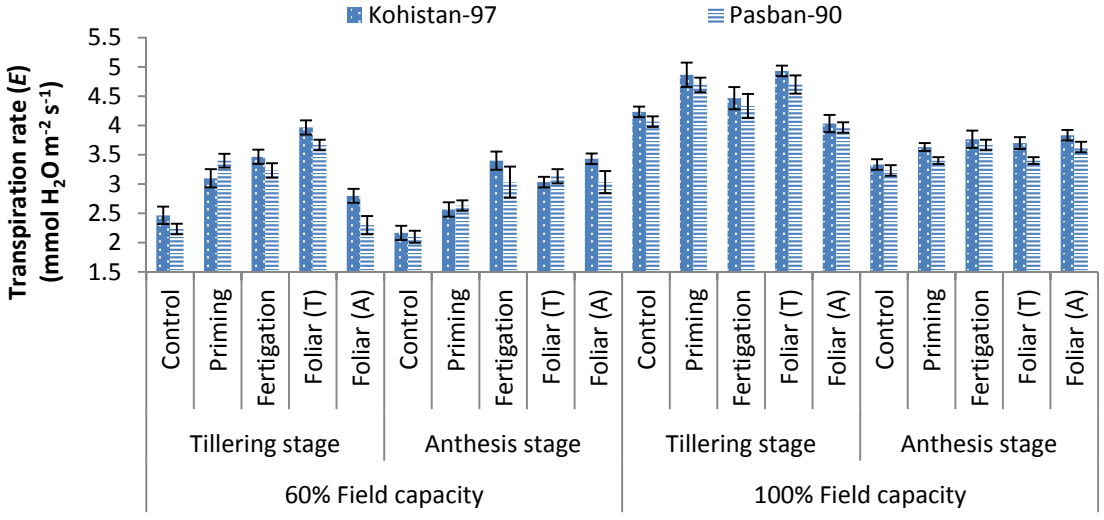

Figure 4.32: Effect of exogenous Se supply on transpiration rate (mmol H_2O m^{-1} s^{-1}) of two wheat (*Triticum aestivum* L.) genotypes at tillering and anthesis stage under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

The exogenous Se application methods also differed significantly (P<0.01) for E (Table 4.8). The foliar application of Se at tillering stage resulted in maximum value (3.82 mmol H_2O m^{-1} s^{-1}) for E with an overall increase of 28% as compared to the plants where Se was not supplied (2.98 mmol H_2O m^{-1} s^{-1}). An increase of 23% and 19% was also observed in E by Se fertigation (3.67 mmol H_2O m^{-1} s^{-1}) and Se seed priming (3.54 mmol H_2O m^{-1} s^{-1}) respectively (Fig. 4.32).

The interaction P×S was also significant. The maximum value (4.18 mmol H_2O m^{-1} s^{-1}) was recorded in plants supplied with Se as foliar treatment at tillering stage while Se fertigation gave the second highest value (3.67 mmol H_2O m^{-1} s^{-1}) for E. No Se application at anthesis stage resulted in the lowest value (2.98 mmol H_2O m^{-1} s^{-1}) in plants (Fig. 4.32). The interaction WxSxPxG was non-significant for E (Table 4.8).

4.6.9. Stomatal conductance (g_s)

The effect of drought stress was statistically significant (P<0.01) for g_s of wheat plants (Table 4.8). The limited water supply (60% FC) reduced it by 27% as compared to normal(100% FC) plants. The reduction in g_s was significantly higher at anthesis (3.16 mmol H_2O m^{-2} s^{-1}) than tillering stage (4.24 mmol H_2O m^{-2} s^{-1}). Drought tolerant wheat genotype Kohistan-97 (3.80 mmol H_2O m^{-2} s^{-1}) maintained higher (5%) g_s than Pasban-90 (3.60 mmol H_2O m^{-2} s^{-1}) (Fig. 4.33).

The highly significant (P<0.01) variation was recorded for g_s among different Se application methods. The foliar spray of Se at tillering stage resulted in maximum value for g_s (4.01 mmol H_2O m^{-2} s^{-1}) and it was 21% higher as compared to plants which were not exposed to Se (3.30 mmol H_2O m^{-2} s^{-1}). The Se fertigation also increased g_s (3.85mmol H_2O m^{-2} s^{-1}) by 17% closely followed by Se seed priming (3.81mmol H_2O m^{-2} s^{-1}) while Se foliar application at anthesis stage increased it by only 7% as compared to no Se supply (Fig. 4.33a).

The highly significant (P<0.01) interaction W×S was recorded for g_s (Table 4.8). The application of Se as foliar spray at tillering stage gave the maximum value (4.48 mmol H_2O m^{-2} s^{-1}) for this variable statistically at par with Se seed priming (4.40 mmol H_2O m^{-2} s^{-1}) and

fertigation (4.36 mmol H_2O m^{-2} s^{-1}) under normal conditions (100%FC) (Fig. 4.33b). Similar trend was recorded under limited water conditions (60% FC) where foliarly applied Se at

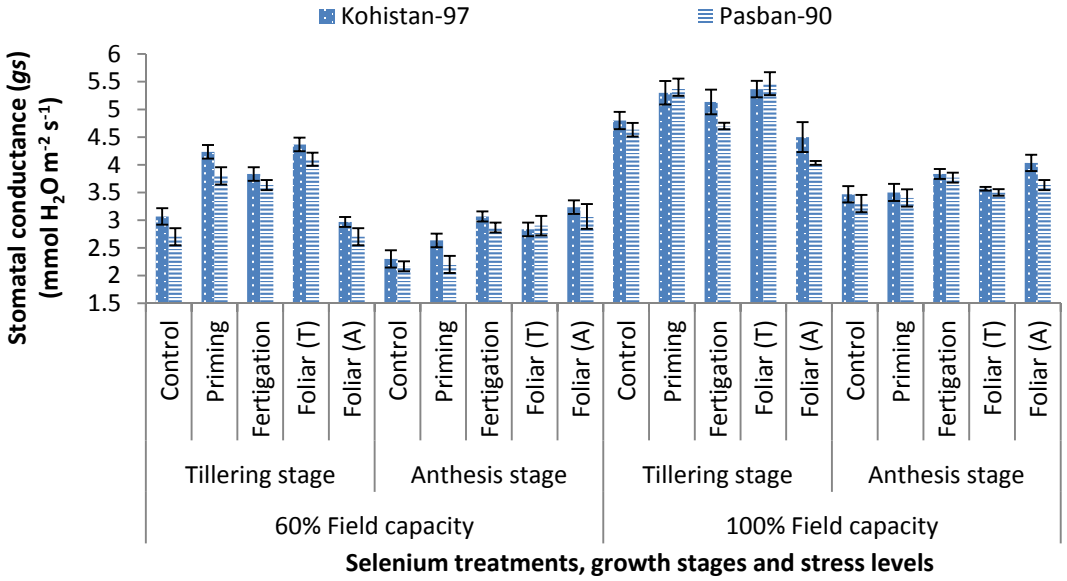

Figure 4.33a: Effect of exogenous Se supply on stomatal conductance (mmol H_2O m^{-1} s^{-1}) of two wheat (*Triticum aestivum* L.) genotypes at tillering and anthesis stage under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

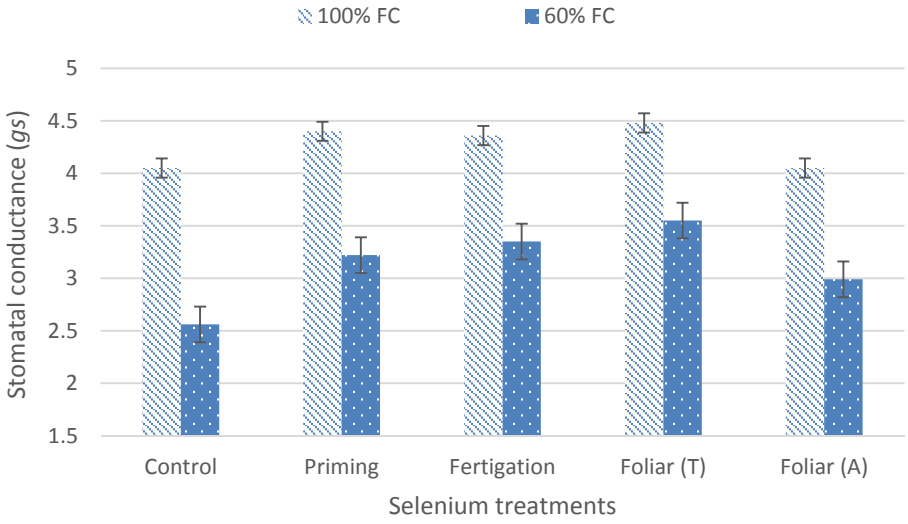

Figure 4.33b: Interaction effect between selenium treatments (S) and water stress levels (W) on stomatal conductance (g_s). Values are mean ± standard error.

tillering stage increased g_s (3.55mmol H_2O m^{-2} s^{-1}) by 39% in respect to plants with no Se supply (2.56mmol H_2O m^{-2} s^{-1}) (Fig. 4.33b). The interaction among different factors (WxSxPxG) was non-significant for this variable (Table 4.8).

4.6.10. Total soluble sugars (TSS)

Analysis of variance regarding TSS revealed that water stress significantly affected this parameter. The leaf TSS increased significantly (P<0.01) due to water deficit conditions (Table 4.9). An increase of 10% was recorded in TSS in water stressed than normal plants. The plants accumulated higher TSS at anthesis (0.55 mg g^{-1} FW) than tillering stage (0.52 mg g^{-1} FW). The accumulation of TSS was (4%) higher in drought sensitive (Pasban-90) than tolerant wheat genotype (Kohistan-97).

Different methods of Se supply varied significantly (P<0.01) for TSS (Table 4.9). The fertigation of plants with Se resulted in the highest value for TSS (0.62 mg g^{-1} FW) closely followed by Se seed priming (0.61 mg g^{-1} FW). The foliar spray of Se at tillering (0.59 mg g^{-1} FW) and anthesis stage (0.52 mg g^{-1} FW) also significantly increased TSS by 64% and 44% respectively as compared to no Se supply that exhibited lowest value for TSS (0.36 mg g^{-1} FW) (Fig. 4.34).

The interaction P×W was also significant (P<0.01). The maximum value for TSS (0.59 mg g^{-1} FW) was recorded at tillering stage under drought stress conditions (60% FC) while it was minimum (0.47 mg g^{-1} FW) in plants grown under normal conditions (100% FC) at the same stage (Fig. 4.28). The highly significant (P<0.001) interaction W×S gave the highest value (0.67 mg g-^{1} FW) for TSS in plants fertigated with Se which was statistically at par with Se applied foliarly(0.65 mg g^{-1} FW) at tillering stage under drought stress. The lowest value (0.33 mg g^{-1} FW) was recorded in plants with no Se supply under water deficit conditions (Fig. 4.34). The interaction among different factors exhibited non-significant difference (P>0.05) regarding accumulation of TSS in leaves (Table 4.9).

Table: 4.9. Analysis of variance of leaf total soluble sugars (mg g^{-1} FW), total soluble proteins (mg g^{-1} FW), and total free amino acids (µmol g^{-1} FW) in two wheat genotypes exposed to exogenous selenium supply under drought stress.

SOV[a]	Total Soluble Sugars (mg g^{-1} FW)	Total Soluble Proteins (mg g^{-1} FW)	Total Free Amino acids (µmol g^{-1} FW)
Growth Stages (P)	*	***	***
Water stress levels (W)	***	***	***
Selenium treatments (S)	***	***	***
Genotypes (G)	*	*	**
P×W	***	**	***
P×S	***	*	***
P×G	**	NS	NS
W×S	***	*	***
W×G	NS	NS	NS
S×G	*	*	**
P×W×S	***	**	***
P×W×G	NS	NS	NS
P×S×G	***	NS	NS
W×S×G	NS	NS	**
W×S×G×P	NS	***	NS
CV[b]	8.54	9.66	12.93

NS= non-significant, *significant at $P< 0.05$, ** significant at $P< 0.01$,

*** significant at $P< 0.001$

[a] Source of variation, [b] Co-efficient of variation

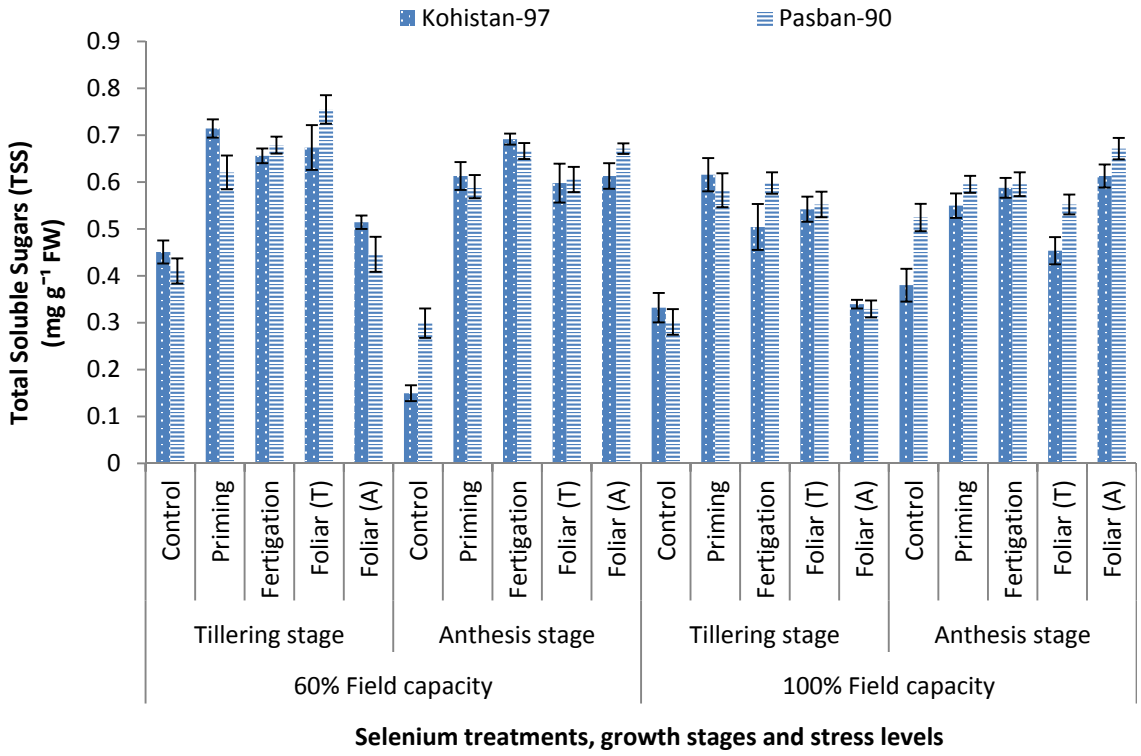

Figure 4.34: Effect of exogenous Se supply on leaf total soluble sugars (mg g^{-1} FW) of two wheat (*Triticum aestivum* L.) genotypes at tillering and anthesis stage under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

4.6.11. Total soluble proteins (TSP)

Imposition of drought stress significantly ($P<0.01$) decreased TSP in wheat genotypes (Table 4.9). A significant reduction of 16% in TSP was recorded in the leaves of plants exposed to drought stress with respect to their normal plants. A more pronounced decrease (8%) was recorded at tillering (7.50 mg g^{-1} FW) than anthesis (8.13 mg g^{-1} FW) stage. Wheat genotype Pasban-90 showed higher reduction (5%) in TSP than Kohistan-97 (Fig. 4.35).

A significant variation ($P<0.05$) among various exogenous Se supply methods for TSP was recorded (Table 4.9). The fertigation of plants resulted in the highest reduction in TSP (7.18 mg g^{-1} FW) as compared to other Se supply methods. The plants grown from seeds primed with Se gave the highest value for TSP (8.25 mg g^{-1} FW) closely followed by Se foliar spray at anthesis stage (8.03 mg g^{-1} FW) and no Se supply (7.97 mg g^{-1} FW).

The interaction W×S was significant ($P<0.05$) for TSP. The lowest value for TSP was noted in normal plants fertigated with Se (7.43 mg g^{-1} FW) while Se seed priming gave maximum value (7.62 mg g^{-1} FW) of TSP in water stressed wheat plants. The interaction among different factors (WxSxPxG) was also significant. The normal plants of genotype Kohistan-97 showed maximum reduction in TSP at tillering stage by Se fertigation whereas Se foliar spray at anthesis stage resulted in highest accumulation of TSP in Kohistan-97 (10.02 mg g^{-1} FW) under normal conditions (Fig. 4.35).

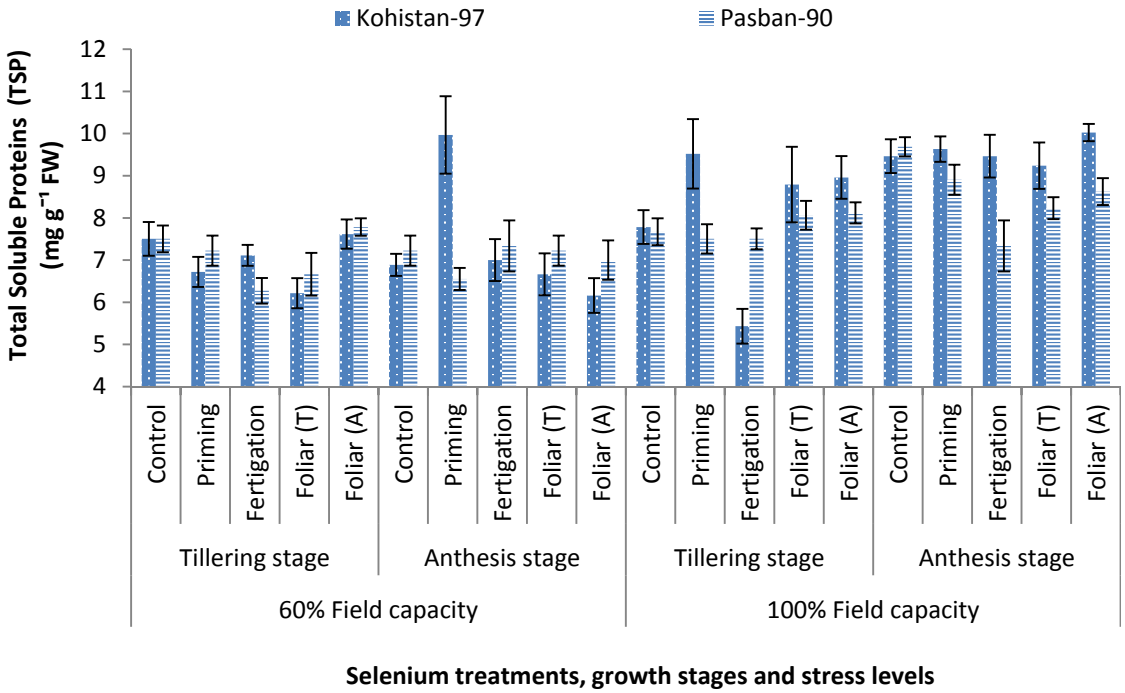

Figure 4.35: Effect of exogenous Se supply on leaf total soluble proteins (mg g^{-1} FW) of two wheat (*Triticum aestivum* L.) genotypes at tillering and anthesis stage under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

4.6.12. Total free amino acids (TFA)

Total free amino acids (TFA) increased significantly ($P<0.01$) by the exposure of wheat genotypes to drought stress (Table 4.9).The water deficit conditions (60% FC) increased TFA concentration by 45% with respect to plants grown under normal conditions (100% FC). The accumulation of TFA was significantly higher (44%) at anthesis (45.21µmol g^{-1} FW) than tillering (31.42 µmol g^{-1} FW) stage (Fig. 4.36). Drought sensitive wheat genotype (Pasban-90) accumulated 9% more TFA than tolerant genotype (Kohistan-97).

The variation among different Se application methods was highly significant ($P<0.01$) for TFA. The plants grown from Se primed seeds accumulated greater TFA (43.93 µmol g^{-1} FW) closely followed by Se foliar spray at tillering stage (43.82 µmol g^{-1} FW) and Se fertigation (43.68 µmol g^{-1} FW) as compared to those with no Se supply (26.41 µmol g^{-1} FW).The foliar application of Se at anthesis stage also increased TFA concentration (33.72 µmol g^{-1} FW) by 28% as compared to control (Fig. 4.36).

The interaction between water stress levels (W) and Se supply methods (S) was also significant (Table 4.9). The maximum value (59.48 µmol g^{-1} FW) was observed by Se seed priming statistically at par with Se fertigation (57.55 µ mol g^{-1} FW) as compared to plants with no Se supply (30.67 µ mol g^{-1} FW) under limited water conditions (Fig. 4.36). Under normal conditions, Se foliar application at tillering stage gave the highest value (32.89 µmol g^{-1} FW) closely followed by Se fertigation (29.81 µmol g^{-1} FW). Non-significant difference was observed between Se foliar spray at anthesis stage (22.36 µmol g^{-1} FW) and no Se supply (22.14 µmol g^{-1} FW) under normal conditions (Fig. 4.36). The interaction among different factors (WxSxPxG) was non-significant for TFA (Table 4.9).

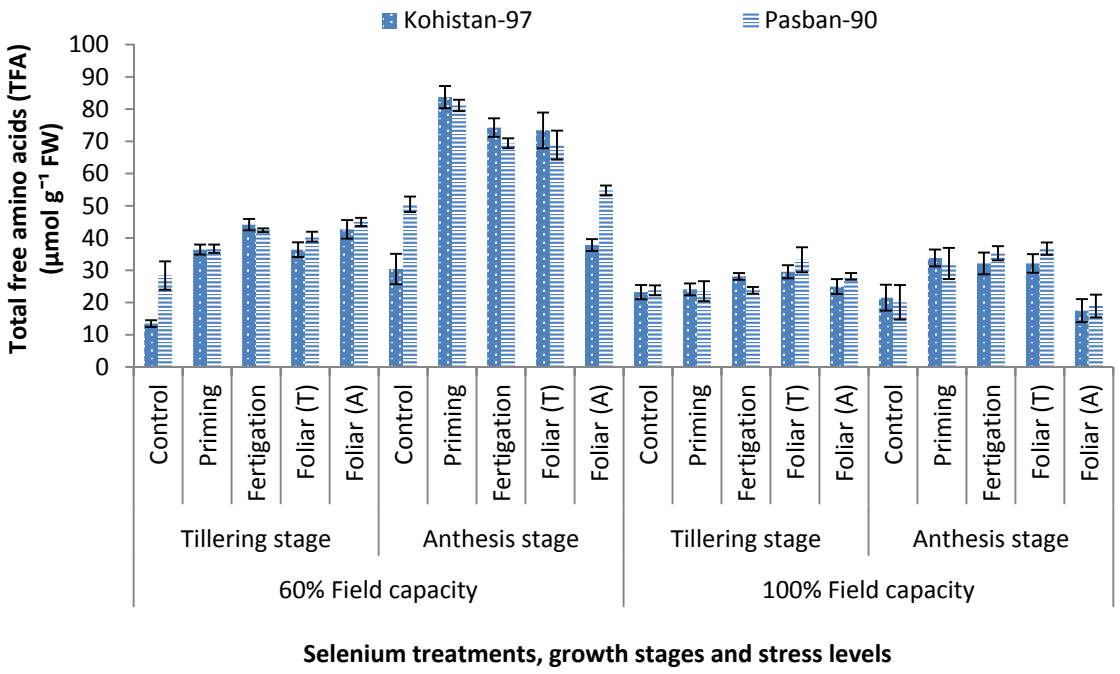

Figure 4.36: Effect of exogenous Se supply on total free amino acids (µmol g^{-1} FW) of two wheat (*Triticum aestivum* L.) genotypes at tillering and anthesis stage under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

4.6.13. Leaf proline contents

The proline content significantly increased (P<0.01) in the leaves of water stressed (60% FC) wheat plants with respect to normal plants (100%FC) (Table (4.10). The proline accumulation was higher under water deficit conditions (0.95 mmol g^{-1} FW) as compared to normal water supply (0.43 mmol g^{-1} FW). The plants accumulated more proline at anthesis (1.15 mmol g^{-1} FW) than tillering stage (0.22 mmol g^{-1} FW) (Fig. 4.37). Non-significant difference was recorded between genotypes for this variable (Table 4.10).

The exogenous Se supply methods differed significantly (P<0.01) for accumulation of proline in leaves. The foliar spray at tillering (0.86 mmol g^{-1} FW) and anthesis (0.79 mmol g^{-1} FW) stages resulted in significantly higher proline contents with an overall increase of 69% and 55% as compared to no Se supply (0.51 mmol g^{-1} FW). A significant proline accumulation was also observed by Se fertigation (0.73 mmol g^{-1} FW) whereas non-significant increase was noted by Se seed priming (0.55 mmol g^{-1} FW) (Fig. 4.37).

The highly significant (P<0.01) interaction was observed between water stress levels (W) and Se supply methods (S) (Table 4.10). The exogenous Se supply as foliar spray at tillering stage resulted in the highest proline contents (1.23 mmol g^{-1} FW) and caused a significant increase of 73% as compared to plants with no Se supply (0.71mmol g^{-1} FW) under drought stress. Similarly, Se fertigation and foliar spray at anthesis stage increased proline accumulation in water deficit plants (60% FC) by 41% and 48% respectively (Fig. 4.37). The highest proline concentration (0.53 mmol g^{-1} FW) was observed in the normal plants (100% FC) foliarly supplied with Se at anthesis stage which was statistically at par with the values obtained by Se fertigation (0.47 mmol g^{-1} FW) and foliar application at tillering stage (0.48 mmol g^{-1} FW). Non-significant difference was recorded between Se seed priming and no Se supply under normal conditions (Fig. 4.37). The interaction WxSxPxG was non-significant (Table 4.10).

Table: 4.10. Analysis of variance of proline content (mmol g^{-1}FW), peroxidase (units min^{-1} g^{-1} FW), catalase (units min^{-1} g^{-1} FW) and ascorbate Peroxidase (ABA digested g^{-1} FW h^{-1}) activity in two wheat genotypes exposed to exogenous selenium (Se) supply under drought stress.

SOV[a]	Proline (mmol g^{-1} FW)	Peroxidase (units min^{-1} g^{-1} FW)	Catalase (units min^{-1} g^{-1} FW)	Ascorbate Peroxidase (ABA digested g^{-1} FW h^{-1})
Growth Stages (P)	***	***	***	***
Water stress levels (W)	***	***	***	***
Selenium treatments (S)	***	***	***	***
Genotypes (G)	NS	NS	*	***
P×W	***	***	NS	***
P×S	***	***	***	***
P×G	NS	NS	NS	NS
W×S	***	***	***	**
W×G	*	NS	***	NS
S×G	NS	*	*	*
P×W×S	NS	***	*	*
P×W×G	**	NS	**	NS
P×S×G	*	NS	NS	NS
W×S×G	NS	NS	**	NS
W×S×G×P	NS	NS	*	*
CV[b]	20.24	8.70	6.05	4.41

NS= non-significant, *significant at $P< 0.05$, ** significant at $P< 0.01$,

*** significant at $P< 0.001$

[a] Source of variation, [b] Co-efficient of variation

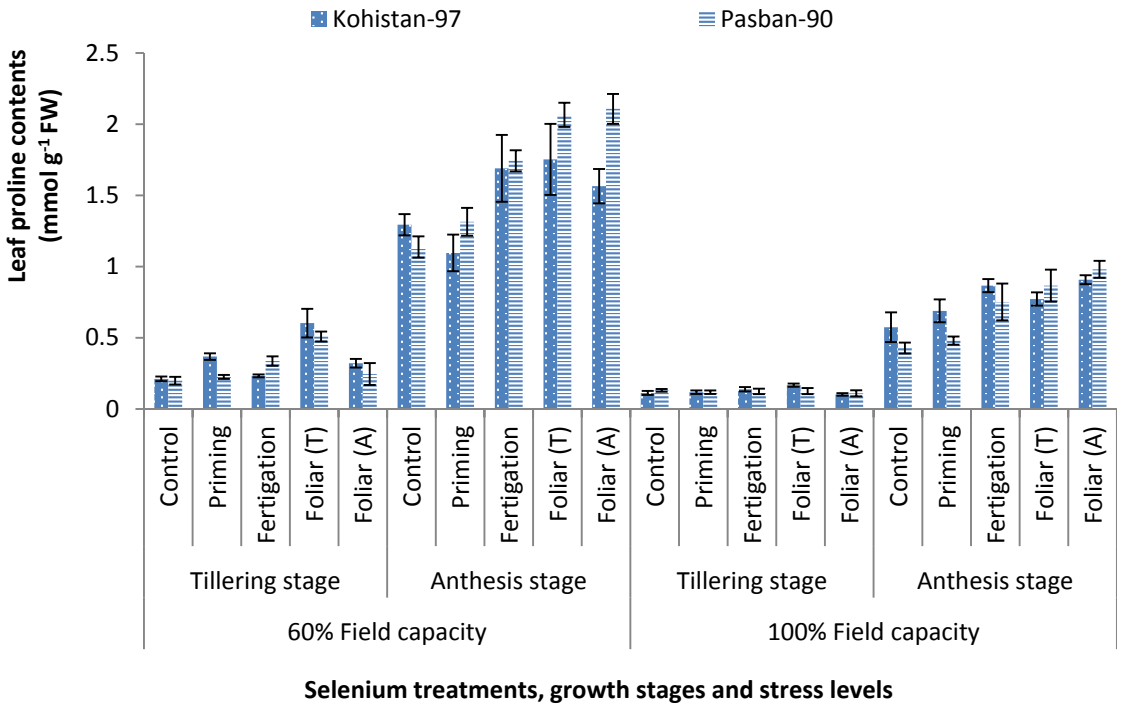

Figure 4.37: Effect of exogenous Se supply on proline content (mmol g^{-1} FW) of two wheat (*Triticum aestivum* L.) genotypes at tillering and anthesis stage under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

4.6.14. Peroxidase activity (POX)

Plants exposed to water stress showed significant (P<0.01) increase in their activity of peroxidase (POX). Plants grown under limited supply of water had higher POX activity (17%) as compared to normal plants (Table 4.10). A pronounced increase in POX activity was recorded at anthesis (1420.41 units min^{-1} g^{-1} FW) than tillering (451.60 units min^{-1} g^{-1} FW) stage (Fig. 4.38). Wheat genotypes (Kohistan-97 and Pasban-90) showed non-significant variation for this variable (Table 4.10).

Different methods of exogenous Se supply varied significantly (P<0.01) for POX activity (Table 4.10). The plants foliarly applied with Se at tillering stage exhibited maximum POX activity (1063.01 units min^{-1} g^{-1} FW). The Se fertigation (974.22 units min^{-1} g^{-1} FW) and Se seed priming (966.51 units min^{-1} g^{-1} FW) non-significantly differed from each other in POX activity while minimum activity (758.9 units min^{-1} g^{-1} FW) was recorded in plants with no Se supply (Fig. 4.38).

The interaction W×S was also significant for POX activity. The highest value (1130.5 units min^{-1} g^{-1} FW) was noted in plants supplied with Se as foliar spray at tillering stage closely followed by Se foliar spray at anthesis stage (1106.12 units min^{-1} g^{-1} FW) and Se fertigation (1070.61 units min^{-1} g^{-1} FW) under drought stress (Fig. 4.38). In normal plants, the maximum POX activity (995.44 units min^{-1} g^{-1} FW) was also recorded in plants sprayed foliarly with Se at tillering stage which was statistically at par with Se seed priming (944.33 units min^{-1} g^{-1} FW). The plants applied with no Se gave the lowest values under both normal (706.50units min^{-1} g^{-1} FW) and water stress (811.21 units min^{-1} g^{-1} FW) conditions (Fig. 4.38).

The different factors (WxSxPxG) exhibited non-significant difference for this variable (Table 4.10).

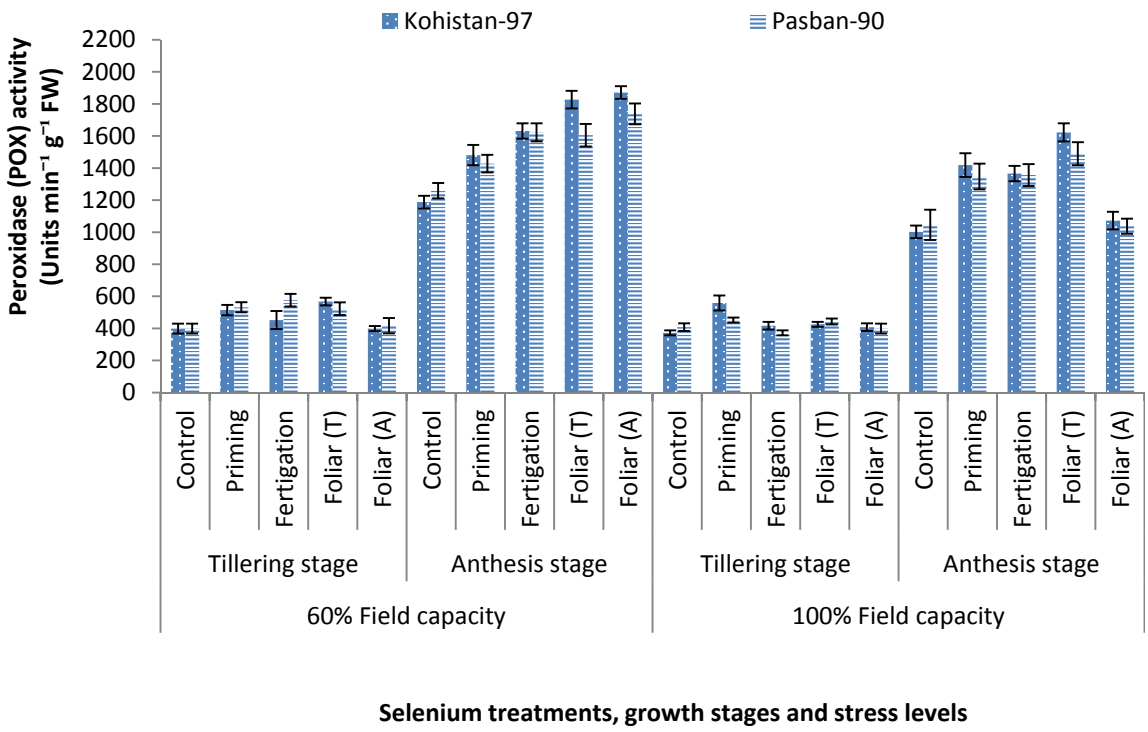

Figure 4.38: Effect of exogenous Se supply on peroxidase activity (units min^{-1} g^{-1} FW) of two wheat (*Triticum aestivum* L.) genotypes at tillering and anthesis stage under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

4.6.15. Catalase activity (CAT)

A highly significant ($P<0.01$) increase in the CAT activity of wheat genotypes was observed under drought stress (Table 4.10). An increase of 7% was recorded in the enzymatic activity of water stressed plants as compared to normal ones. At anthesis stage, plants (512.74 units min^{-1} g^{-1} FW) exhibited 27% higher CAT activity than at tillering (404.8 units min^{-1} g^{-1} FW) stage (Fig. 4.39). Drought tolerant genotype (Kohistan-97) maintained higher CAT activity (3%) than drought sensitive genotype (Pasban-90).

The plants subjected to various Se supply methods responded significantly ($P<0.05$) for CAT activity (Table 4.10).The maximum activity (482.50 units min^{-1} g^{-1} FW) was noted in plants grown from Se primed seeds closely followed by Se fertigation (473.41 units min^{-1} g^{-1} FW). Foliar spray of Se at tillering and anthesis stages increased CAT activity by 6% and 3% respectively compared to no Se supply (431.62 units min^{-1} g^{-1} FW) (Fig. 4.39).

The interaction W×S was significant and the highest CAT activity (502.70 units min^{-1} g^{-1} FW) was recorded in plants foliarly sprayed with Se at tillering stage which was statistically at par with the values obtained by Se fertigation (491.52 units min^{-1} g^{-1} FW) and Se seed priming (491.01 units min^{-1} g^{-1} FW) under drought stress (Fig. 4.39). A significant effect of Se supply was also recorded under normal conditions. The priming of seeds with Se resulted in the maximum CAT activity (474.03 units min^{-1} g^{-1} FW) in normal plants whereas foliar spray of Se at tillering stage gave the minimum value (415.20 units min^{-1} g^{-1} FW) statistically related to the value (419.50 units min^{-1} g^{-1} FW) noted in plants with no Se supply under normal supply of water (Fig. 4.39).

The interaction among different factors (WxSxPxG) was also significant for CAT activity. The maximum activity (584.02 units min^{-1} g^{-1} FW) was recorded in wheat genotype Pasban-90 at anthesis stage in Se fertigated plants grown under water deficit conditions whereas no Se supply resulted in minimum CAT activity (326.04 units min^{-1} g^{-1} FW) in normal plants at tillering stage (Fig. 4.39).

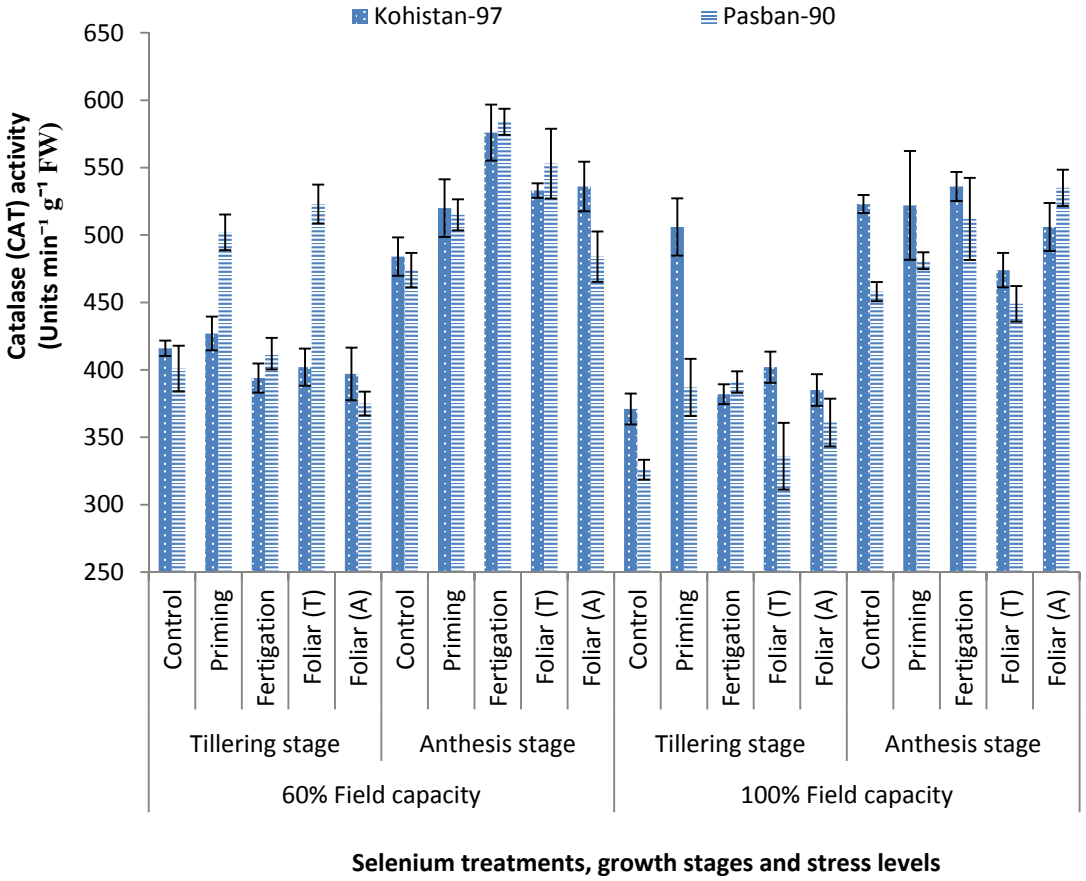

Figure 4.39: Effect of exogenous Se supply on catalase activity (mg g^{-1} FW) of two wheat (*Triticum aestivum* L.) genotypes at tillering and anthesis stage under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

4.6.16. Ascorbate peroxidase activity (APX)

The APX activity increased significantly ($P<0.01$) in the leaves of wheat plants exposed to drought stress (Table 4.10). A significant increase of 20% was recorded in APX activity under water deficit conditions with respect to normal plants. The activity was significantly higher at anthesis (3.49 ABA digested g^{-1} FW h^{-1}) than tillering (1.52 ABA digested g^{-1} FW h^{-1}) stage (Fig. 4.40).Wheat genotypes also differed significantly ($P<0.05$), Kohistan-97 maintained 7% higher APX activity than Pasban-90.

Exogenous Se supply methods varied significantly ($P<0.01$) for this variable. The fertigation of plants resulted in maximum APX activity (2.67 ABA digested g^{-1} FW h^{-1}) closely followed by Se foliar spray at tillering stage. The plants grown from Se primed seeds and foliarly applied with Se at anthesis stage also exhibited 23% and 22% higher APX activity respectively than control (no Se supply) plants (2.09 ABA digested g^{-1} FW h^{-1}) (Fig. 4.40).

The interaction W×S revealed highly significant ($P<0.01$) effect of different Se supply methods on APX activity of both normal and water stressed plants (Table 4.10). The application of Se as foliar spray at tillering stage gave maximum value (2.96 ABA digested g^{-1} FW h^{-1}) for this variable statistically at par with the value obtained by Se fertigation (2.89 ABA digested g^{-1} FW h^{-1}) under water deficit conditions. Non-significant difference ($P>0.05$) was observed between plants foliarly applied with Se at anthesis stage (2.76 ABA digested g^{-1} FW h^{-1}) and Se seed priming (2.74 ABA digested g^{-1} FW h^{-1}). Similarly, Se seed priming (2.39 ABA digested g^{-1} FW h^{-1}), Se fertigation (2.45 ABA digested g^{-1} FW h^{-1}) and Se foliar application at tillering stage (2.37 ABA digested g^{-1} FW h^{-1}) were statistically similar in response to APX activity under normal conditions (Fig. 4.40).The plants with no Se supply showed minimum APX activity under both normal (1.85 ABA digested g^{-1} FW h^{-1}) and water stressed conditions (2.33 ABA digested g^{-1} FW h^{-1}).

The interaction among factors such as growth stages (P), genotypes (G), water stress levels (W) and Se supply methods (S) was also significant (Table 4.10). The water stressed plants of Kohistan-97 foliarly applied with Se at anthesis stage exhibited the highest APX activity (4.05 ABA digested g^{-1} FW h^{-1}) while the lowest activity (0.93 ABA digested g^{-1} FW

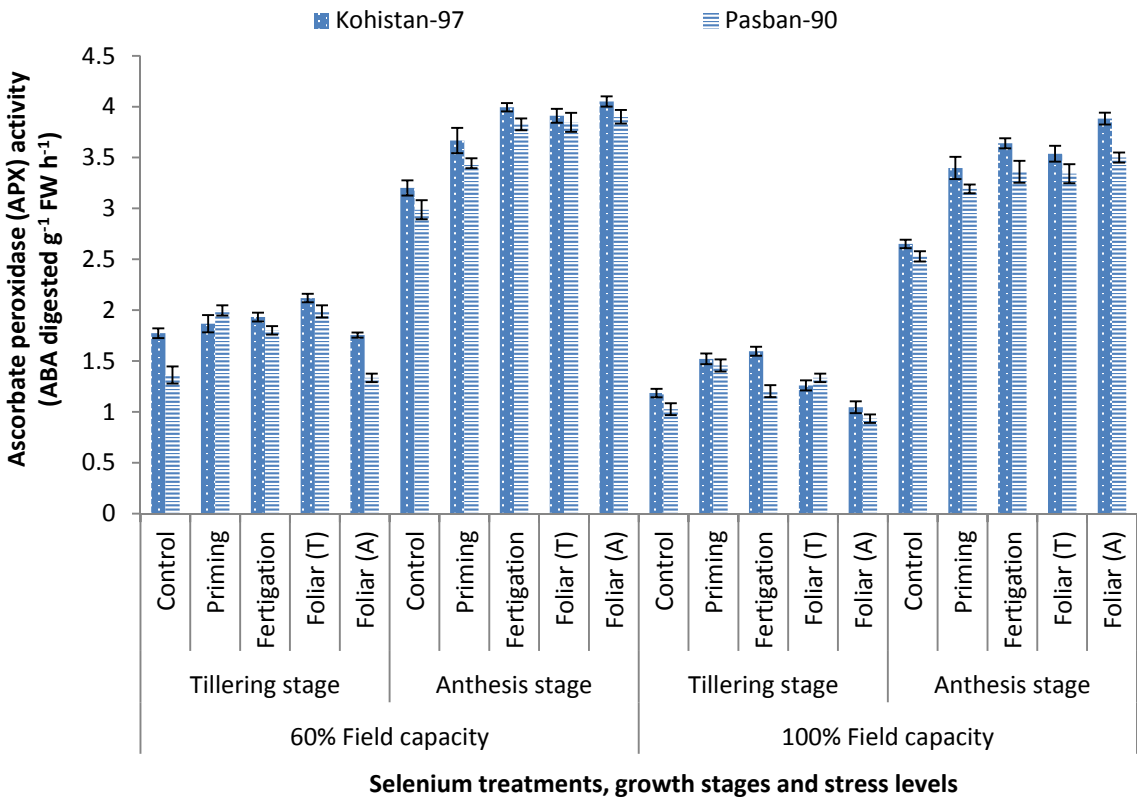

Figure 4.40: Effect of exogenous Se supply on ascorbate peroxidase activity (ABA digested g^{-1} FW h^{-1}) of two wheat (*Triticum aestivum* L.) genotypes at tillering and anthesis stage under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

h^{-1}) was recorded in normal plants of Pasban-90 at tillering stage with no Se supply (Fig. 4.40).

4.6.17. Concentration of nutrients in shoot

The water deficit conditions had a significant effect (P<0.05) on the concentration of nutrients in shoot (Table 4.11). The effect of exogenous Se application on Se concentration in shoot was examined and the results showed 77% higher accumulation in shoot of water stressed wheat plants (60% FC) than normal plants (100% FC). The maximum accumulation (19.87 µg g^{-1}) of Se was observed in plants fertigated with Se. The plants foliarly supplied with Se at tillering and anthesis stage accumulated 15.66 µg g^{-1} and 4.10 µg g^{-1} Se in shoot while negligible amount was recorded in plants applied with no Se or exogenously treated with Se, as seed priming (Fig. 4.41). Non-significant difference (P>0.05) was observed between Kohistan-97 and Pasban-90 for shoot Se concentration (Table 4.11).

The interaction among stress levels (W), Se treatments (S) and genotypes (G) was also significant (P<0.05). Wheat genotype Kohistan-97 accumulated maximum Se (26.00 µg g^{-1}) by fertigation of plants with Se under water deficit conditions statistically at par with plants foliarly applied with Se at anthesis stage (24.47 µg g^{-1}) and plants of Pasban-90 supplied with Se through fertigation under drought stress (22.40 µg g^{-1}) (Fig. 4.41).

Drought stress significantly reduced (P<0.05) shoot iron (Fe) concentration of both wheat genotypes (Table 4.11). The water stressed plants grown at 60% FC accumulated 35% less Fe in shoot as compared to normal plants (100%FC). The shoot Fe concentration also decreased significantly by exogenous Se supply. The highest value (1709.5 µg g^{-1}) was recorded in plants without Se supply (control). Overall, less Fe accumulation in shoot of plants applied with Se through any method showed inhibitory effect of Se on Fe uptake in plants. Se foliar application at tillering and anthesis stage caused significantly less reduction (19% and 16%) than Se fertigation (43%) and Se seed priming (38%) with respect to control (Fig. 4.42).

The interaction among different factors (W×S×G) was also highly significant (Table 4.11). The maximum shoot Fe accumulation (2605.30 µg g^{-1}) was recorded in Pasban-90 plants sprayed with Se at tillering stage which was statistically at par with no Se supply in

Table: 4.11. Analysis of variance of shoot selenium (Se), phosphorous (P), potassium (K) and Zinc (Zn) concentration (µg g^{-1} DW) in two wheat genotypes by exogenous selenium supply under drought stress.

SOV[a]	Shoot Selenium (Se) Conc. (µg g^{-1} DW)	Shoot Iron (Fe) Conc. (µg g^{-1} DW)	Shoot Zinc (Zn) Conc. (µg g^{-1} DW)	Shoot Phosphorous (P) Conc. (µg g^{-1} DW)
Water stress levels (W)	***	***	***	***
Selenium treatments (S)	***	***	***	***
Genotypes (G)	NS	***	NS	NS
W×S	***	***	***	NS
W×G	*	***	NS	NS
S×G	NS	***	NS	NS
W×S×G	*	***	NS	NS
CV[b]	29.96	13.55	31.91	13.23

NS= non-significant, *significant at $P< 0.05$, ** significant at $P< 0.01$,

*** significant at $P< 0.001$

[a] Source of variation

[b] Co-efficient of variation

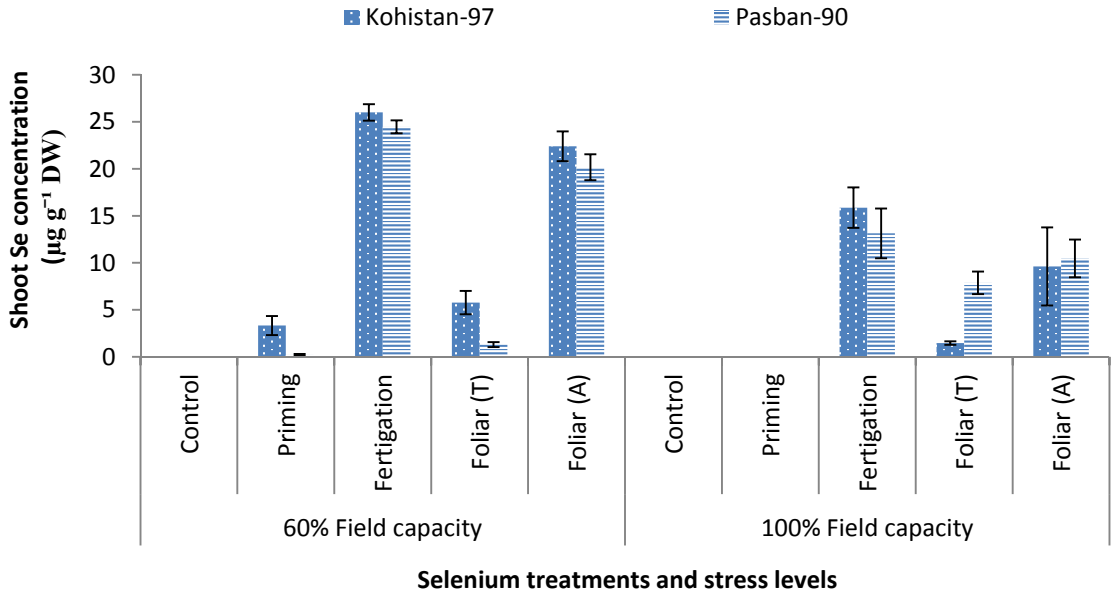

Figure 4.41: Effect of exogenous Se supply on shoot selenium (Se) concentration (µg g^{-1} DW) of two wheat (*Triticum aestivum* L.) genotypes under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

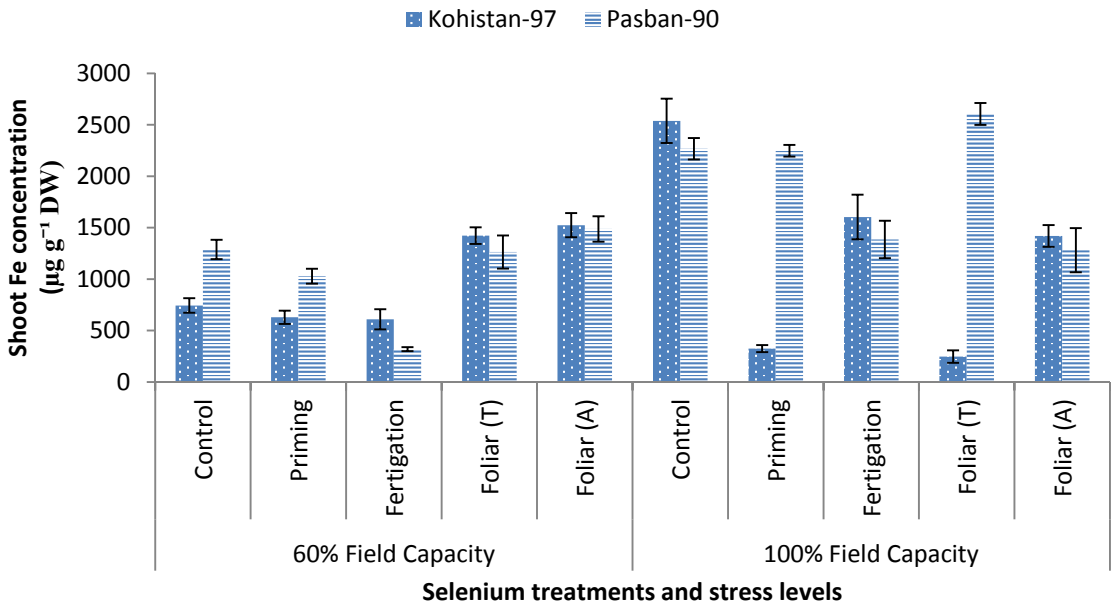

Figure 4.42: Effect of exogenous Se supply on shoot iron (Fe) concentration (µg g^{-1} DW) of two wheat (*Triticum aestivum* L.) genotypes under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

Kohistan-97 under normal conditions (2538.80 µg g^{-1}). Drought tolerant genotype Kohistan-97 showed minimum Fe accumulation (247.00 µg g^{-1}) in shoot by Se foliar application at tillering stage (Fig. 4.42).

The exposure to drought stress significantly increased (P<0.001) accumulation of zinc (Zn) in the shoot of wheat plants (Table 4.11). An overall increase of 48% was recorded in water stressed plants in comparison to normal plants. A significant decrease (P<0.001) in shoot Zn concentration was recorded by exogenous Se supply. The minimum reduction of 39% was recorded in plants foliarly applied with Se at anthesis stage (29.98 µg g^{-1}) while all other methods viz. Se foliar application at tillering stage (21.10 µg g^{-1}), Se seed priming (21.16 µg g^{-1}) and Se fertigation (28.17 µg g^{-1}), decreased shoot Zn accumulation by 57%, 57% and 43% respectively as compared to plants applied with no Se (49.13 µg g^{-1}) (Fig. 4.43). Wheat genotype Kohistan-97 and Pasban-90 showed non-significant difference for this variable. The interaction among different factors i.e. water stress levels (W), Se treatments (S) and genotypes (G) was also non-significant (Table 4.11).

The shoot phosphorus (P) concentration significantly reduced (P<0.01) in plants grown under water stress conditions (Table 4.11). The plants exposed to drought stress accumulated 31% less P in shoot than normal plants. The exogenous Se supply showed antagonistic effect on P uptake in wheat plants and decreased its accumulation in shoot by Se fertigation (34%), Se foliar application at anthesis (26%), Se seed priming (25%) and Se foliar application at tillering stage (23%) as compared to normal plants (100% FC) which accumulated maximum P (833.04 µg g^{-1} P) in shoot (Fig. 4.44). Both genotypes showed non-significant difference for this variable. All the interactions among different factors were also non-significant (Table 4.11).

Analysis of variance for the data regarding shoot potassium (K) concentration showed highly significant effect (P<0.05) of drought stress on K uptake in wheat (Table 4.12). The plants exposed to drought stress accumulated 11% more K in shoot than normal plants (Fig. 4.45). The different methods of exogenous Se supply also varied significantly (P<0.01) for this variable. Foliar application of Se at tillering stage resulted in maximum shoot K concentration (2166.70 µg g^{-1}) in comparison to control i.e.no Se supply (666.72 µg

g^{-1}). Fertigation with Se was the second best method to enhance K accumulation in wheat shoot

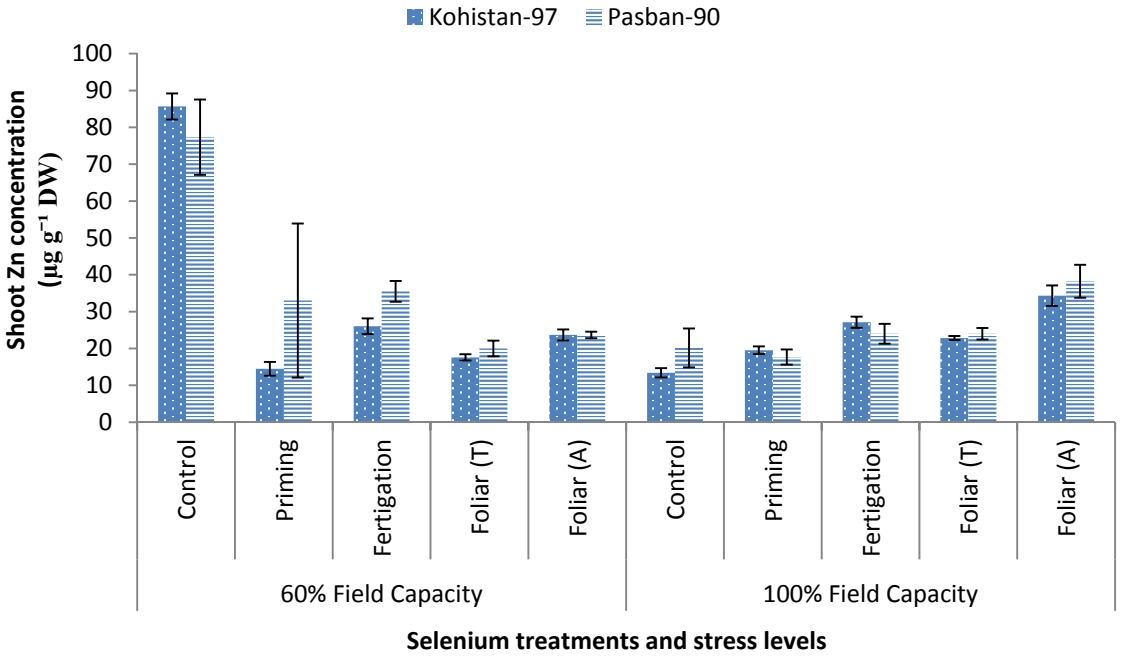

Figure 4.43: Effect of exogenous Se supply on shoot zinc (Zn) concentration (µg g^{-1} DW) of two wheat (*Triticum aestivum* L.) genotypes under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

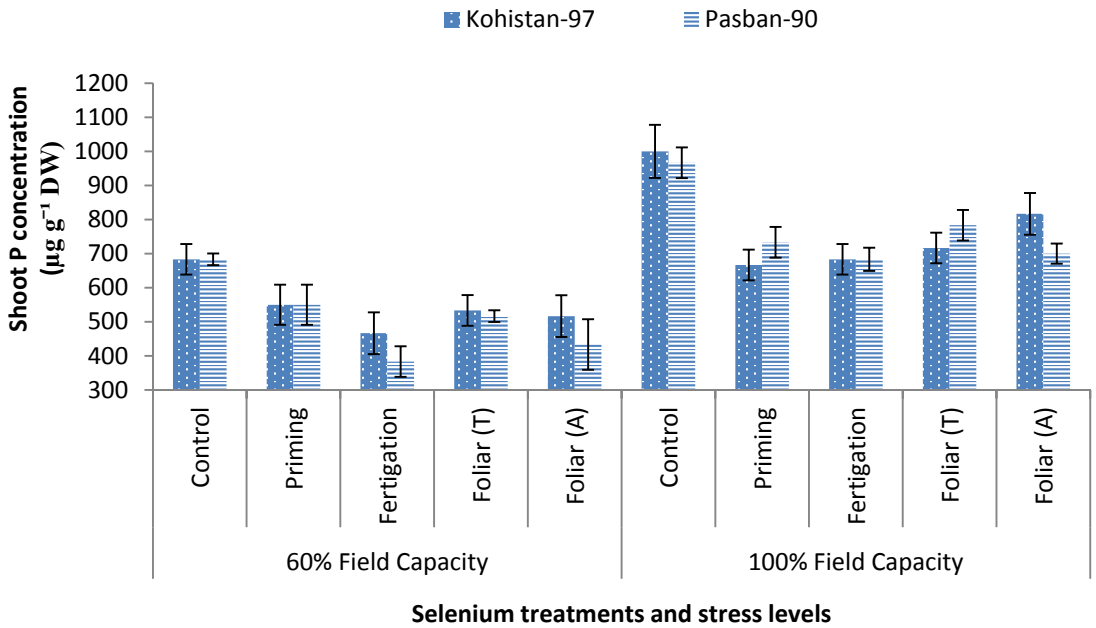

Figure 4.44: Effect of exogenous Se supply on shoot phosphorous (P) concentration (µg g^{-1} DW) of two wheat (*Triticum aestivum* L.) genotypes under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

(1762.51 µg g^{-1}) while non-significant difference was recorded between Se seed priming (1283.30 µg g^{-1}) and Se foliar application at anthesis stage (1316.70 µg g^{-1}) (Fig. 4.45).

The interaction among water stress levels (W), genotypes (G) and Se treatments (S) was also significant (P<0.05). The foliar application of Se at tillering stage gave the highest values for shoot K concentration in water stressed plants of both Pasban-90 (2466.70 µg g^{-1}) and Kohistan-97 (2266.71µg g^{-1}) while no Se supply resulted in the lowest K accumulation in both genotypes under normal conditions (Fig. 4.45).

The shoot accumulation of magnesium (Mg) was reduced by 15% in plants exposed to drought stress as compared to normal plants (Fig 4.46). The exogenous Se supply enhanced the uptake of Mg in wheat plants under both normal and water deficit conditions. The Se fertigation of plants resulted in maximum accumulation of Mg in shoot (971.08 µg g^{-1}) and had non-significant difference with the Se foliar application at tillering stage (955.17 µg g^{-1}). The foliar application of Se at anthesis stage was recognized as the second best method regarding shoot Mg concentration with an overall accumulation of 824.24 µg g^{-1}. The minimum concentration (600.92 µg g^{-1}) was recorded in control plants i.e. no Se supply (Fig. 4.46). Both genotypes showed non-significant difference between them for this variable (Table 4.12).

The interaction between water stress levels (W) and Se methods (S) was also highly significant. Se fertigation of normal plants (100% FC) gave the highest value i.e. 1146.00µg g^{-1} Mg while no Se supply under water deficit conditions resulted in the lowest value (472.20 µg g^{-1} Mg) for this variable. The plants foliarly applied with Se at tillering stage showed maximum shoot Mg concentration (1016.00 µg g^{-1} Mg) under drought stress (Fig. 4.46). The interaction W×S×G was non-significant for this variable (Table 4.12).

Analysis of variance for the data showed highly significant effect (P<0.01) of drought stress on shoot calcium (Ca) concentration in wheat plants (Table 4.12). The

exposure to drought stress reduced Ca uptake in plants by 32%. Among different Se application methods, only plants which were foliarly sprayed with Se at tillering stage showed significantly higher shoot Ca concentration i.e. 3234.50 µg g^{-1} while non-significant difference was observed among other Se supply methods viz. control (2593.70µg g^{-1}), Se foliar application at anthesis stage (2536.70µg g^{-1}), Se seed priming (2437.20µg g^{-1}) and Se fertigation (2377.70µg g^{-1}) (Fig. 4.47).

The interaction among W×S×G was also highly significant ($P<0.01$). The normal plants of Pasban-90 showed maximum shoot Ca accumulation where Se was applied foliarly at tillering stage (5140.30 µg g^{-1}) statistically similar to that of no Se supply (4668.00 µg g^{-1}) and Se seed priming (4501.30 µg g^{-1}) under normal conditions while minimum value (783.9 µg g^{-1} Ca) was also recorded in plants of Pasban-90 fertigated with Se under drought stress (Fig. 4.47).

Table: 4.12. Analysis of variance of shoot magnesium (Mg), iron (Fe) and calcium (Ca) concentration (µg g^{-1} DW) in two wheat genotypes by exogenous selenium supply under drought stress

SOV[a]	Shoot Potassium (K) concentration (µg g^{-1} DW)	Shoot Magnesium (Mg) concentration (µg g^{-1} DW)	Shoot Calcium (Ca) concentration (µg g^{-1} DW)
Water stress levels (W)	*	***	***
Selenium treatments (S)	***	***	**
Genotypes (G)	NS	NS	***
W×S	NS	***	***
W×G	NS	NS	***
S×G	NS	***	***
W×S×G	*	NS	**
CV[b]	18.56	8.16	18.16

NS= non-significant, *significant at $P< 0.05$, ** significant at $P< 0.01$,

*** significant at $P< 0.001$

[a] Source of variation, [b] Co-efficient of variation

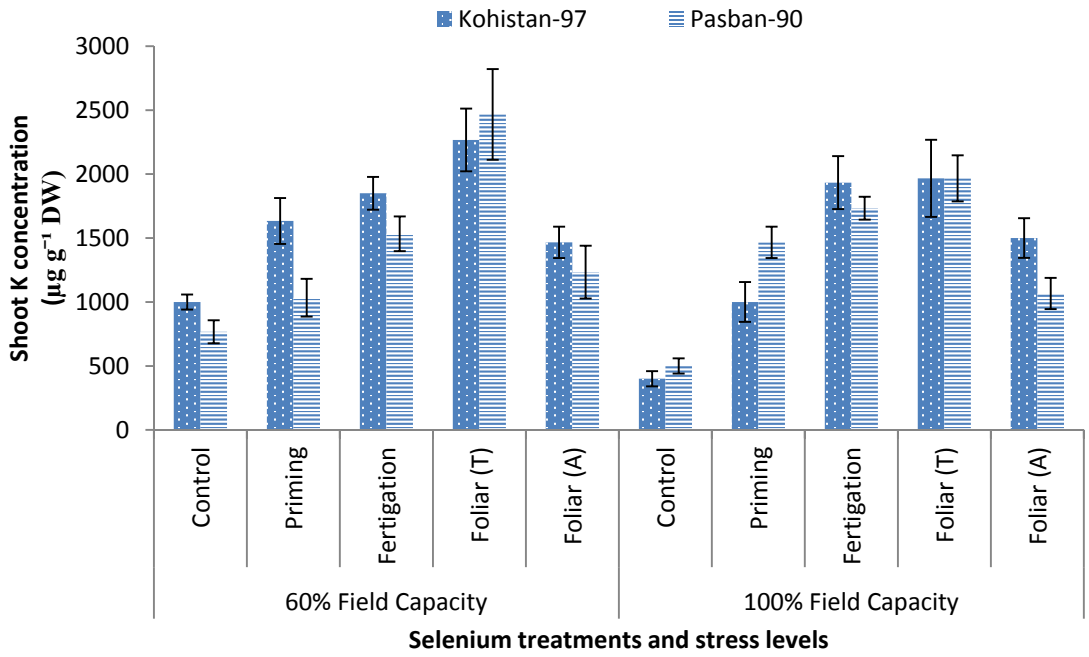

Figure 4.45: Effect of exogenous Se supply on shoot potassium (K) concentration (µg g^{-1} DW) of two wheat (*Triticum aestivum* L.) genotypes under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

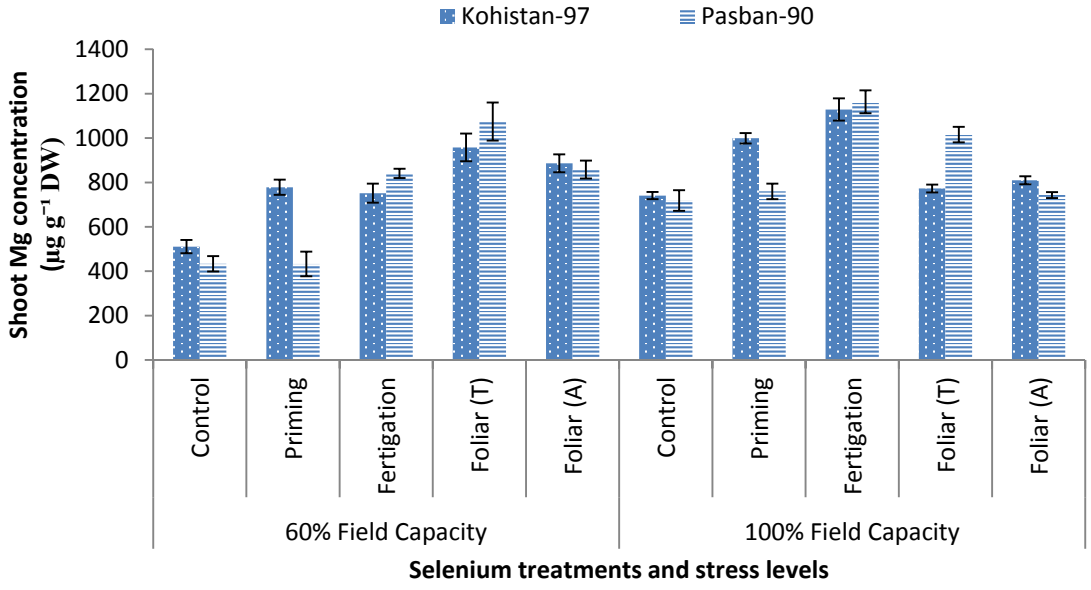

Figure 4.46: Effect of exogenous Se supply on shoot magnesium (Mg) concentration (µg g^{-1} DW) of two wheat (*Triticum aestivum* L.) genotypes under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

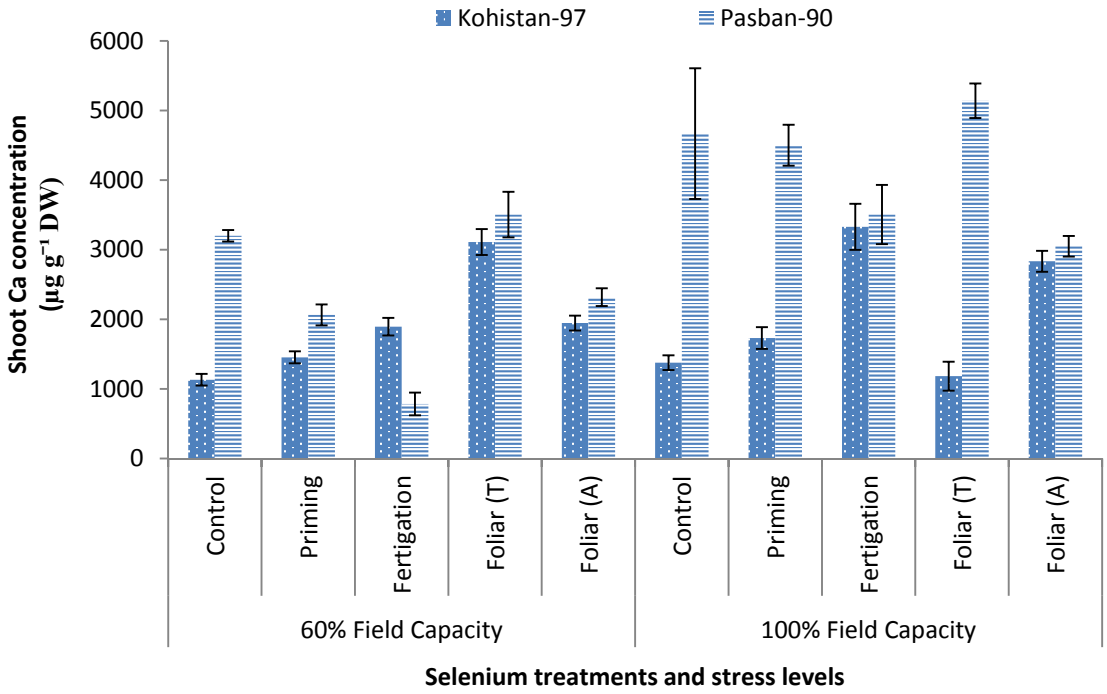

Figure 4.47: Effect of exogenous Se supply on shoot calcium (Ca) concentration (µg g^{-1} DW) of two wheat (*Triticum aestivum* L.) genotypes under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

4.6.18. Concentration of nutrients in grain

Analysis of variance for the data regarding grain Se concentration in wheat showed highly significant effect ($P<0.001$) of drought stress (Table 4.13). It was significantly higher (3.17 µg g^{-1} Se) in plants grown under limited water supply in comparison to normal plants (1.46 µg g^{-1} Se). The different methods of exogenous Se supply also varied significantly ($P<0.01$) for this variable. The foliar application of Se at anthesis resulted in maximum grain Se accumulation (4.02 µg g^{-1} Se) statistically at par with Se fertigation (3.89 µg g^{-1} Se). Foliar application of Se at tillering was the second best method that resulted in higher grain Se concentration (2.49 µg g^{-1}) while no Se application resulted in minimum accumulation (0.20 µg g^{-1}). Kohistan-97 accumulated 45% higher Se in grain (2.74 µg g^{-1}) than Pasban-90 (1.89 µg g^{-1}) for this variable (Fig. 4.48).

The interaction among water stress levels (W), Se treatments (S) and genotypes (G) was also highly significant ($P<0.01$) (Table 4.13). The highest value for grain Se (8.67 µg g^{-1} Se) was recorded in plants of Kohistan-97 fertigated with Se under limited water conditions while no Se supply under normal conditions resulted in minimum value (0.63µg g^{-1} Se) in Pasban-90 (Fig. 4.48).

The data regarding analysis of variance for grain iron (Fe) concentration revealed highly significant effect ($P<0.001$) of drought stress on accumulation of Fe in wheat grain (Table 4.13). The exposure to limited water conditions reduced Fe in grains by 43% as compared to normal plants. The application of Se significantly improved grain Fe accumulation only in plants foliarly applied with Se at anthesis stage and gave the highest value (494.20 µg g^{-1}) for this variable while non-significant difference ($P>0.05$) was observed among Se seed priming (52.20 µg g^{-1}), no Se supply (51.02 µg g^{-1}) and Se fertigation (33.63 µg g^{-1}). The minute or negligible amount of Se was recorded in grains of plants foliarly applied with Se at tillering stage. Wheat genotype Pasban-90 accumulated 35% more Fe in grains than Kohistan-97 (Fig. 4.49).

The interaction W×S×G was also highly significant ($P<0.01$) for this variable (Table 4.13). The normal plants of Pasban-90 accumulated maximum Fe in grains (824.40 µg g^{-1}) by foliar application of Se at anthesis stage while the water stressed plants of same genotype

Table: 4.13. Analysis of variance of grain selenium (Se), Iron (Fe) and Zinc (Zn) concentration (µg g^{-1} DW) of two wheat genotypes by exogenous selenium (Se) supply under drought stress.

SOV[a]	Grain Selenium (Se) concentration (µg g^{-1} DW)	Grain Iron (Fe) concentration (µg g^{-1} DW)	Grain Zinc (Zn) concentration (µg g^{-1} DW)
Water stress levels (W)	***	***	***
Selenium treatments (S)	***	***	***
Genotypes (G)	***	***	NS
W×S	***	*	**
W×G	***	***	***
S×G	***	***	NS
W×S×G	***	***	NS
CV[b]	34.13	33.58	21.58

NS= non-significant, *significant at $P< 0.05$, ** significant at $P< 0.01$,

*** significant at $P< 0.001$

[a] Source of variation, [b] Co-efficient of variation

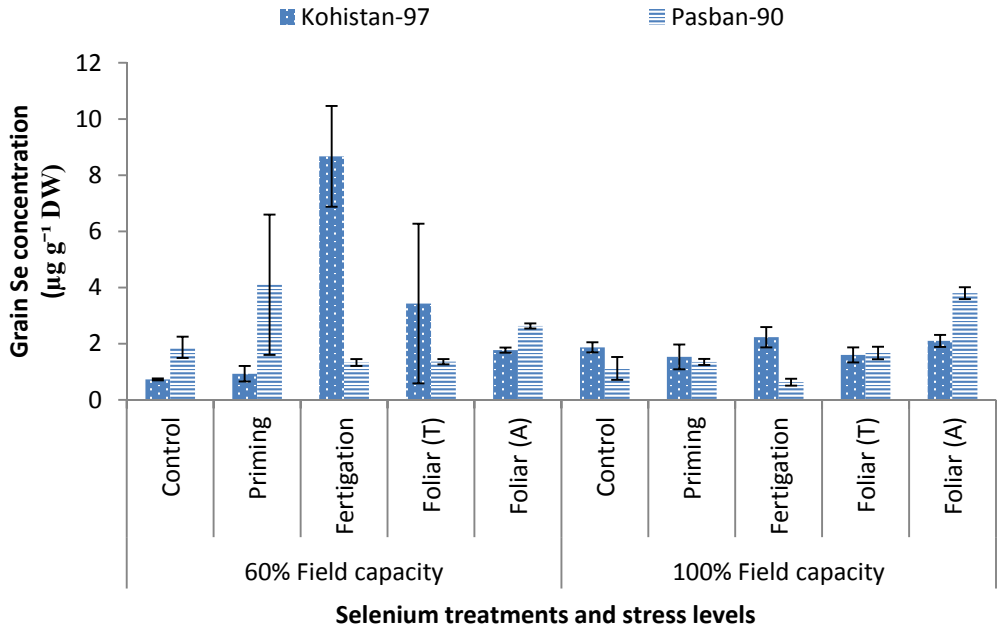

Figure 4.48: Effect of exogenous Se supply on grain selenium (Se) concentration (µg g^{-1} DW) of two wheat (*Triticum aestivum* L.) genotypes under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

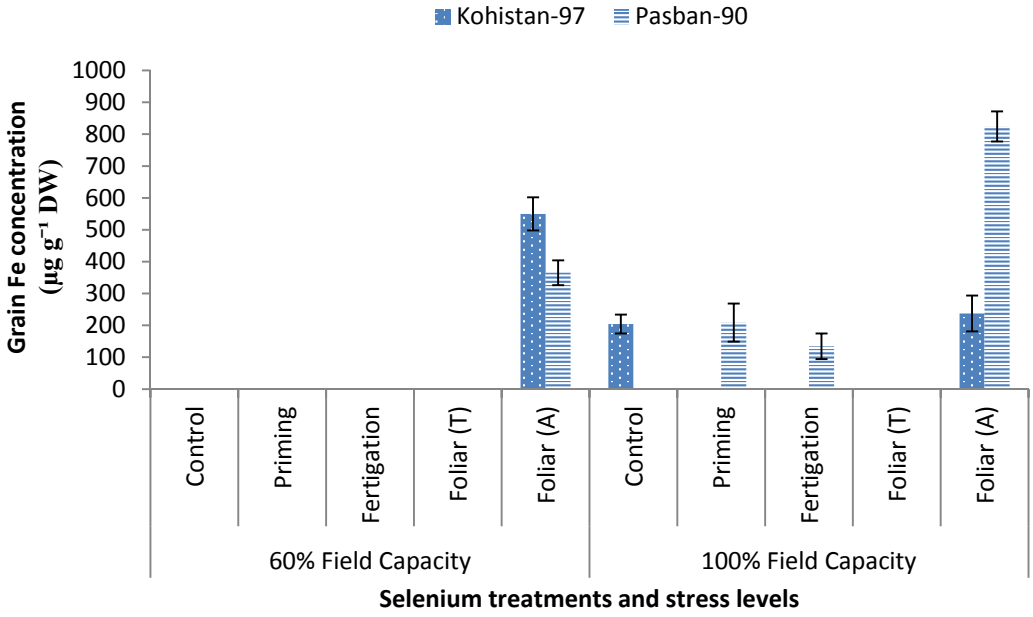

Figure 4.49: Effect of exogenous Se supply on grain iron (Fe) concentration (µg g^{-1} DW) of two wheat (*Triticum aestivum* L.) genotypes under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

foliarly applied with Se at tillering stage gave the minimum value (0.00 µg g^{-1}) for this variable (Fig. 4.49).

A significant decrease (P<0.05) in grain zinc (Zn) concentration was observed under limited water conditions (Table 4.13). The plants exposed to drought stress exhibited 24% decrease in the concentration with respect to normal plants. A highly significant effect (P<0.001) of different exogenous Se application methods was recorded on accumulation of Zn in grains (Table 4.13). The plants fertigated with Se gave maximum value (17.82 µg g^{-1}) statistically at par with Se foliar spray at anthesis (16.84 µg g^{-1}) and tillering (16.42 µg g^{-1}) stages. The priming of seeds with Se resulted in minimum grain Zn concentration (11.70 µg g^{-1}) closely followed by no Se supply (13.18 µg g^{-1}) (Fig. 4.50). Wheat genotypes differed non-significantly (P>0.05) for this variable. The interaction between water stress levels (W) and Se treatments (S) was significant (P<0.01). Under normal conditions, Se fertigation gave the highest value (22.87 µg g^{-1}) for this variable which was statistically related to Se foliar spray at anthesis stage (18.87 µg g^{-1}) whereas the water stressed plants accumulated maximum Zn in grains (17.08 µg g^{-1}) by Se foliar spray at tillering stage. The control plants (no Se supply) gave minimum value for this variable under both normal (10.68 µg g^{-1}) and water deficit conditions (10.27 µg g^{-1}) (Fig. 4.50). The interaction (W×S×G) was non-significant for this variable (Table 4.13).

The limited water supply significantly reduced (P<0.05) grain phosphorous (P) concentration in wheat (Table 4.14). The normal plants showed 33% higher accumulation of P in grain than water stressed plants. The exogenous Se supply significantly reduced grain P concentration. The plants grown under normal water supply gave the highest value (908.33 µg g^{-1} P) for this variable (Fig. 4.51). The plants accumulated lesser P in grain by Se fertigation (595.83 µg g^{-1}Se) and Se foliar application at tillering stage (629.17 µg g^{-1}Se) than Se seed priming (704.17 µg g^{-1}Se) and Se foliar application at anthesis stage (712.50 µg g^{-1}Se). The interaction between water stress levels (W) and selenium treatments (S) was also significant (P<0.05). The normal plant applied with no Se accumulated maximum P in grain (975.00 µg g^{-1}) while minimum was (433.33 µg g^{-1}) in plants fertigated with Se under limited water conditions. All other interactions were non-significant for this variable (Fig. 4.51).

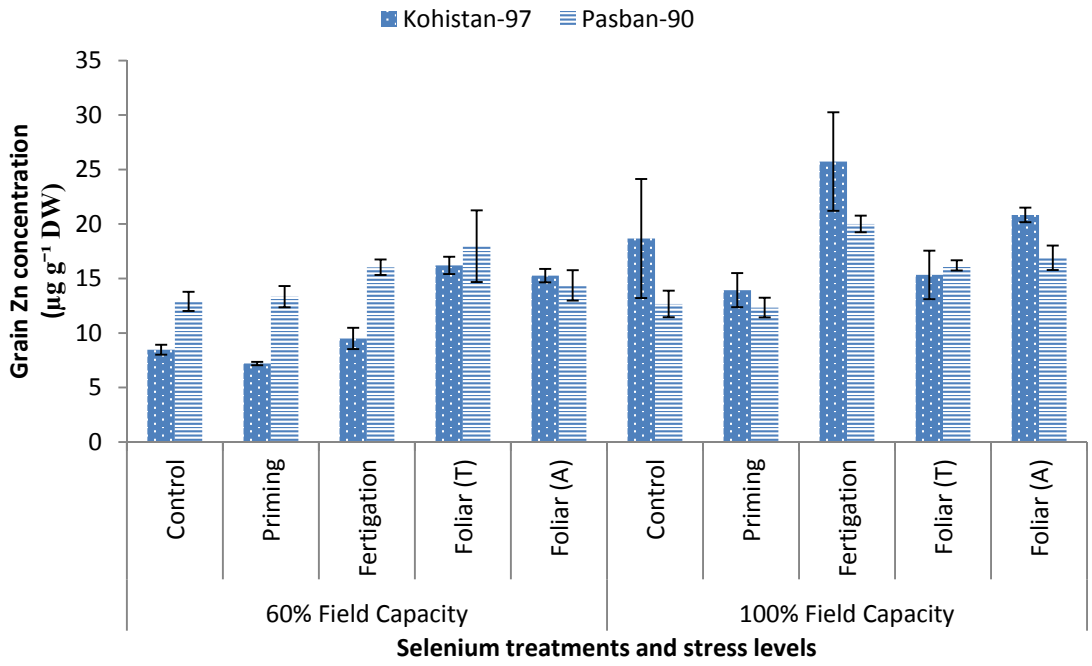

Figure 4.50: Effect of exogenous Se supply on grain zinc (Zn) concentration (µg g^{-1} DW) of two wheat (*Triticum aestivum* L.) genotypes under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

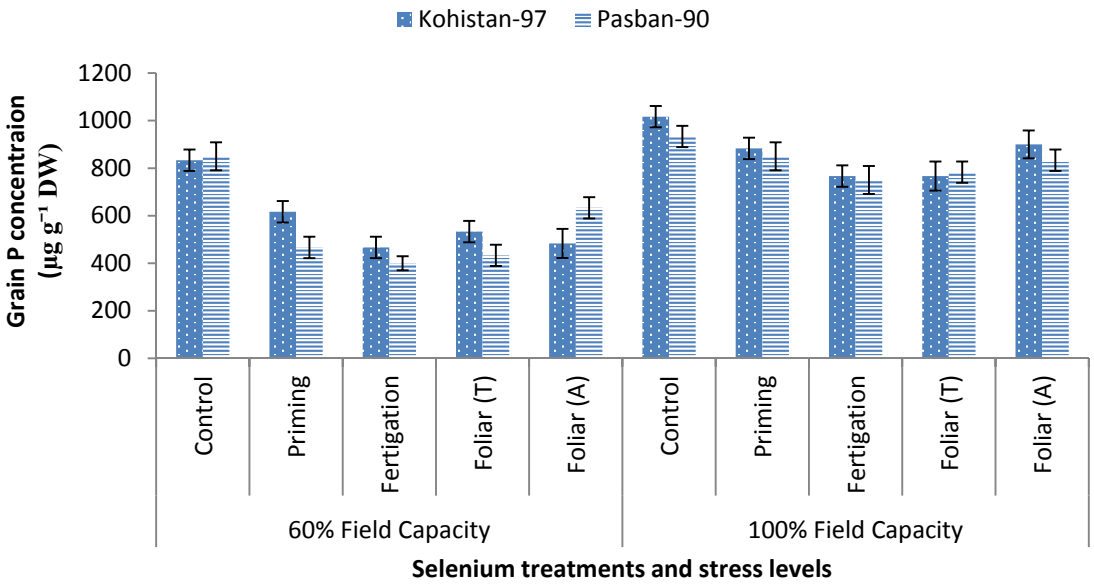

Figure 4.51: Effect of exogenous Se supply on grain phosphorous (P) concentration (µg g^{-1} DW) of two wheat (*Triticum aestivum* L.) genotypes under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

Table: 4.14. Analysis of variance of grain Phosphorous (P), Potassium (K) and magnesium (Mg) concentration (µg g^{-1} DW) in two wheat genotypes by exogenous selenium (Se) supply under drought stress.

SOV[a]	Grain Phosphorous (P) concentration (µg g^{-1} DW)	Grain Potassium (K) concentration (µg g^{-1} DW)	Grain Magnesium (Mg) concentration (µg g^{-1} DW)
Water stress levels (W)	***	NS	***
Selenium treatments (S)	***	**	***
Genotypes (G)	NS	**	***
W×S	*	***	***
W×G	NS	**	NS
S×G	NS	NS	**
W×S×G	NS	NS	**
CV[b]	11.89	6.73	6.31

NS= non-significant, *significant at $P< 0.05$, ** significant at $P< 0.01$,

*** significant at $P< 0.001$

[a] Source of variation, [b] Co-efficient of variation

The water deficit conditions did not significantly affect (P>0.05) grain K concentration in wheat. However, significant difference (P<0.05) was recorded among various Se application methods for variable (Table 4.14). The plants accumulated maximum K in grains by Se fertigation and Se foliar application at anthesis stage (3691.7 µg g^{-1} each). However non-significant difference was observed among other Se supply methods viz. Se foliar application at tillering stage (3450.00 µg g^{-1}), Se seed treatment (3433.33 µg g^{-1}) and no Se supply (3375.00 µg g^{-1}). The interaction among W×G×S was also significant (P<0.05). The maximum K grain concentration (4033.3 µg g^{-1}) was recorded in Kohistan-97 by Se foliar application at tillering stage and Se fertigation under both normal and water stress conditions respectively. While Se seed treatment of Pasban-90 resulted in minimum value (3033.3 µg g^{-1}) under normal conditions (Fig. 4.52).

The exposure to drought stress significantly reduced (P<0.01) grain Mg concentration in wheat plants (Table 4.14). The water stressed plants accumulated 11% less Mg in grains as compared to normal plants. Kohistan-97 exhibited higher value (742.14 µg g^{-1} Mg) than Pasban-90 (677.40 µg g^{-1} Mg) for this variable. Highly significant difference (P<0.01) was also observed among different methods of exogenous Se supply. The application of Se as foliar treatment at tillering stage resulted in maximum value (764.15 µg g^{-1} Mg) and had non-significant difference with the values obtained by Se fertigation (752.68 µg g^{-1}) and Se foliar application at anthesis stage (727.38µg g^{-1} Mg). The minimum value (596.53µg g^{-1} Mg) was recorded in control plants applied with no Se (Fig. 4.53).

The interaction W×S×G was also highly significant (P<0.01) for this variable. The Se fertigation of plants resulted in maximum grain Mg accumulation (877.40 µg g^{-1}) in Kohistan-97 under normal conditions (100% FC) statistically at par with the value (803.67 µg g^{-1} Mg) obtained by Se foliar application at tillering stage in the same genotype under drought stress (60% FC). The water stressed plants of Pasban-90 applied with no Se gave minimum value (404.87 µg g^{-1} Mg) for this variable (Fig. 4.53).

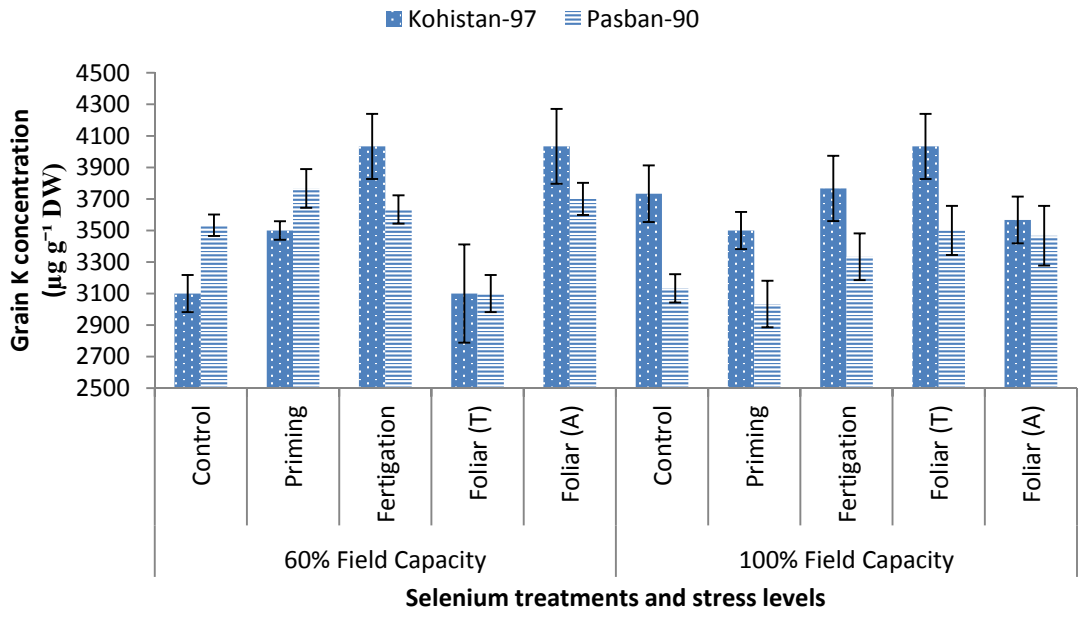

Figure 4.52: Effect of exogenous Se supply on grain potassium (K) concentration (µg g^{-1} DW) of two wheat (*Triticum aestivum* L.) genotypes under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

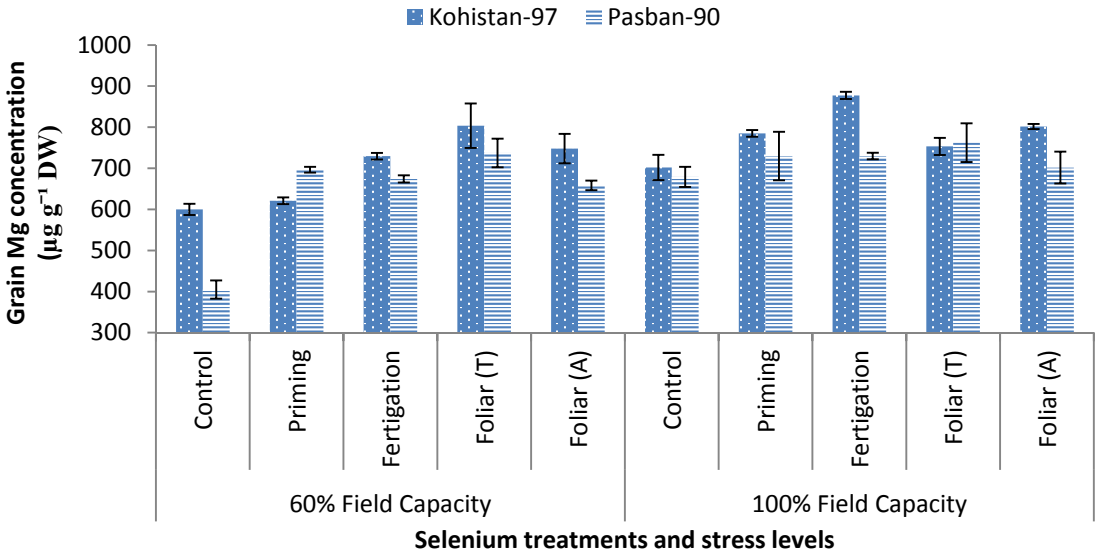

Figure 4.53: Effect of exogenous Se supply on grain magnesium (Mg) concentration (µg g^{-1} DW) of two wheat (*Triticum aestivum* L.) genotypes under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

4.6.19. Total number of tillers

Analysis of variance revealed highly significant effect ($P<0.01$) of drought stress on total number of tillers m^{-2} in wheat plants. The limited water conditions caused a significant decrease of 16% in number of tillers m^{-2} in both genotypes as compared to normal conditions. There was no significant difference between Kohistan-97 and Pasban-90 for this variable (Table 4.15).

The highly significant differences ($P<0.01$) were recorded among various methods of exogenous Se supply. The plants foliarly sprayed with Se at tillering stage produced maximum number of tillers (484.33). A significant increase in number of tillers was also observed by Se fertigation (417.83) closely followed by Se foliar spray at anthesis stage (402.83). The plants applied with no Se produced minimum number of tillers (323.67) (Fig. 4.54).

The interaction W×S was also significant (Table 4.15). The highest value (516.33) was recorded in normal plants (100% FC) under foliar application of Se at tillering stage that improved number of tillers by 44% as compared to no Se supply (358.33) under normal conditions. Similar trend was recorded in plants grown under water deficit conditions, where maximum tillers (452.33) were recorded in plants foliarly sprayed with Se at tillering stage with an increase of 56% in respect to plants with no Se supply (289.00). The other methods of exogenous Se supply viz. Se foliar application at anthesis stage, Se seed priming and Se fertigation were statistically related to each other and increased the number of tillers by 34%, 21% and 21% respectively under drought stress (Fig. 4.54).The interaction among different factors such as water stress levels (W), Se treatments (S) and genotypes (G) was non-significant for this variable (Table 4.15).

4.6.20. Total number of fertile tillers

The number of fertile tillers were significantly ($P<0.01$) reduced by limited water supply that directly influenced the final grain yield of wheat crop at harvest (Table 4.15). Drought stress decreased the number of productive tillers by 27% in both genotypes as compared to normal conditions. Non-significant difference was recorded between genotypes for this variable (Table 4.15).

Table: 4.15. Analysis of variance of total number of tillers m^{-2}, total number of fertile tillers m^{-2}, spike length (cm), number of spikelets $spike^{-1}$ and number of grains $spike^{-1}$ in two wheat genotypes exposed to exogenous selenium supply under drought stress.

SOV[a]	Total no. of tillers	Total no. of fertile tillers	Spike Length (cm)	No. of Spikelets $spike^{-1}$	No. of grains $spike^{-1}$
Water stress levels (W)	***	***	**	***	***
Selenium treatments (S)	***	***	***	***	***
Genotypes (G)	NS	NS	***	*	***
W×S	***	***	NS	NS	NS
W×G	**	**	NS	NS	***
S×G	***	***	NS	NS	NS
W×S×G	NS	NS	NS	*	NS
CV[b]	5.56	6.79	7.70	3.55	5.30

NS= non-significant, *significant at $P< 0.05$, ** significant at $P< 0.01$,

*** significant at $P< 0.001$

[a] Source of variation, [b] Co-efficient of variation

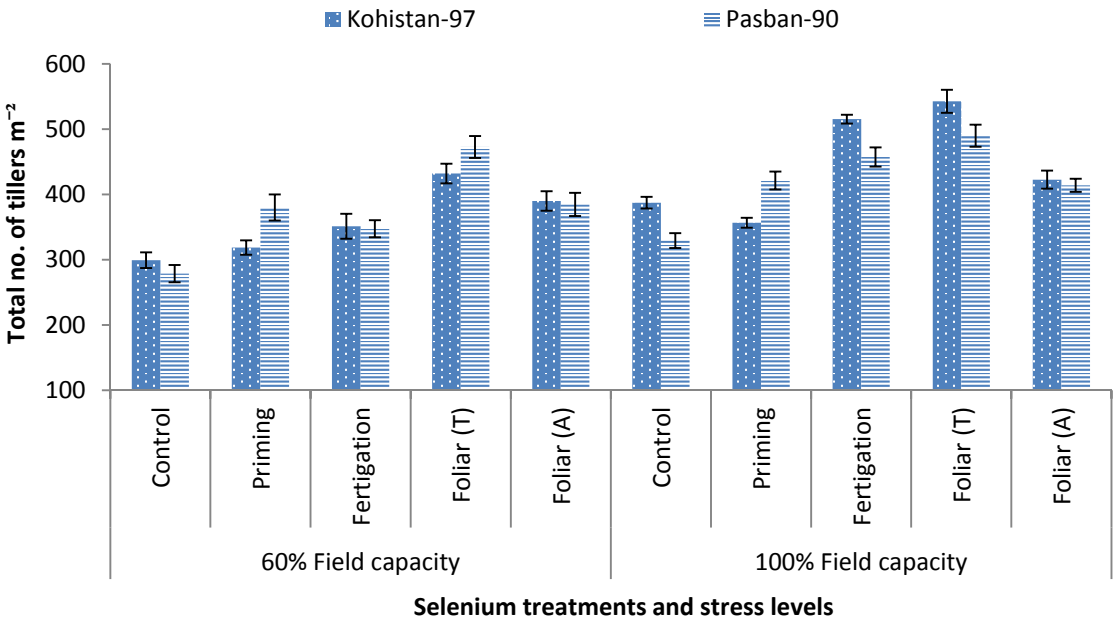

Figure 4.54: Effect of exogenous Se supply on total number of tillers m^{-2} of two wheat (*Triticum aestivum* L.) genotypes under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

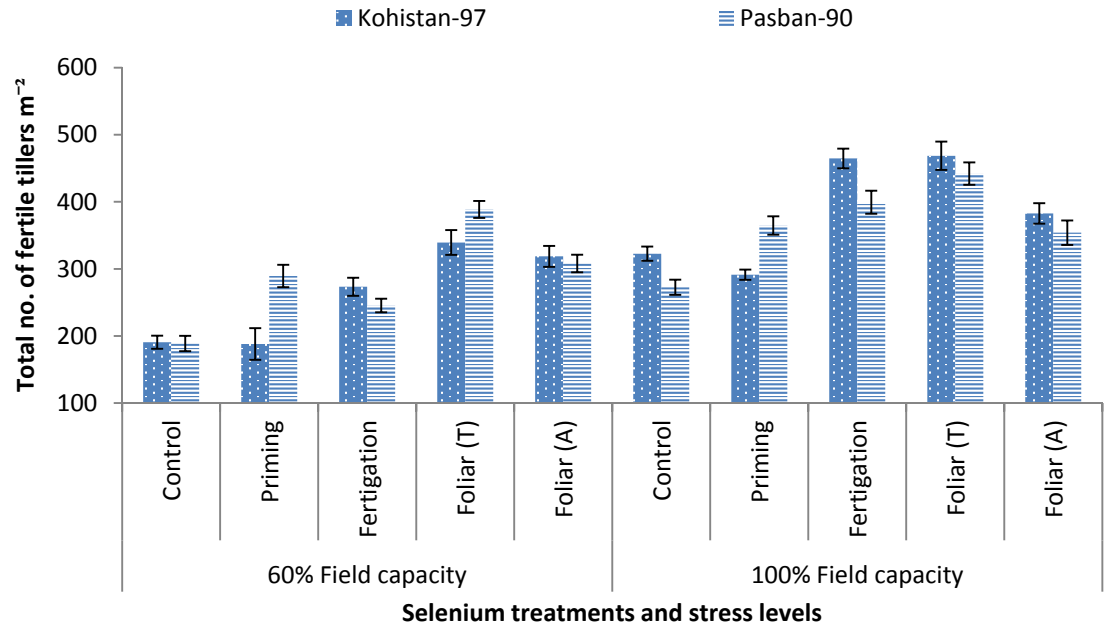

Figure 4.55: Effect of exogenous Se supply on number of fertile tillers m^{-2} of two wheat (*Triticum aestivum* L.) genotypes under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

The different methods of exogenous Se supply varied significantly (P<0.01) for this variable. The foliar spray of Se at tillering stage resulted in the production of maximum number of productive tillers (409.67) in plants. The fertile tillers were also increased by Se fertigation (345.67) and Se foliar spray at anthesis stage (340.83). The control plants (no Se supply) produced minimum number (243.67) of fertile tillers (Fig. 4.55).

The highly significant (P<0.01) interaction W×S revealed that application of Se, as foliar spray at tillering gave the highest value (455.33) for this variable statistically at par with Se fertigation (432.00) under normal conditions. The water stressed plants also produced maximum number of fertile tillers (364.00) with Se foliar spray at tillering stage while no Se supply resulted in minimum number of productive tillers (189.67) under drought stress (Fig. 4.55). The interaction among different factors (W×S×G) was non-significant (Table 4.15).

4.6.21. Spike length

The exposure to drought stress significantly (P<0.05) affected the spike length of both the wheat genotypes and reduced it by 7% as compared to normal plants (Table 4.15). The reduction was significantly higher (8%) in drought sensitive (Pasban-90) than tolerant genotype (Kohistan-97) (Fig. 4.56).

The various methods of exogenous Se supply showed highly significant (P<0.01) differences for spike length. The plants fertigated with Se exhibited maximum spike length (5.86 cm) which was statistically related to Se foliar spray at tillering (5.60 cm) and anthesis stages (5.82 cm). The minimum spike length (4.75 cm) was recorded in control plants and non-significantly differed from Se seed priming (5.07 cm) (Fig. 4.56).

All the interactions among different treatments were non-significant for spike length (Table 4.15).

4.6.22. Number of spikelets spike^{-1}

The limited water conditions (60% FC) induced a significant (P<0.01) decrease of 7% in the number of spikelets spike^{-1} in wheat plants as compared to their normal ones. A

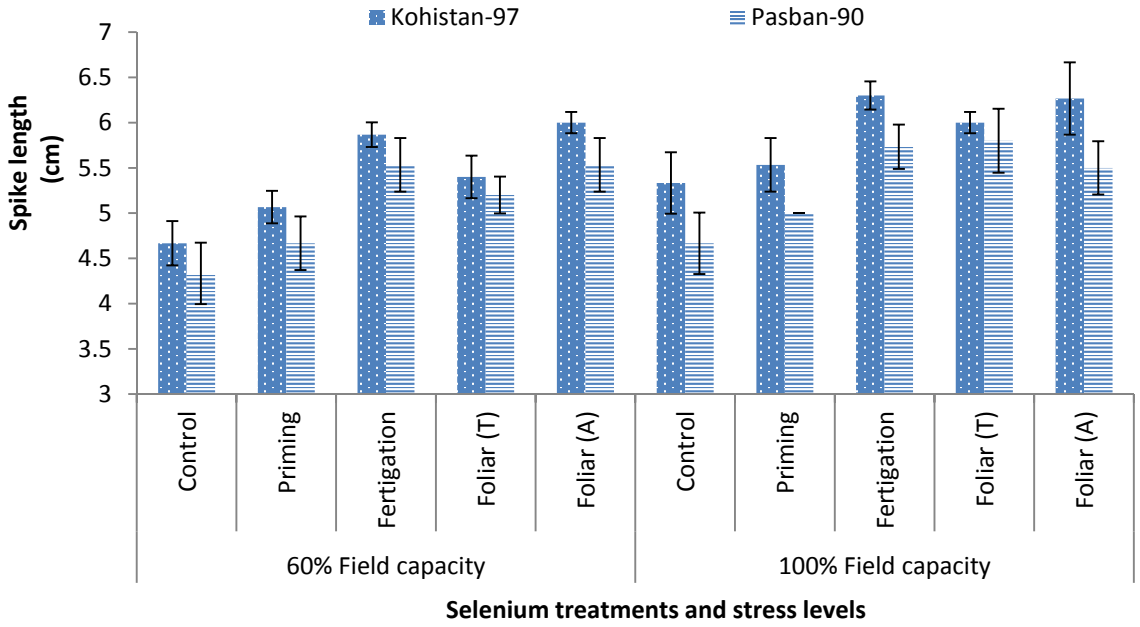

Figure 4.56: Effect of exogenous Se supply on spike length (cm) of two wheat (*Triticum aestivum* L.) genotypes under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

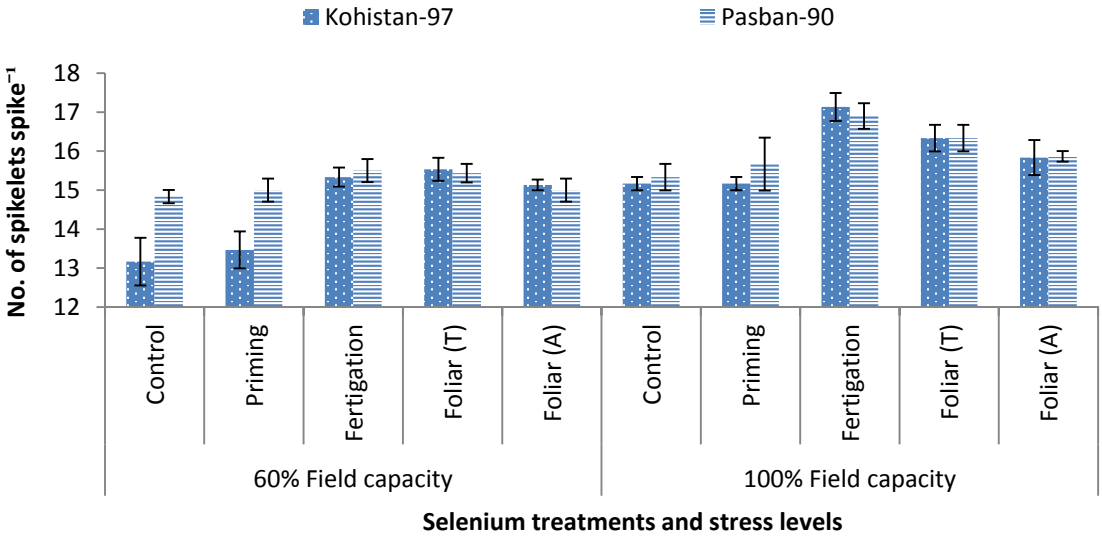

Figure 4.57: Effect of exogenous Se supply on number of spikelets spike^{-1} of two wheat (*Triticum aestivum* L.) genotypes under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

significant difference (P<0.05) was observed between genotypes; Pasban-90 maintained significantly higher number of spikelets (15.59) than Kohistan-97 (15.23) (Table 4.15).

The various methods of exogenous Se supply exhibited highly significant differences (P<0.01) for this variable. The highest value was recorded by Se fertigation (16.22) closely followed by Se foliar spray at tillering stage (15.91). The plants foliarly applied with Se at anthesis stage also produced significantly higher number of spikelets spike^{-1} (15.46) than control plants that gave the lowest value (14.62) for this variable (Fig. 4.57).

The interaction among different factors (W×S×G) was significant (Table 4.15). The highest value (17.13) was recorded in normal plants of Kohistan-97 fertigated with Se while the lowest value (13.17) was observed in the same genotype with no Se supply under drought stress (Fig. 4.57). All other interactions were non-significant for this variable (Table 4.15).

4.6.23. Number of Grains Spike^{-1}

There were highly significant differences (P<0.01) in both water stress treatments (100% and 60% FC) for number of grains spike^{-1}. The limited water supply caused a decrease of 23% as compared to normal plants. The grain production spike^{-1} was higher (6%) in Kohistan-97 than Pasban-90 (Table 4.15).

Significant differences (P<0.01) were also observed among different Se application methods (Table 4.15). The plants foliarly applied with Se at tillering stage produced highest number of grains spike^{-1} (38.83). The fertigation (36.50) and foliar spray of Se at anthesis stage (36.42) also significantly enhanced the number of grains spike^{-1} (Fig. 4.58). The plants sprayed with no Se produced minimum number of grains spike^{-1}(11.78) and differed non-significantly from those grown from Se primed seeds (12.15).

The interaction W×G was significant. Under normal conditions, the wheat genotype Pasban-90 produced maximum number of grains spike^{-1} (40.13) statistically at par with Kohistan-97 (39.93). However, number of grains spike^{-1} was significantly higher in

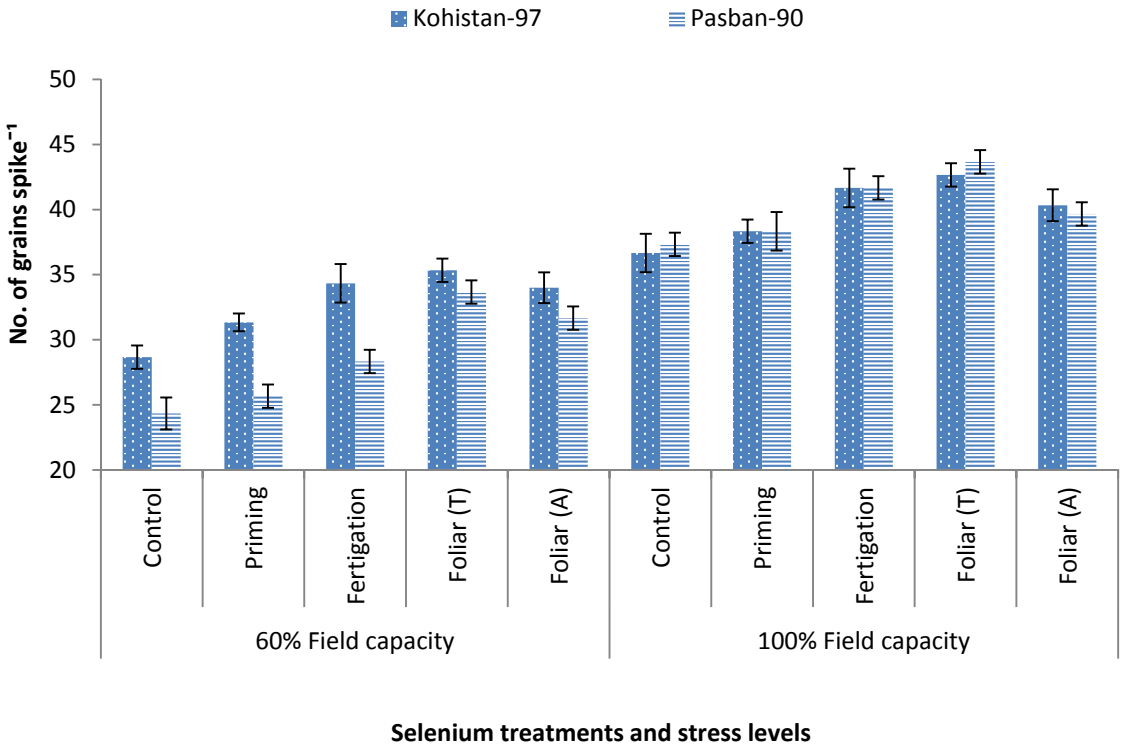

Figure 4.58: Effect of exogenous Se supply on number of grains spike^{-1} of two wheat (*Triticum aestivum* L.) genotypes under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

Kohistan-97 (32.73) than Pasban-90 (28.73) under water stress conditions (Fig. 4.58). All other interactions were non-significant for this variable (Table 4.15).

4.6.24. Thousand grain weight

The exposure of plants to drought stress had highly significant effect (P<0.01) on the 1000-grain weight of wheat plants (Table 4.16). Drought stress reduced it by 34% in respect to normal ones. Significant difference was also observed between genotypes for this variable. The reduction in weight was 8% more in Pasban-90 than Kohistan-97.

The data regarding 1000-grain weight revealed highly significant difference (P<0.01) among various Se supply methods (Table 4.12). The grain weight was maximum (44.60 g) in Se fertigated plants. The foliar spray at tillering and anthesis stages also increased 1000-grain weight by 18% and 21% respectively with respect to control plants i.e. applied with no Se (Fig. 4.59).

The interaction W×S was also significant (P<0.05). The highest value (53.75 g) was obtained by Se fertigation in normal plants statistically at par with Se foliar application at anthesis stage (51.67 g) under normal supply of water (Fig. 4.59). Likewise, Se fertigation gave the maximum value (35.45 g) for 1000-grain weight and non-significantly differed from Se foliar application at tillering and anthesis stages (33.63 g and 32.97 g respectively). The minimum value (27.27 g) was recorded in water stressed plants applied with no Se (Fig. 4.59). All other interactions were non-significant for this variable (Table 4.16).

4.6.25. Biological yield

Analysis of variance showed highly significant effect (P<0.05) of drought stress on BY of wheat plants (Table 4.16). The water deficit conditions reduced it by 33% as compared to normal conditions. Wheat genotype Kohistan-97 maintained 17% higher BY than Pasban-90 (Fig. 4.60).

The various methods of exogenous Se supply differed significantly (P<0.01) for BY (Table 4.16). The plants foliarly sprayed with Se at tillering stage produced maximum BY (11.79 t ha^{-1}). The other methods of exogenous Se supply viz. Se

Table: 4.16. Analysis of variance of 1000-grain weight (g), biological yield (t ha^{-1}), grain yield (t ha^{-1}) and harvest index (%) in two wheat genotypes exposed to exogenous selenium supply under drought stress.

SOV[a]	1000-grain weight (g)	Biological yield (t ha^{-1})	Grain yield (t ha^{-1})	Harvest Index (%)
Water stress levels (W)	***	***	***	*
Selenium treatments (S)	***	***	***	*
Genotypes (G)	***	***	***	NS
W×S	*	***	***	NS
W×G	NS	***	*	NS
S×G	NS	***	***	NS
W×S×G	NS	***	*	NS
CV[b]	5.60	5.18	8.62	8.94

NS= non-significant, *significant at $P< 0.05$, ** significant at $P< 0.01$,

*** significant at $P< 0.001$

[a] Source of variation, [b] Co-efficient of variation

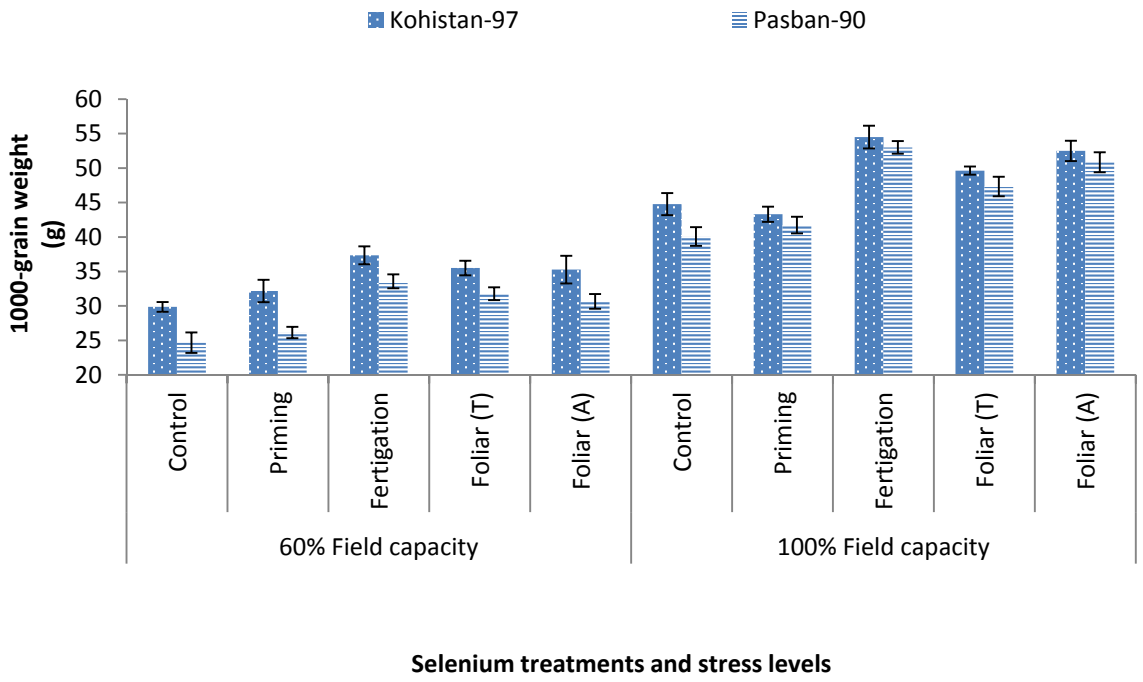

Figure 4.59: Effect of exogenous Se supply on 1000-grain weight (g) of two wheat (*Triticum aestivum* L.) genotypes under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

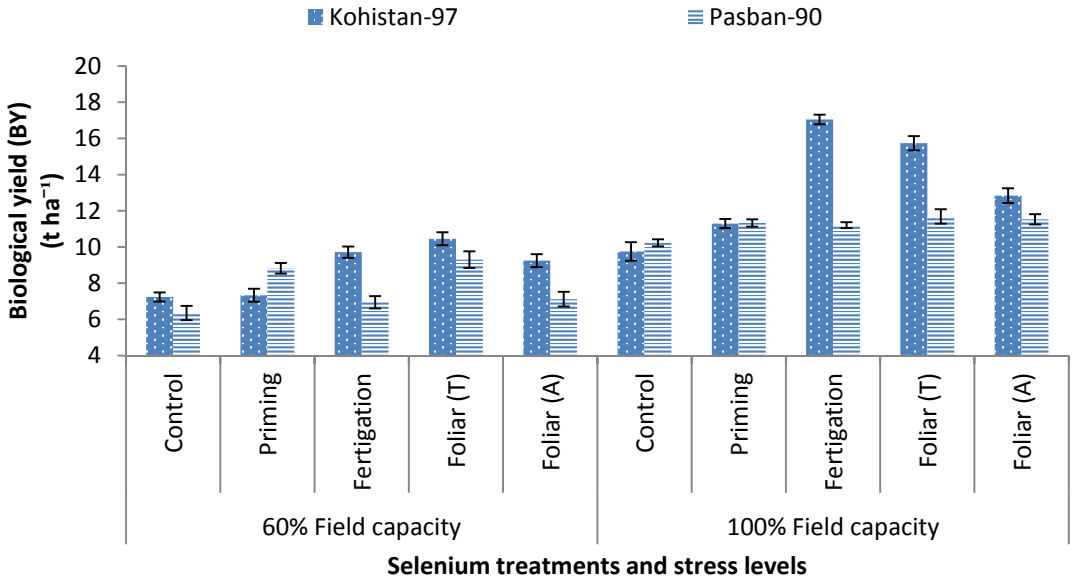

Figure 4.60: Effect of exogenous Se supply on biological yield (t ha^{-1}) of two wheat (*Triticum aestivum* L.) genotypes under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

fertigation, Se foliar spray at anthesis stage and Se seed priming also increased BY of plants by 34%, 21% and 16% as compared to no Se supply for this variable (Fig. 4.60).

The interaction among different factors (W×S×G) was also highly significant (P<0.01). The normal plants of Kohistan-97 fertigated with Se maintained the highest BY (17.05 t ha^{-1}). Under drought stress, the maximum BY (10.45 t ha^{-1}) was also recorded in genotype Kohistan-97 foliarly sprayed with Se at tillering stage whereas no Se supply resulted in the lowest BY (6.35 t ha^{-1}) in water stressed plants of Pasban-90 (Fig. 4.60).

4.6.26. Grain yield

The effect of drought stress on GY of wheat plants was highly significant (P<0.01) and caused a reduction of 36% as compared to normal plants (Table 4.16). The decrease in GY was 17% more in drought sensitive (Pasban-90) than drought tolerant (Kohistan-97) wheat genotype (Fig. 4.61).

The various exogenous Se supply methods also varied significantly for GY (Table 4.16). The plants foliarly sprayed with Se at tillering stage produced maximum GY (4.12 t ha^{-1}) closely followed by Se fertigation (3.92 t ha^{-1}). The foliar spray of Se at anthesis stage and Se seed priming also increased GY by 33% and 19% with respect to no Se supply (Fig. 4.61).

The significant interaction W×S×G revealed that wheat genotype Kohistan-97 produced maximum GY (6.15 t ha^{-1}) by Se fertigation under normal conditions. The foliar spray of Se at tillering stage was observed as best method for increasing BY under drought stress and gave the highest value (3.52 t ha^{-1}) in the plants of Kohistan-97. The minimum value (1.88 t ha^{-1}) was recorded in water stressed plants of Pasban-90 applied with no Se (Fig. 4.61).

4.6.27. Harvest index

The data regarding HI showed significant effect (P<0.05) of drought stress on HI of wheat plants. The plants exposed to water deficit conditions exhibited 6% lower HI than

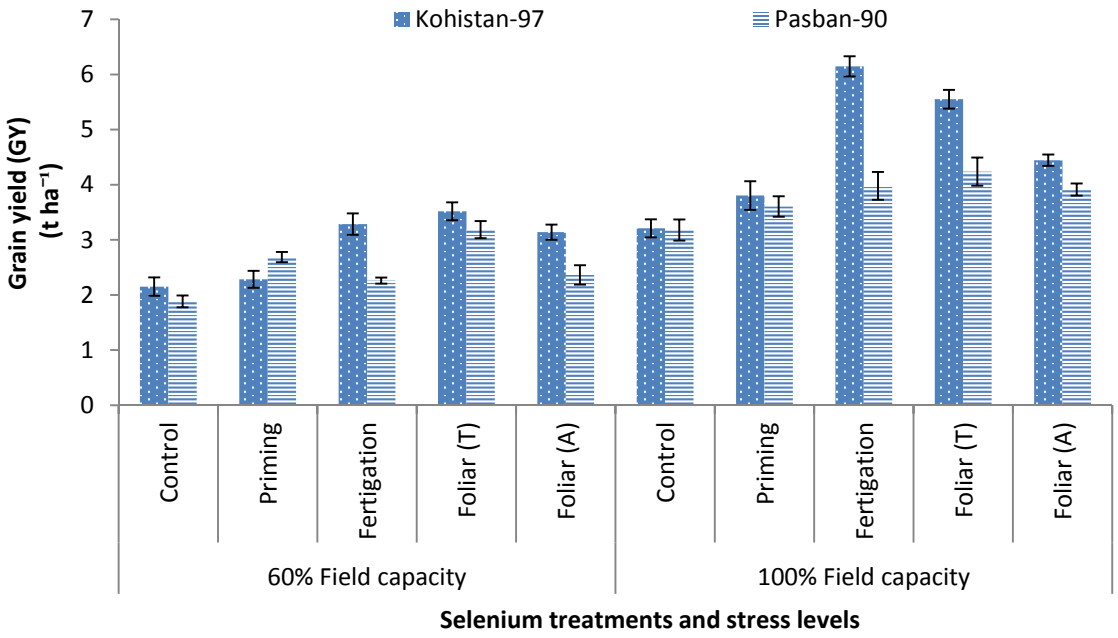

Figure 4.61: Effect of exogenous Se supply on grain yield (t ha^{-1}) of two wheat (*Triticum aestivum* L.) genotypes under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

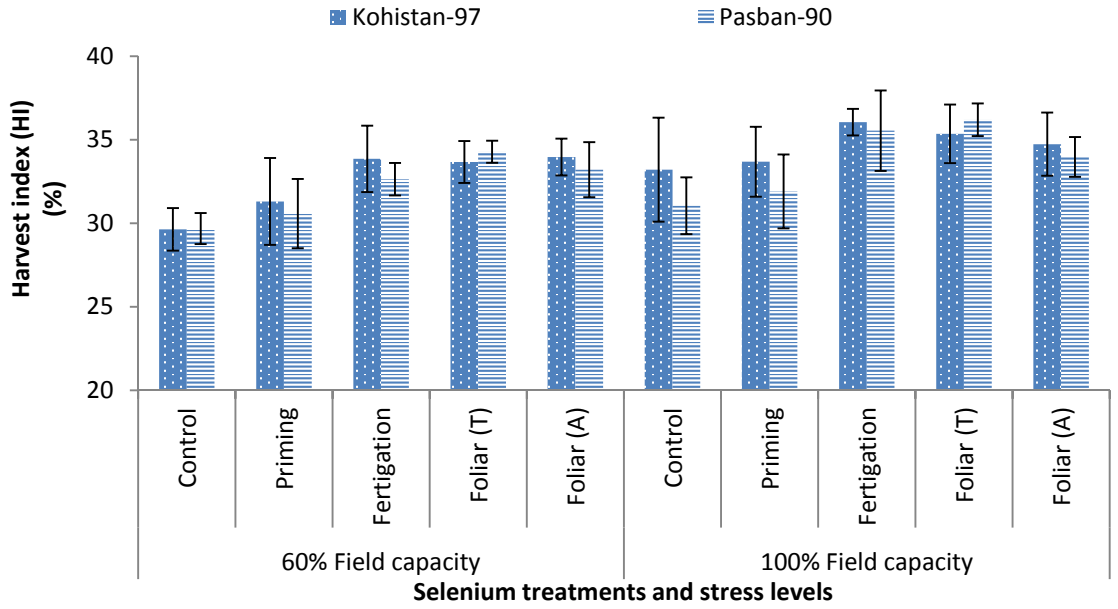

Figure 4.62: Effect of exogenous Se supply on harvest index (%) of two wheat (*Triticum aestivum* L.) genotypes under normal (100% FC) and drought stress (60% FC) conditions. Values are mean ± standard error.

normal plants. However, non-significant difference was recorded between genotypes (Kohistan-97 and Pasban-90) for this variable (Table 4.16).

The various methods of exogenous Se supply differed significantly (P<0.05) for HI. The foliar spray of Se at tillering stage resulted in maximum HI (34.88%) closely followed by Se fertigation (34.52%) and Se foliar spray at anthesis stage (33.97). The plants applied with no Se had minimum HI (30.89%) closely followed by those grown from Se primed seeds (31.89%) (Fig. 4.62).

All the interactions were non-significant for HI (Table 4.16)

4.7. Field experiments

4.7.1. Total number of tillers

Analysis of variance for the data regarding total number of tillers showed that drought stress significantly (P<0.01) decreased number of tillers in both wheat genotypes during 2011-12 and 2012-13 (Table 4.17a, b). The decrease was significantly higher in plants exposed to drought stress at tillering than anthesis stage. Water deficit conditions reduced number of tillers by 24% in 2011-12 and 27% during 2012-13 in plants water stressed at tillering stage while production of tillers was 20% less in plants subjected to drought stress at anthesis stage in both the years with respect to their control (normal supply of water). Wheat genotype Kohistan-97 produced higher number of tillers (389.09 and 431.11) than Pasban-90 (373.33 and 381.13) during 2011-12 and 2012-13 respectively (Fig. 4.63).

The different methods of exogenous Se supply significantly (P>0.01) increased number of tillers in wheat. During 2011-12, the plants fertigated with Se gave the maximum value (406.22) statistically related to Se foliar application at tillering stage (403.06) and Se seed priming (398.61). In 2012-13, the highest value (430.61) was recorded in plants foliarly sprayed with Se at tillering stage closely followed by Se fertigation (430.17) and Se seed treatment (418.0). No Se application gave the minimum values (327.17 and 351.94) for this variable in both years i.e. 2011-12 and 2012-13 respectively (Fig. 4.63).

The significant interaction between stress levels (W) and Se supply methods(S) of plants grown during first and second year (2011-12 and 2012-13) revealed that Se fertigation was the best treatment because it maintained the highest values of 474.33 and 502.0 respectively statistically at par with Se foliar application at tillering stage (459.33 and 492.67) and Se seed priming (448.50 and 481.83) under normal conditions. The water stressed plants foliarly applied with Se at tillering stage produced maximum number of tillers (430.17) in 2011-12; however, Se fertigation gave the highest value (424.83) in plants exposed to limited water conditions at anthesis stage during 2012-13.

Table: 4.17a. Analysis of variance of total number of tillers, total number of fertile tillers, spike length (cm), number of spikelets spike^{-1} and number of grains spike^{-1} in two wheat genotypes exposed to exogenous selenium (Se) supply under drought stress in the year 2011-12.

SOV[a]	Total no. of tillers	Total no. of fertile tillers	Spike Length (cm)	No. of Spikelets spike^{-1}	No. of grains spike^{-1}
Water stress levels (W)	***	***	NS	***	***
Selenium treatments (S)	***	***	***	**	***
Genotypes (G)	**	***	**	*	***
W×S	***	***	**	***	***
W×G	NS	NS	NS	NS	*
S×G	NS	***	NS	NS	NS
W×S×G	*	**	NS	NS	NS
CV[b]	6.56	5.87	6.69	6.66	6.62

NS= non-significant, *significant at $P< 0.05$, ** significant at $P< 0.01$,

*** significant at $P< 0.001$

[a] Source of variation, [b] Co-efficient of variation

Table: 4.17b. Analysis of variance of total number of tillers, total number of fertile tillers, spike length (cm), number of spikelets spike^{-1} and number of grains spike^{-1} in two wheat genotypes exposed to exogenous selenium supply under drought stress in the year 2012-13.

SOV[a]	Total no. of tillers	Total no. of fertile tillers	Spike Length (cm)	No. of Spikelets spike^{-1}	No. of grains spike^{-1}
Water stress levels (W)	***	***	***	***	***
Selenium treatments (S)	***	***	**	**	***
Genotypes (G)	***	***	***	***	***
W×S	***	***	***	**	***
W×G	*	***	NS	NS	NS
S×G	NS	**	NS	NS	NS
W×S×G	NS	NS	NS	NS	NS
CV[b]	5.19	6.26	4.36	8.84	5.89

NS= non-significant, *significant at $P< 0.05$, ** significant at $P< 0.01$,

*** significant at $P< 0.001$

[a] Source of variation, [b] Co-efficient of variation

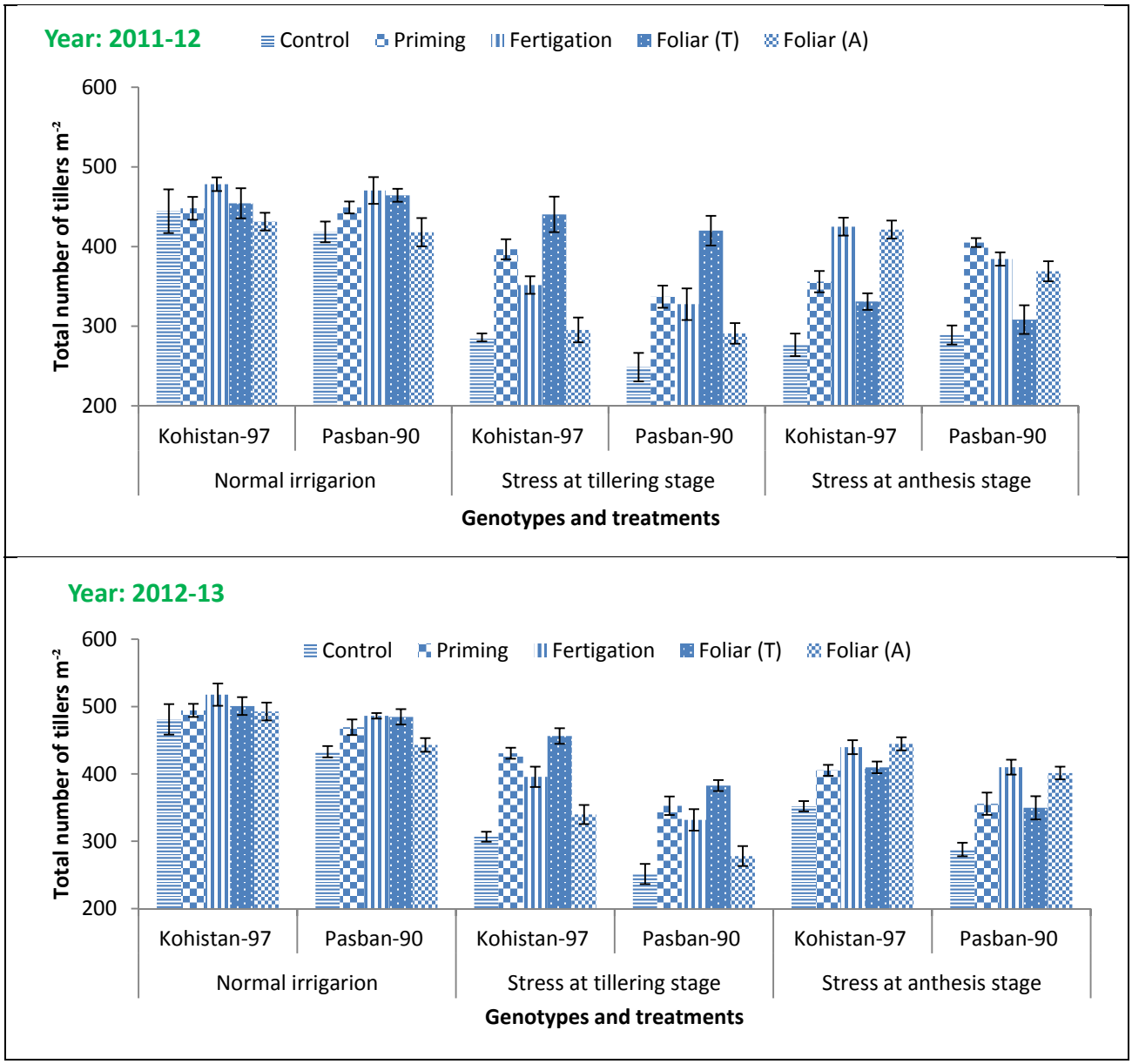

Figure 4.63: Effect of exogenous Se supply on total number of tillers/m² of two wheat (*Triticum aestivum* L.) genotypes under normal water supply, stress at tillering stage and stress at anthesis stage during two consecutive years i.e. 2011-12 and 2012-13. Values are mean ± standard error.

Plants with no Se application at tillering stage gave the minimum values (267.33 and 279.0) for this variable under limited water conditions in both the years (Fig. 4.63).

4.7.2. Total number of fertile tillers

Water deficit conditions significantly (P<0.01) reduced number of productive tillers in both the years (Table 4.17a, b). During the first year (2011-12), the limited water supply at tillering and anthesis stage decreased the number of fertile tillers by 31% and 25% respectively, while in 2012-13, it was reduced by 33% and 29% respectively as compared to normal supply of water. Wheat genotype Kohistan-97 produced 10% and 19% more productive tillers than Pasban-90 during 2011-12 and 2012-13 respectively (Fig. 4.64).

Highly significant difference (P<0.01) was recorded among different Se application methods for productive tillers in both years. During 2011-12, the maximum value (332.22) was recorded for plants fertigated with Se statistically at par with Se seed priming (327.78) and foliar application at tillering stage (323.67). Similar trend was observed during 2012-13, when plants fertigated with Se maintained the highest value (353.33) statistically related to the values obtained for Se foliar application at tillering (348.83) and anthesis (341.89) stages. The plants applied with no Se gave the minimum values i.e. 248.67 and 278.06 for this variable in 2011-12 and 2012-13 respectively (Fig. 4.64).

During the first year 2011-12, the interaction among water stress levels (W), Se treatments (S) and genotypes (G) was also significant. The normal plants Kohistan-97 produced maximum number of fertile tillers (412.33) under Se fertigation. The plants exposed to drought stress at anthesis stage resulted in minimum number of productive tillers (180.33) in Kohistan-97 (Fig. 4.64). The interaction W×S×G was non-significant in 2012-13 (Table 4.17 b).

4.7.3. Spike length

The exposure to drought stress did not significantly (P>0.05) affect the spike length of both wheat genotypes during first year (2011-12), however, a significant reduction (P<0.01) of 7% and 10% was noted for this variable in 2012-13 under water deficit conditions at tillering and anthesis stage respectively (Table 4.17a, b) in comparison to

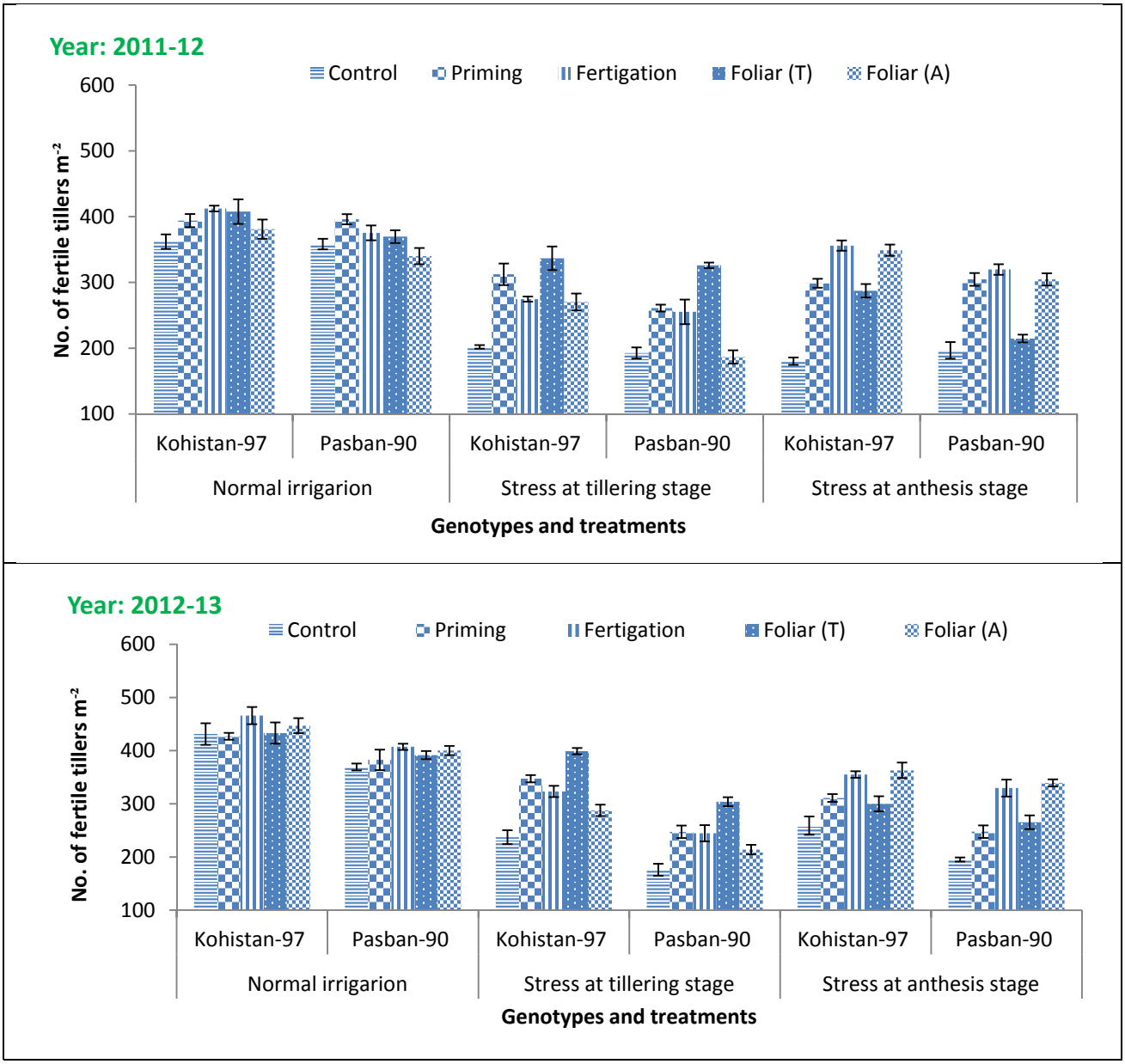

Figure 4.64: Effect of exogenous Se supply on total number of fertile tillers/m² of two wheat (*Triticum aestivum* L.) genotypes under normal water supply, stress at tillering stage and stress at anthesis stage during two consecutive years i.e. 2011-12 and 2012-13. Values are mean ± standard error.

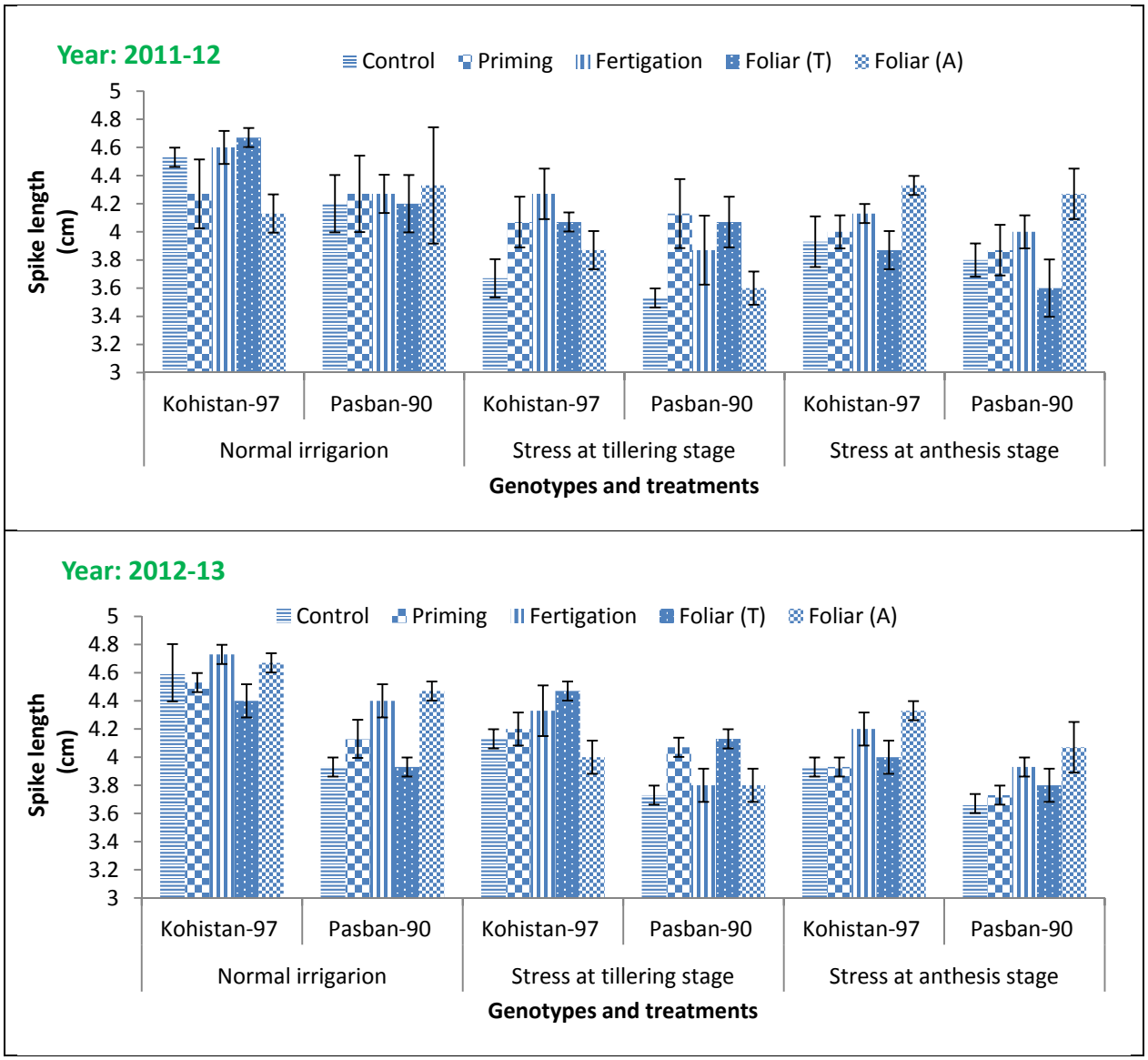

Figure 4.65: Effect of exogenous Se supply on spike length (cm) of two wheat (*Triticum aestivum* L.) genotypes under normal water supply, stress at tillering stage and stress at anthesis stage during two consecutive years i.e. 2011-12 and 2012-13. Values are mean ± standard error.

normal conditions. The plants of Kohistan-97 maintained significantly higher (P<0.01) spike length than Pasban-90 in both the years.

Among different Se application methods, Se fertigation gave the maximum values (4.19 cm and 4.23 cm) for this variable during 2011-12 and 2012-13 respectively (Fig. 4.65). However, non-significant difference (P>0.05) was observed between other Se application methods viz. Se seed treatment, Se fertigation and Se foliar application at tillering and anthesis stage in 2012-13.

The interaction among water stress levels (W), genotypes (G) and Se treatments (S) was non-significant during both the years (Table 4.17a, b).

4.7.4. Number of spikelets spike^{-1}

Analysis of data for both the years showed highly significant effect (P<0.01) of drought stress on number of spikelets spike^{-1} (Table 4.17a, b). The exposure of plants to water deficit conditions at anthesis stage caused significantly higher reduction (22% and 26%) in number of spikelets than the plants water stressed at tillering stage (12% and 14%) during 2011-12 and 2012-13 respectively. Drought tolerant genotype (Kohistan-97) produced 4% and 20% more spikelets spike^{-1} than drought sensitive genotype (Pasban-90) in 2011-12 and 2012-13 respectively (Fig. 4.66).

A non-significant difference (P>0.05) was observed among different methods of exogenous Se supply in the first year (2011-12) however highly significant difference (P<0.01) was recorded among these methods in the year 2012-13. The plants foliarly applied with Se at anthesis stage gave the highest value (15.72) for this variable statistically at par with the value (15.39) obtained by Se fertigation (Fig. 4.66). The other Se treatments viz. no Se supply, Se seed priming and Se foliar application at tillering stage exhibited non-significant differences (P>0.05) for this variable.

The interaction among different factors i.e. water stress levels (W), genotypes (G) and Se treatments (S) was non-significant in both the years (Table 4.17a, b).

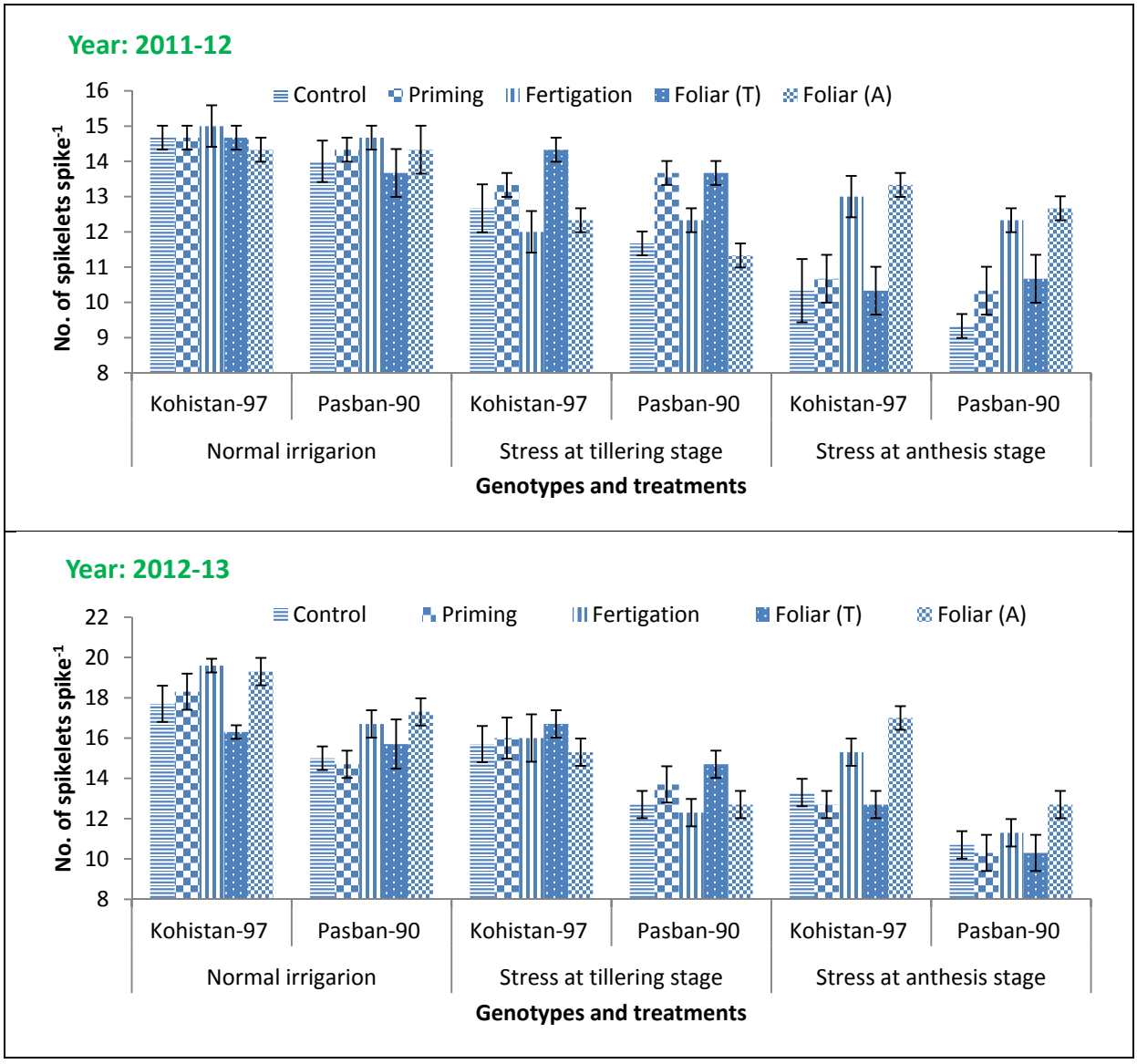

Figure 4.66: Effect of exogenous Se supply on no. of spikelets spike^{-1} of two wheat (*Triticum aestivum* L.) genotypes under normal water supply, stress at tillering stage and stress at anthesis stage during two consecutive years i.e. 2011-12 and 2012-13. Values are mean ± standard error.

4.7.5. Number of grains spike^{-1}

The number of grains per spike were significantly (P<0.01) reduced by drought stress in wheat genotypes during the year 2011-12 and 2012-13 (Table 4.17a, b). The decrease in number of grains was more pronounced in water stressed plants at later stages of growth i.e. anthesis stage. During 2011-12, the limited water supply at tillering stage reduced number of grains by 16% while it was reduced by 20% at anthesis stage as compared to normal conditions. Similar trend was observed in the year 2012-13 where plants exposed to drought stress at anthesis stage exhibited higher reduction (35%) in this variable than those water stressed at tillering stage (21%) with respect to normal plants. Wheat genotype Pasban-90 produced 6% and 5% less number of grains spike^{-1} than Kohistan-97 in 2011-12 and 2012-13 respectively (Fig. 4.67).

Highly significant variations (P<0.01) for different Se treatments were recorded for this variable. In 2011-12, plants foliarly applied with Se at anthesis stage produced maximum number of grains spike^{-1} (33.33). The production of grains was also significantly increased by Se fertigation (31.67) closely followed by Se foliar spray at tillering stage (31.61). During 2012-13, the plants foliarly sprayed with Se produced maximum number of grains spike^{-1} (35.72) and were statistically at par with those foliarly applied with Se at anthesis stage (35.44) and fertigated with Se (35.11). Non-significant differences (P>0.05) were recorded among plants grown from Se primed seeds and those applied with no Se that gave the minimum values for this variable during both the years (Fig. 4.67).

The interaction W×S was also significant in both the years. During 2011-12, the plants foliarly applied with Se at anthesis stage produced maximum number of grains spike^{-1} (37.33) closely followed by Se fertigation (35.83) under normal conditions. However, Se foliar spray at tillering stage gave highest value (31.33) for this variable in plants water stressed at tillering stage whereas the plants exposed to drought stress at anthesis stage maintained maximum number of grains (32.83) by Se foliar spray at anthesis stage (Fig. 4.67). Similarly in 2012-13, the foliar spray Se at tillering stage resulted in maximum number of grains spike^{-1} (44.00) closely followed by Se fertigation (43.17) and Se foliar spray at anthesis stage (43.00) under normal conditions. Likewise, the plants

water stressed at tillering stage maintained highest value (35.83) for this variable by Se foliar spray at

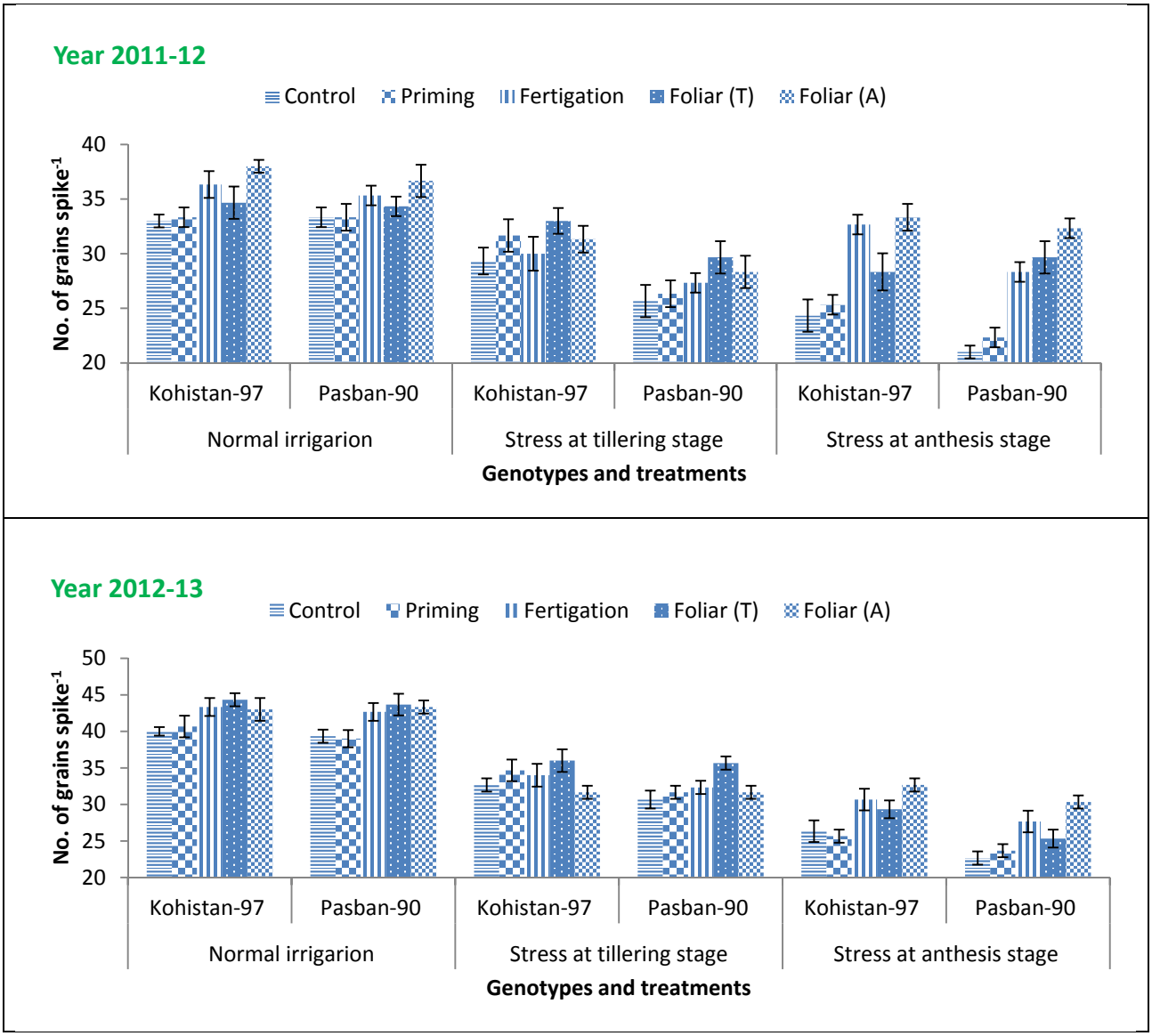

Figure 4.67: Effect of exogenous Se supply on number of grains spike^{-1} of two wheat (*Triticum aestivum* L.) genotypes under normal water supply, stress at tillering stage and stress at anthesis stage during two consecutive years i.e. 2011-12 and 2012-13. Values are mean ± standard error.

tillering stage. However, the application of Se as foliar spray at anthesis stage resulted in the production of maximum grains spike^{-1} (31.50) in plants exposed to drought stress at anthesis stage (Fig. 4.67). The interaction W×S×G was non-significant during both the years (Table 4.17a, b).

4.7.6. Thousand grain weight

Drought stress significantly reduced (P<0.01) the 1000-grain weight during both the years (Table 4.18a, b). In 2011-12, the water deficit conditions at tillering and anthesis stage significantly decreased it by 18% and 26% respectively while during the second year (2012-13), reduction was 18% and 27% respectively as compared to control. Wheat genotype Kohistan-97 maintained 7% and 9% higher grain weight than Pasban-90 in both the years i.e. 2011-12 and 2012-13 respectively (Fig. 4.68).

The effect of different Se treatments was also significant (P<0.01) for this variable. During 2011-12, exogenous Se supply as fertigation gave the maximum value (41.09 g) and had no significant difference with Se seed priming (40.14 g) and Se foliar application at tillering stage (39.84 g) (Fig. 4.68). The interaction among different factors (W×S×G) was also significant (Table 4.18 a). The highest grain weight (48.83 g) was recorded in Kohistan-97 under Se fertigation in plants grown in normal conditions. Likewise Se foliar application at tillering and anthesis stages gave maximum values i.e. 44.30 g and 41.17 g respectively in Kohistan-97 under limited water conditions. The minimum value (29.17 g) was recorded in Pasban-90 in no Se supplied plants stressed at anthesis stage (Fig. 4.68).

In the second year (2012-13), the plants where Se foliar spray was done at anthesis stage exhibited the highest grain weight (44.24 g) and it was statistically related to the values obtained for Se fertigation (43.76 g) and Se foliar application at tillering stage (43.36 g). The plants applied with no Se gave the minimum values of 36.97 g and 39.58 g for this variable during 2011-12 and 2012-13 respectively (Fig. 4.68).The interaction W×S×G was non-significant during 2012-13 (Table 4.18 b).

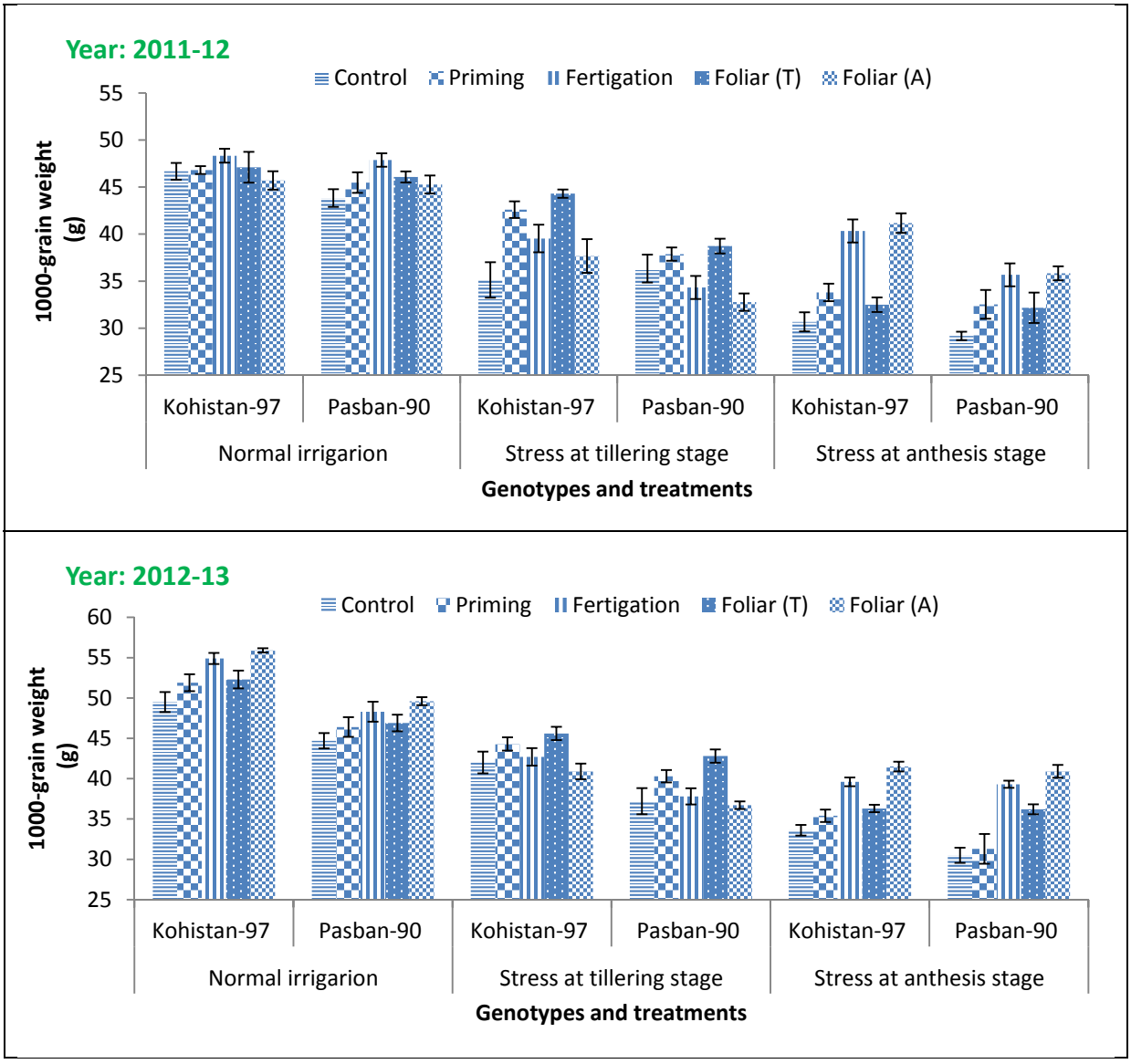

Figure 4.68: Effect of exogenous Se supply on 1000-grain weight of two wheat (*Triticum aestivum* L.) genotypes under normal water supply, stress at tillering stage and stress at anthesis stage during two consecutive years i.e. 2011-12 and 2012-13. Values are mean ± standard error.

4.7.7. Biological Yield

A highly significant effect (P<0.01) of limited water supply was recorded on BY in wheat genotypes during 2011-12 and 2012-13 (Table 4.18a, b). During 2011-12, a non-significant difference was observed between plants water stressed at tillering and anthesis stages. However during 2012-13, the plants exposed to drought stress at tillering stage gave significantly higher BY (8%) than those water stressed at anthesis stage. Significant variations (P<0.01) were also observed between genotypes for this variable. Kohistan-97 produced 7% and 8% more BY than Pasban-90 during 2011-12 and 2012-13 respectively (Fig. 4.69).

The different methods of exogenous Se supply varied significantly (P<0.01) in improving the BY (Table 4.18a, b). In 2011-12, the application of Se as foliar spray resulted in the highest BY (10.04 t ha^{-1}) and was statistically at par with Se fertigation (9.90 t ha^{-1}). A significant increase in BY was also observed by Se foliar spray at anthesis stage (9.01 t ha^{-1}) and Se seed priming (8.89 t ha^{-1}) whereas plants applied no Se exhibited lowest BY (7.98 t ha^{-1}). During the year 2012-13, plants fertigated with Se maintained maximum BY (10.77 t ha^{-1}) closely followed by those foliarly sprayed with Se tillering stage (10.56 t ha^{-1}). A non-significant difference was observed between Se foliar spray at anthesis stage (10.34 t ha^{-1}) and Se seed priming (10.31 t ha^{-1}) that increased BY by 10% as compared to no Se supply (9.414 t ha^{-1}) (Fig. 4.69).

The interaction W×S was significant (P<0.05) in both the years. During 2011-12, the fertigation of plants with Se resulted in maximum BY (12.34 t ha^{-1}) and differed non-significantly from Se foliar spray at tillering stage (11.66 t ha^{-1}) and Se seed priming (11.50 t ha^{-1}) under normal supply of water. The plants exposed to drought stress at tillering stage maintained highest BY (10.12 t ha^{-1}) by foliar spray of Se at tillering stage whereas, Se fertigation resulted in maximum BY (9.47 t ha^{-1}) in plants water stressed at anthesis stage (Fig. 4.69). The different methods of exogenous Se supply (Se seed priming, Se fertigation and Se foliar spray at tillering and anthesis stages) did not differ significantly under normal conditions in 2012-13. However, in plants water stressed at tillering stage, maximum BY

(10.12 t ha^{-1}) was recorded by Se foliar spray at tillering stage whereas Se fertigation resulted in highest BY (9.47 t ha^{-1}) in plants exposed to drought stress at anthesis (Fig.

Table: 4.18a. Analysis of variance of 1000-grain weight (g), biological yield (t ha^{-1}), grain yield (t ha^{-1}) and harvest index (%) in two wheat genotypes exposed to exogenous selenium supply under drought stress in the year 2011-12.

SOV[a]	1000-grain weight (g)	Biological yield (t ha^{-1})	Grain yield (t ha^{-1})	Harvest Index (%)
Water stress levels (W)	***	***	***	***
Selenium treatments (S)	***	***	***	**
Genotypes (G)	***	***	***	**
W×S	***	***	***	**
W×G	*	NS	***	***
S×G	NS	NS	NS	NS
W×S×G	*	NS	*	*
CV[b]	4.78	8.43	7.11	11.67

NS= non-significant, *significant at $P< 0.05$, ** significant at $P< 0.01$,

*** significant at $P< 0.001$

[a] Source of variation, [b] Co-efficient of variation

Table: 4.18b. Analysis of variance of 1000-grain weight (g), biological yield (t ha^{-1}), grain yield (t ha^{-1}) and harvest index (%) in two wheat genotypes exposed to exogenous selenium supply under drought stress in the year 2012-13.

SOV[a]	1000-grain weight (g)	Biological yield (t ha^{-1})	Grain yield (t ha^{-1})	Harvest Index (%)
Water stress levels (W)	***	***	***	***
Selenium treatments (S)	***	***	***	***
Genotypes (G)	***	***	***	NS
W×S	***	***	***	***
W×G	***	NS	*	NS
S×G	NS	NS	NS	NS
W×S×G	NS	NS	NS	NS
CV[b]	3.85	4.84	6.38	7.82

NS= non-significant, *significant at $P< 0.05$, ** significant at $P< 0.01$,

*** significant at $P< 0.001$

[a] Source of variation, [b] Co-efficient of variation

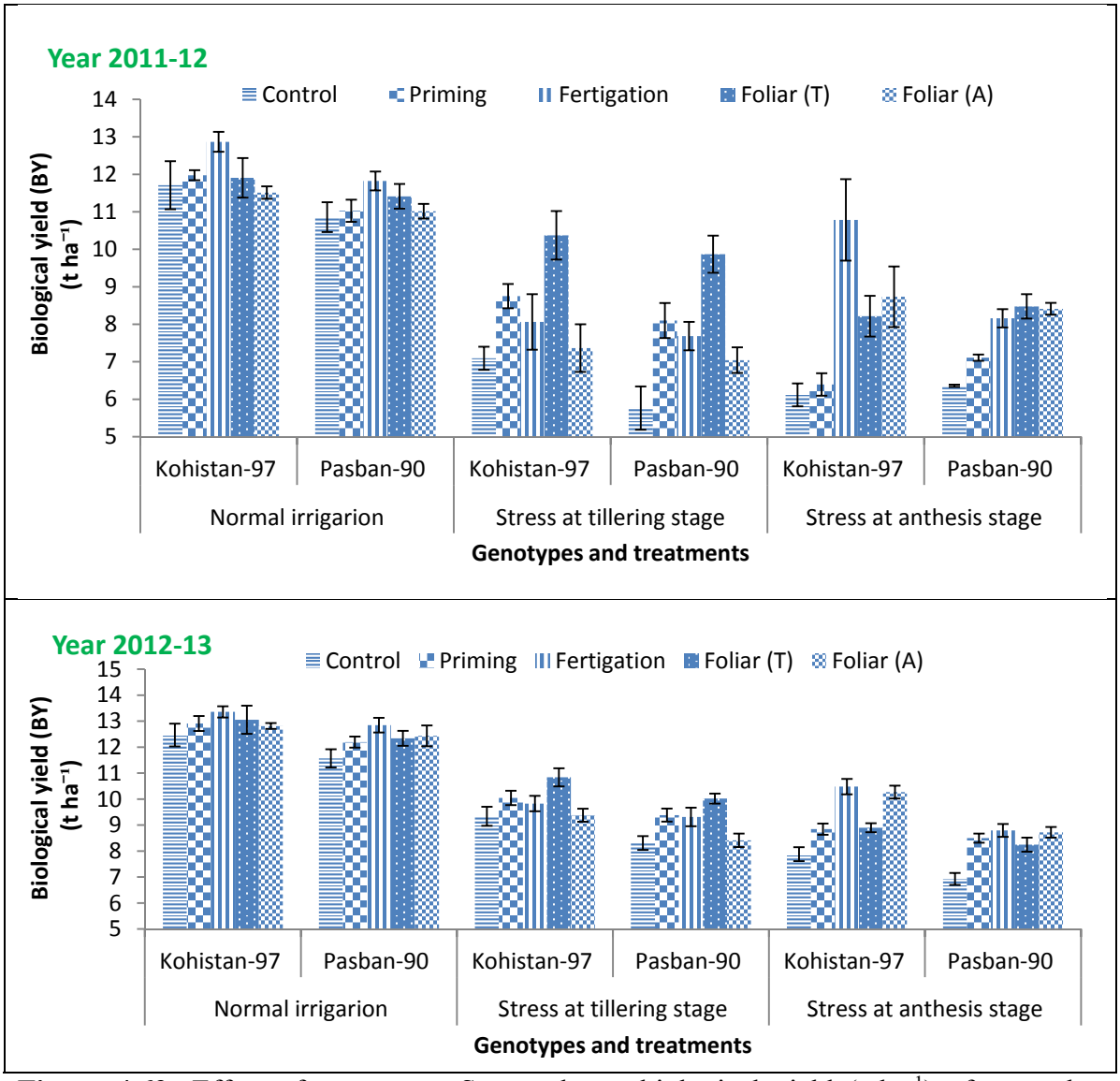

Figure 4.69: Effect of exogenous Se supply on biological yield (t ha^{-1}) of two wheat (*Triticum aestivum* L.) genotypes under normal water supply, stress at tillering stage and stress at anthesis stage during two consecutive years i.e. 2011-12 and 2012-13. Values are mean ± standard error.

4.69). All other interactions were non-significant for BY during both the years (Table 4.18a, b).

4.7.8. Grain Yield

Analysis of variance for the data for grain yield revealed highly significant effect ($P<0.01$) of drought stress on GY of both wheat genotypes during both the years (Table 4.18a, b). During 2011-12, the plants exposed to water deficit conditions at tillering stage showed significantly higher reduction (41%) in GY than plants stressed at anthesis stage (35%) as compared to normal plants. Although limited water conditions also significantly decreased ($P<0.05$) grain yield in the second year (2012-13) but there was a non-significant difference ($P>0.05$) in GY within growth stages i.e. tillering and anthesis stages. A significant reduction of 10% was recorded in drought sensitive (Pasban-90) than tolerant genotype (Kohistan-97) during both the years (Fig.4.70).

The various methods of exogenous Se supply varied significantly ($P<0.01$) for GY. During 2011-12, the plants foliarly sprayed with Se at tillering stage exhibited maximum GY (3.74 t ha^{-1}) statistically at par with Se fertigation (3.65 t ha^{-1}) and Se foliar spray at anthesis stage (3.62 t ha^{-1}). The different methods of Se supply differed non-significantly for GY during 2012-13. The control plants (no Se supply) exhibited minimum values for GY during 2011-12 (2.76 t ha^{-1}) and 2012-13 (3.10 t ha^{-1}) (Fig.4.70).

The interaction W×S×G was significant ($P<0.01$) in 2011-12. The normal plants of Pasban-90 fertigated with Se maintained highest GY (4.79 t ha^{-1}) and were statistically at par with Kohistan-97 for other methods of exogenous Se supply under normal conditions. The maximum GY (3.75 t ha^{-1}) in plants water stressed at tillering stage was recorded in wheat genotype Kohistan-97 by Se foliar application at this stage whereas Se fertigation of plants of Kohistan-97, exposed to drought stress at anthesis stage, gave highest GY (4.11 t ha^{-1}) in same genotype closely followed by Se foliar spray at anthesis stage (4.06 t ha^{-1}) (Fig. 4.70). The interaction among different factors (W×S×G) was non-significant during 2012-13 (Table 4.18 b).

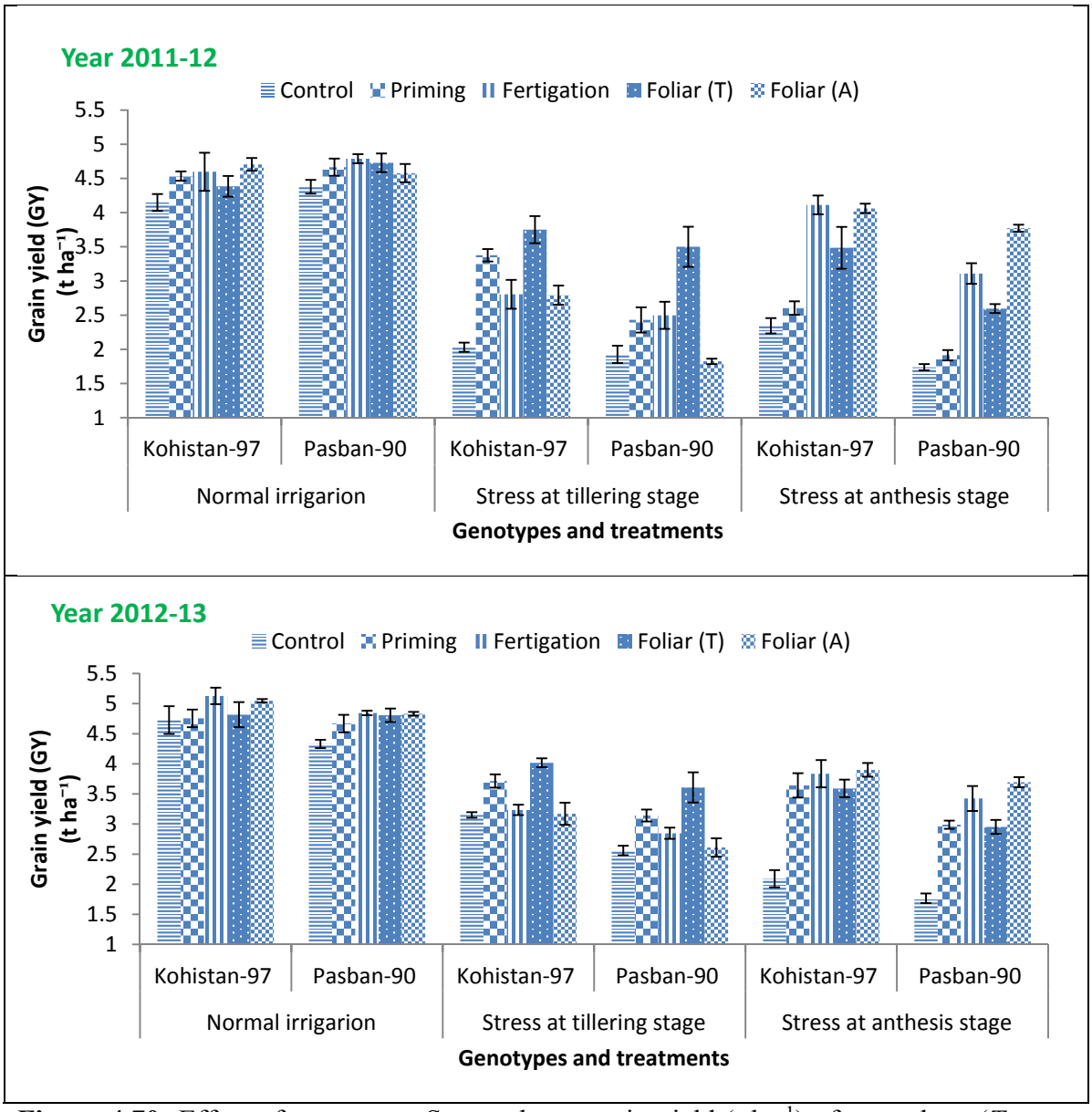

Figure 4.70: Effect of exogenous Se supply on grain yield (t ha^{-1}) of two wheat (*Triticum aestivum* L.) genotypes under normal water supply, stress at tillering stage and stress at anthesis stage during two consecutive years i.e. 2011-12 and 2012-13. Values are mean±standard error.

4.7.9. Harvest Index

Analysis of variance for the data showed highly significant effect ($P<0.01$) of drought stress on HI of plants water stressed at tillering stage in the year 2011-12, while a non-significant difference was observed between normal plants and those exposed to drought stress at anthesis stage (Table 4.18 a). Drought stress reduced HI by 15% in plants water stressed at tillering stage as compared to normal plants (Fig. 4.71). During 2012-13, an overall reduction of 12% and 5% was recorded in plants exposed to drought stress at tillering and anthesis stages respectively with respect to normal plants (Fig. 4.71). Wheat genotype Kohistan-97 maintained higher (7%) HI than Pasban-90 in 2011-12 however, a non-significant difference ($P>0.05$) was recorded between genotypes in 2012-13 (Table 4.18a, b).

A significant ($P<0.01$) increase in HI of wheat genotypes was observed by different methods of exogenous Se supply. During 2011-12, the plants foliarly supplied with Se at anthesis stage gave maximum value (39.88%) for HI which was statistically related to the values obtained by Se foliar application at tillering stage (37.36%). The fertigation of plants with Se (36.84%) and Se seed priming (36.17%) also increased HI by 8% and 6% as compared to no Se supply (34.15%) (Fig. 4.71). The interaction among water stress levels (W), Se treatments (S) and genotypes (G) was also significant ($P<0.05$) during 2011-12 (Table 4.18 a).

In the year 2012-13, exogenous Se supply significantly increased ($P<0.01$) HI in comparison to control plants (applied with no Se) however, a non-significant difference was observed between different methods of Se application viz. Se seed priming, Se fertigation and Se foliar application at tillering and anthesis stage. The interaction W×S×G was also non-significant for this variable (Table 4.18 b).

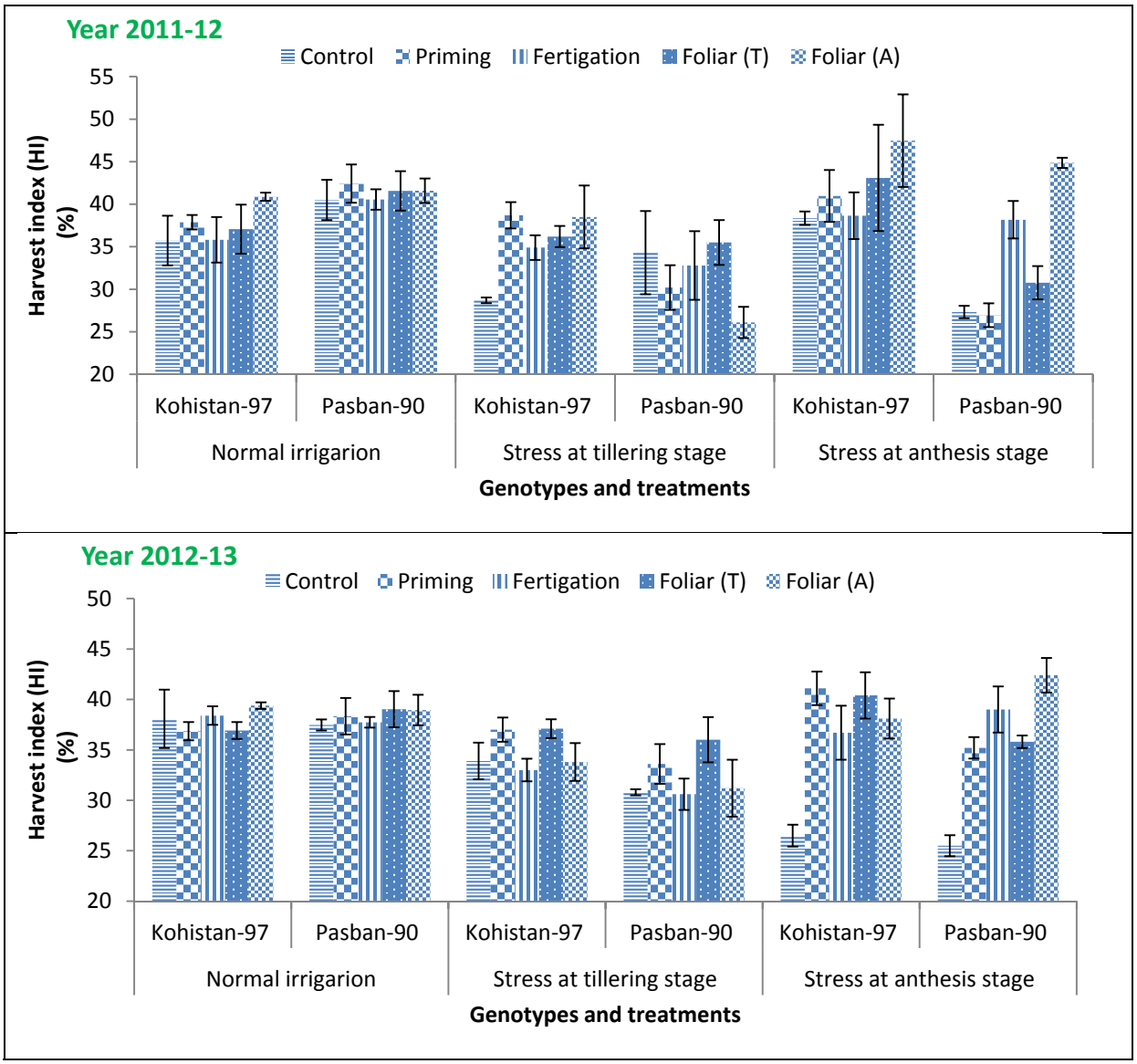

Figure 4.71: Effect of exogenous Se supply on harvest index (%) of two wheat (*Triticum aestivum* L.) genotypes under normal water supply, stress at tillering stage and stress at anthesis stage during two consecutive years i.e. 2011-12 and 2012-13. Values are mean ± standard error.

The results of the present study revealed that drought stress is one of the major limiting factors to achieve sustainability in agricultural crop production. It was observed that exogenous selenium (Se) supply is a shot gun and effective approach in mitigating the adverse effects of water stress on wheat. Recent reports indicated that Se counteracts the detrimental effects of various environmental stresses, such as drought (Hasanuzzaman and Fujita, 2011), heavy metals (Kumar *et al.,* 2012), UV-B (Yao *et al.,* 2010a,b), excess water (Wang, 2011), salt (Hasanuzzaman *et al.,* 2011), cold (Chu *et al.,* 2010) and high temperature (Djanaguiraman *et al.,* 2010). Limited information is available in the literature about the method of Se application and effective crop stage, however, the present investigation has generated applicable information about all these. The results obtained in the present study regarding different aspects i.e., screening of wheat germplasm, effective Se concentration and method as seed priming, fertigation and foliar application, are discussed below:

5.1. Screening of Wheat Genotypes/Lines

The screening of available wheat germplasm before Se application studies provided a useful insight into the drought tolerance potential of selected genotypes/lines. The genotypes were selected on the basis of variation in their response to water stress. Lab screening was carried out in growth chamber using PEG-6000 as an osmotic stress inducing agent.

Several methods are used for screening of genotypes for drought tolerance however, such approaches should be easy, rapid and inexpensive (Austin, 1993). Polyethylene glycol (PEG) is one of the most widely used drought simulator in laboratories (Mohammadi *et al.,* 2003; Dhanda *et al.,* 2004). It is well established that PEG-6000 is a useful osmotic medium for the imposition of drought stress to screen genotypes for drought tolerance (Raza *et al*., 2012; Ahmad *et al.,* 2009; Guttieri *et al*., 2001; Ashraf and Naqvi, 1995 and Blum *et a1*., 1986). Various useful traits such as coleoptile length (Rebetzke *et al.,* 2007; Bayoumi *et al.,* 2008), higher germination rate (Mohammadi *et al.,* 2003; Zarei *et al.,* 2007) rapid seedling growth and establishment (Rebetzke and Richards 1999; Rosyara *et al.,* 2008), root length at

early growth stages (Dhanda *et al.,* 2004), and survival of seedlings after desiccation (Bhutta, 2006) are used for the screening of wheat genotypes.

The results of screening experiments indicated significant variations among tested wheat genotypes/lines for different physiological indices used to estimate the stress tolerance potential. A significant decrease ($P<0.001$) in germination stress tolerance index (GSI), promptness index (PI) and emergence index (EI) was observed in all genotypes by the application of polyethylene glycol (PEG-6000) (Table 4.1). However, Kohistan-97 maintained highest PI and EI while the highest GSI was recorded in Manthar-03. Dodd and Donovan (1999) reported reduction in germination due to limited water uptake by the seeds under water deficient conditions while Almansouri *et al.* (1999) observed that moderate stress only delays, whereas high concentration of PEG reduces final germination percentages. Literature indicated that physiological indices are an effective, efficient and inexpensive tool for the screening of genotypes and the genotypes that maintain high GSI, GP, RLSI and PHSI can been categorized as drought tolerant (Zarei *et al.*, 2007; Khodarahmpour, 2011; Khayatnezhad *et al.,* 2010; Raza *et al.*, 2012). The reduction in germination indices might be attributed to PEG-induced decreased imbibition of water in seeds, hence due to lack of water hydrolytic enzyme activity did not work properly as a consequent germination was reduced (Taiz and Zeiger, 2006). The results of present study are in accordance with the findings of other researchers in various crops/plants such as sorghum (Gill *et al*., 2002), lentil (Haq *et al.,* 2010), corn (Gharoobi *et al.,* 2012), sugar beet (Sadeghian and Yavari, 2004), cotton (Basal *et al*., 2005), sunflower (Ahmad *et al.,* 2009), *Agropyron trichophorum* (Rahmati and Farshadfar, 2012), canola (Giancarla *et al.,* 2012) and wheat (Dhanda *et al*., 2004; Raza *et al.,* 2012)

The seedling growth was characterized as an efficient and reliable procedure for screening due to variation among wheat genotypes at seedling stage under drought stress (Ashraf *et al.,* 2008; 1996). Wheat genotype Kohistan-97 exhibited maximum plant height stress tolerance (PHSI) (Fig. 4.5) and dry matter stress tolerance index (DMSI) (Fig. 4.7). The decrease in PHSI under drought stress might be due to reduction in cell expansion and elongation which is the most sensitive physiological aspect of a plant under water deficit conditions that ultimately affect plant height (Larson, 1992). Root length and seedling dry

weight are major traits for the selection of drought tolerant genotypes (Nazeri, 2005). Wheat genotypes Pak-81 and Chakwal-86 maintained maximum root length stress tolerance index (RLSI) (Fig. 4.6). These results are in accordance with the findings of Yamur and Kaydan (2008) who reported an important and significant relation between root and seedling dry weight with germination rate, root length, and shoot dry weight under drought stress. The dehydration of protoplasm (Hussain *et al.,* 2008) or hindrance in translocation of photosynthates due to blockage of xylem and phloem vessels (Abdalla, 2011) might have attributed to reduced PHSI. The changes in cell wall extensibility due to hormonal imbalance (cytokinin, abscisic acid) under water deficit conditions may also be responsible for decrease in plant height (Banon *et al.,* 2006). The decrease in DMSI might be the result of loss of turgor due to closure of stomata (Yordanov *et al.,* 2003) that disturbs the partitioning and translocation of photosynthates and consequently reduces dry matter (Miao *et al.,* 2006).

The results of the present study revealed that Kohistan-97 maintained the highest physiological indices and was categorized as the most tolerant genotype. Manthar-03, Chakwal-86, Pak-81 and Chakwal-50 were second best in their performance and can be categorized as moderately drought tolerant genotypes. Genotype Pasban-90 was found to be the most sensitive genotype under limited water conditions (Table 4.2). The variation among genotypes may be due to suppression of genetic variability under osmotic conditions which results in the reduction of genetic variance and heritability under stress environment (Ludlow and Muchow 1990). This variation under water deficit conditions can be helpful in breeding for drought tolerance (Dhanda *et al.,* 2004).

5.2. Optimization of Rate and Duration for Selenium (Se) Seed Priming

The results of the study confirmed that Se seed priming enhances seed germination and growth of wheat seedlings exposed to drought stress. The duration of seed priming was kept ½ h and 1 h to avoid Se toxic (Brown and Shrift, 1981) and inhibitory effects (Kahle, 1988) to plant systems at low and high concentrations respectively. A significant increase in seedlings growth at higher doses of Se seed treatment under limited water conditions depicts its role in lowering osmotic potential to improve water relations of moisture stressed seedlings (Hartikainen, 2005). Both genotypes differed significantly in their response to Se seed treatment and genetic potential may be responsible for variation between genotypes i.e.

Kohistan-97 and Pasban-90 which comply with the findings of Eurola *et al.* (1991). The increase in PHSI of both genotypes (Fig. 4.8a, b) specifies Se role in regulation of water status of moisture stressed seedlings (Djanaguiraman *et al.,* 2005) and activation of plant hormones responsible for cell expansion and enlargement (Larson, 1992). Kahakachchi *et al.* (2004) and Smrkolj *et al.* (2006b) reported that actively growing plant parts such as young leaves and seeds accumulate the largest amounts of Se. The Se treatment also increased DMSI of seedlings (Fig 4.10a, b) which suggests its positive role in increasing plant dry weight, however, RLSI only increased at higher doses of Se (75 µM and 100 µM) in 1 h priming (Fig. 4.9a, b) and comply with the findings of Yao *et al.* (2009) that Se supply enhances root activity of wheat seedlings grown under limited water conditions. The increase in root length and dry weight at higher doses of Se supports the fact that a significant relation exists between root and dry biomass of water stressed seedlings (Okçu *et al.,* 2005; Yamur and Kaydan, 2008) and Se treated radicles are more vigorous and healthier than normal ones (Carlson *et al.,* 1989).

5.3. Optimization of Selenium (Se) Level for Fertigation and Foliar Spray

The results indicate that water stress adversely influences wheat growth due to poor germination and seedling establishment. It was observed that fertigation and foliar spray are efficient, viable and effective approaches for the application of fertilizers and improving their efficiency (Latif and Iqbal, 2001). An increase in physiological indices with Se supply confirmed the hypothesis that Se plays a positive role in improving drought tolerance of wheat seedlings.

The increase in PHSI specifies Se role in regulation of water status of moisture stressed seedlings (Djanaguiraman *et al.,* 2005) and activation of plant hormones responsible for cell expansion and enlargement (Larson, 1992). The maximum PHSI observed by Se fertigation treatment of 7.35 µM (Fig. 4.13) and Se foliar application @ 7.06 µM (Fig. 4.18) might be attributed to the Se-regulated decrease in osmotic potential that increases the water relations of water stressed seedlings (Hartikainen, 2005). The actively growing plant parts

such as young leaves and seeds accumulate large amounts of Se which affects osmoregulation in plants (Kahakachchi *et al*., 2004; Smrkolj *et al.*, 2006b).

The growth and development of plants is directly influenced by root activity (Bai *et al.,* 1994) so it can serve as an important index of plant resistance (Yao *et al.,* 2009). The increase in root length is an adaptive response of wheat plants exposed to drought stress. The highest RLSI recorded in plants fertigated (Fig. 4.14) and foliarly sprayed (Fig. 4.19) with Se treatments of 7.35 µM and 7.06 µM, respectively indicates the effectiveness of Se in improving plant resistance against drought stress. The radicles treated with Se are healthier and vigorous with extensive root hairs (Carlson *et al.,* 1989). Yao *et al.* (2009) reported an increase in root activity (growth and uptake) of water stressed seedlings by extra Se supply resulting in an increase in dry matter which improved DMSI of seedlings (Fig. 4.17; 4.22). Similarly, Valadabadi *et al.* (2010) noted significant increase in total dry weight of rapeseed cultivars foliarly sprayed with Se under water stress conditions The increase in root length and dry weight by Se application supports the fact that a significant relation exists between root and seedling dry weight of water stressed seedlings (Okçu *et al.,* 2005; Yamur and Kaydan, 2008).

The fertigation of seedlings by Se treatment of 7.35 µM resulted in the highest biomass accumulation (Fig 4.15; 4.16). However, low Se fertigation doses did not significantly improve the biomass (SFSI and RFSI). These results are in line with the findings of Nawaz *et al.* (2013) who observed that Se significantly increased growth of water stressed wheat seedlings and were of the view that Se regulates water status under drought stress. Non-significant effect of lower Se doses on biomass has been reported in wheat (Yao *et al.,* 2009), perennial ryegrass and strawberry clover (Hopper and Parker, 1999). The high Se fertigation dose (14.70 µM) caused a significant reduction in biomass (Fig. 4.15; 4.16). Similar results were reported by Ximenez-Embun *et al.* (2004) in white lupine (20%) and sunflower (40%) at high Se fertilization doses. High levels of Se inhibit photosynthesis by decreasing the light energy absorbed by the antenna system and impairs photosynthetic machinery of wheat (Łabanowska *et al.*, 2012) which results in the lower production of starch (Vítová *et al.,* 2011; Wang *et al.,* 2012) that may lead to a decrease in the biomass production.

The highest biomass accumulation (SFSI and RFSI) with Se foliar spray (Fig 4.20; 4.21) may be due to the diffusion of Se ions that takes place from the surface of leaves to epidermal cells. The foliar application of Se has been reported to stimulate growth in lettuce (Xue *et al.,* 2001), green tea (Hu *et al.,* 2003) and potato (Turakainen *et al.,* 2004). Germ (2008) observed significant increase in mass of only water stressed potato tubers supplemented with Se, whereas the mass of tubers was reduced by Se application in well-watered plants. Similar results were reported by Habibi (2013) in barley. The increased Se efficiency by foliar application may be due to its direct absorption and accumulation in the plants by diffusion from the surface of leaves to epidermal cells (Wójcik, 2004) but its high concentration can cause damage to leaf surface (Marschner, 1995). Therefore, concentration of solution at fertigation and foliar application of Se should be chosen with care, based on recommendations.

From the results of experiments conducted for optimization of Se for seed priming, fertigation and foliar application in wheat it was concluded that Se seed priming for one hour @ 75 µM, Se fertigation @ 7.35µM and Se foliar application @ 7.06 µM were optimum Se doses for its exogenous supply to mitigate adverse effects of drought.

5.4. Optimization of Method and Time of Selenium (Se) Application

The identification of ideal selenium application method and period is crucial for better understanding of selenium translocation into crop plants (Ducsay *et al.,* 2008).

5.4.1. Physiological Parameters

5.4.1.1. Water Relations

Osmotic adjustment helps the plants in the maintenance of water uptake under drought stress (Chen and Jiang, 2010; Abdelmalek and Khaled, 2011). The maintenance of turgor by active lowering of osmotic potential (Ψ_s) is generally considered as an adaptation of plants under water limited environment (Ludlow and Muchow, 1990). The plants exposed to drought stress had more negative leaf water potential (Ψ_w) than the normal plants. The plants tend to maintain favorable water relations that help to develop resistance against drought stress (Passioura, 1992; Kaldenhoff *et al.,* 2008). The treatment of plants by Se

significantly enhanced Ψ_w under both normal and limited water conditions (Table 4.6). The water retention in the plant tissues is increased significantly by Se through increasing uptake of water by the root system without decreasing transpiration rate (*E*) (Kuznestov *et al.,* 2003). The Ψ_w was least negative at tillering stage in plants fertigated and foliarly applied with Se @ 7.35µM and 7.06 µM respectively (Fig. 4.23). The results showed that exogenous Se supply enhanced Ψ_w and confirm the findings of Germ *et al.* (2007) who reported higher Ψ_w in Se treated potato plants under drought stress. Likewise Sajedi *et al.* (2009) also reported a significant increase in yield and water use efficiency of maize plants by Se foliar application. The addition of Se in growth medium improved leaf Ψ_w of maize plants under severe drought stress conditions (Qiang-yun, 2008)

It was observed that a decrease in Ψ_w caused a parallel reduction in Ψ_S which helps the plants to maintain pressure potential (Ψ_p) (Serraj and Sinclair, 2002). The minimum reduction (3%) in Ψ_S was recorded in plants fertigated with Se @ 7.35 µM and was statistically related to Ψ_S observed by Se seed priming @ 75 µM for one hour and Se foliar application at tillering stage (7.06 µM) (Fig. 4.24). Higher reduction in Ψ_S at later stages of growth i.e. anthesis stage may be due to loss of (Ψ_p) and increase in metabolic activity, related to senescence during maturity. The maintenance of Ψ_S by Se may be attributed to its positive role in increasing root activity and decreasing *E* under limited water conditions (Kuznestov *et al.,* 2003; Yao *et al.,* 2009). The results suggest that Se influences the net accumulation of osmolytes or simple passive concentration of solutes during drought stress that results in the reduction of Ψ_S. The increased Se efficiency by foliar application may be due to the reason that it could be directly transferred and accumulated in the plants. Diffusion of Se ions to leaf epidermal cells increases by foliar application (Wójcik, 2004) but its high concentrations may lead to toxicity and cause damage to leaf surface (Marschner, 1995). Therefore, concentration of solution at foliar application of Se should be chosen with care.

The loss in Ψ_p under drought stress can be attributed due to reduction in Ψ_w, lowering of photosynthetic rate (*Pn*) and stomatal conductance (g_s) (Nawaz *et al.,* 2012). Turgor maintenance is a prime defense mechanism in plants that helps to cope with environmental stresses particularly drought stress. The disturbance in osmotic adjustment causes reduction in Ψ_p, resulting decrease in cell elongation (Vassilev and Yordanov, 1997). A non-significant

difference (P>0.05) among various Se application methods for leaf Ψ_p (Table 4.6) suggest that all tested methods are equally good in maintaining osmotic adjustment, water uptake, growth, photosynthesis and protection of metabolic structures under water deficit conditions (Zhang *et al.,* 1999; Kuznestov *et al.,* 2003; Yang and Lu, 2006). Reports have shown that drought induced reduction in Ψ_w results in reduced relative water contents (RWC) in wheat (Živčák *et al.,* 2009; Nawaz *et al.,* 2012; Raza *et al.,* 2013). The foliar applied Se at tillering stage increased the RWC by 10% and gave the maximum value (79.5%) for this variable statistically at par with Se fertigation (77.8%) under drought stress (Fig. 4.26). The positive role of Se in increasing RWC has been reported by Wang (2011) who observed that PEG+Se treated *Trifolium repens* L. plants maintained higher RWC than PEG-treated plants. Habibi (2013) observed the highest RWC in barley plants treated with Se under well watered conditions. The stimulating effect of Se may be attributed to the increase in membrane integrity (Hartikainen *et al.,* 2000) or decrease in photo-oxidation (Seppänen *et al.,* 2003).

Available literature indicated variation between drought tolerant and susceptible genotypes which may be due to the maintenance of tissue turgor, physiological activities, water uptake from soil and reduction in water loss through stomata (Song *et al.,* 1995; Siddique *et al.,* 2000; Terzi and Kadioglu, 2006). However in contrast, non-significant differences were recorded between droughts tolerant (Kohistan-97) and sensitive (Pasban-90) genotypes in present study which indicate that drought susceptible genotype may be more responsive to exogenous Se supply for the maintenance of leaf water relations as compared to tolerant one.

5.4.1.2. Pigments

Pigments especially chlorophyll contents are necessary to maintain optimum photosynthetic capacity in plant (Wright *et al.,* 1994; Nageswara *et al.,* 2001). Drought stress inhibits photosynthesis by reducing chlorophyll contents (Chl) and affecting its synthesis, and damaging the photosynthetic machinery of plants (Iturbe Ormaetxe *et al.,* 1998). The severity and duration of drought stress determines the extent of damage to photosynthetic apparatus and Chl of plants (Rensburg and Kruger, 1994; Kyparissis *et al.,* 1995; Jagtap *et al.,* 1998). The results of this study showed a higher decrease (36%) in Chl_b content as

compared to Chl_a (4%) and Chl_t (15%) in water stressed plants. Literature indicated that limited water availability reduces Chl in sunflower (Manivannan *et al.,* 2007), wheat (Fotovat *et al.,* 2007), chickpea (Mafakheri *et al.,* 2010) and corn (Khayatnezhad *et al.,* 2011). Drought stress reduces leaf Chl (Ommen *et al.,* 1999) mainly due to damage to chloroplasts caused by reactive oxygen species (ROS) (Smirnoff, 1995). Contrasting results were reported by Schelmmer *et al.* (2005) that drought stress had no significant effect on Chl in maize. Recent developments have shown that decrease in chlorophyll pigments increases the reflectance of the incident radiation (Schelmmer *et al.,* 2005) that can protect photosynthetic system against stress (Arjenaki *et al.,* 2012).

The application of Se reduces the damage to the chloroplasts and increases the Chl (Filek *et al.,* 2009; Hawrylak-Nowak, 2009; Chu *et al.,* 2010; Wang, 2011; Yao *et al.,* 2011; Malik *et al.,* 2012). Our study confirmed these findings as Se seed treatment resulted in maximum leaf Chl_a contents under normal conditions and was statistically at par with Se fertigation. Similar, trend was noted under water deficit conditions (Fig. 4.27). The observations followed the pervious trend in case of leaf Chl_b contents in water stressed plants which was maximum in Se fertigation and at par with Se foliar application at tillering and anthesis stages (Fig. 4.28). The plants maintained higher Chl_a contents by Se application at anthesis than tillering stage while non-significant difference was recorded between stages for Chl_b contents (Table 4.7). In contrast, Kumar and Paul (1997) observed that water stress significantly decreases Chl_a and Chl_b contents at later growth stages (flowering and seed filling) in rape. Se seed treatment showed the highest increase in leaf Chl_t contents under both normal and limited water conditions (Fig. 4.29). Zahedi *et al.* (2012) reported similar results in Se supplemented canola cultivars exposed to drought stress. The increase in photosynthetic pigments by Se under water deficit conditions can be attributed to scavenging of ROS by production of antioxidants to prevent lipid peroxidation that leads to chlorophyll destruction (Mirnoff, 1993; Foyer *et al.,* 1994; Habibi, 2013). Low Se concentrations can increase Chl by altering its biosynthetic pathway (Djanaguiraman *et al.,* 2005). Much higher increase in pigments in Kohistan-97 (drought tolerant) than Pasban-90 (drought sensitive) confirmed the findings of Alaei (2011) in wheat and of Khayatnezhad *et al.* (2011) in maize that drought tolerant genotypes had higher Chl that can be used in breeding programs for drought resistance.

Low molecular antioxidant compounds such as anthocyanins, carotenoids (Car), flavonoids and proline (Radyuk *et al.,* 2009) play an important role in stress tolerance in plants. Studies have proven their ability to scavenge free radicals and inhibit membrane lipid peroxidation of seedlings (Steyn *et al.,* 2002; Peng and Zhou, 2009). Car biosynthesis is a genetic characteristic in plants (Bojović and Stojanović, 2005) but is strongly influenced by environmental conditions. Chu *et al.* (2010) observed that Se treatments significantly increased Car, anthocyanins, flavonoids, and phenolic content of wheat seedlings subjected to cold stress. In our study, the limited water conditions reduced Car content by 10% as compared to normal supply of water (Fig. 4.30). Priming of seeds with Se was the most effective method in increasing Car contents under both normal and limited water conditions. Se fertigation was statistically related to seed treatment under drought stress (Fig. 4.30). Increase in Car content by Se application might be attributed to decrease in chloroplast destruction (Habibi, 2013) or alteration in biosynthetic pathway (Djanaguiraman *et al.,* 2004) and is in line with the findings of Yao *et al.* (2009) in wheat seedlings under drought stress. Dong *et al.* (2013) found photosynthetic pigments in *Lycium chinense* leaves to be Se dependent and reported an increase of 200-400% in them by Se supplementation. In contrast, Hawrylak-Nowak (2009) witnessed non-significant change in Car content after Se supplementation of cucumber plants. Low doses of Se (3 and 6 mg L^{-1}) significantly increased photosynthetic pigments while higher dose of 12 mg L^{-1} caused a significant reduction in Car content in leaves of sorghum plants exposed to cold stress (Abbas, 2012). However, Se toxicity decreased photosynthetic pigments in *Chlorella vulgaris* (Tian-Feng *et al.,* 2005) which may be due to negative effect on the production of porphobilinogen synthetase (Padmaja *et al.,* 1995) or replacement of sulphur (S) atoms by Se in S-containing amino acids, cysteine and methionine (Terry *et al.,* 2000).

5.4.1.3. Gas Exchange

Drought stress limits photosynthesis which determines the crop production (Tognetti *et al.,* 2005; Bacelar *et al.,* 2006; Ben Ahmed *et al.,* 2009), through metabolic impairment and stomatal closure (Tezara *et al.,* 1999; Lawson *et al.,* 2003; Pieters and El Souki, 2005). Decrease in CO_2 assimilation rate (P_n), g_s and E under drought stress has been reported in many crops such as wheat (Moud and Yamagishi, 2007), maize (Ashraf *et al.,* 2007),

Brassica napus (Kauser *et al.,* 2006) and mungbean genotypes (Ahmed *et al.,* 2002). Se-induced protection of photosynthetic apparatus has been reported in rape (*Brassica napus* L.) seedlings (Filek *et al.,* 2010), potato (Germ, 2008), garlic (Yong-xiang *et al.,* 2012), barley (Habibi, 2013) and lettuce (Feng *et al.,* 2007). Our study confirmed similar results in wheat as foliar application of Se at tillering stage significantly increased P_n and was statistically at par with Se seed treatment (Fig. 4.31) in normal plants (100% FC). Under water deficit conditions (60% FC), the foliarly applied Se at tillering stage increased P_n by 34% in respect to plants with no Se supply. Contrary studies showed that Se application did not increase P_n in Se-supplemented water deficit barley plants (Habibi, 2013). The decrease in P_n in water stressed plants may be due to decrease in Chl (as observed in this study), chlorophyll degradation, fragmentation and suppression of rubisco, stomata closure (Hajduch *et al.,* 2001; Pietrini *et al*., 2003). All these reduced the photochemical efficiency of PSII (Pieters and El Souki, 2005) with the possible net loss of D1 protein of PSII reaction centers (Baker, 1993; Cornic, 1994). Application of Se was beneficial in maintenance of photosynthetic rate that might be due to protective role of Se to minimize the damage to chloroplast structure and PSII reaction centers under limited water conditions. Drought tolerant genotypes have been reported to show higher P_n than sensitive ones (Rahbarian *et al.,* 2011). However, non-significant difference was observed between Kohistan-97 (drought tolerant) and Pasban-90 (drought sensitive) in present study (Table 4.8) which may due to variation in response to Se utilization in metabolic activities.

Stomatal regulation is an early response of plants to water stress in order to reduce leaf transpiration rate and maintain cell turgor for normal biochemical reactions (Liang *et al.,* 2002; Moud and Yamagishi, 2007; Bogale *et al.,* 2011). Studies have shown reduction in g_s in wheat (Moud and Yamagishi, 2007), chickpea (Rahbarian *et al.,* 2011), barley (Habibi, 2013) and potato (Kawakami *et al.,* 2005) under limited water conditions. Reduced inhibition in P_n under limited water conditions is necessary for drought tolerance in plants (Zlatev and Yordanov, 2004). In present study, g_s was reduced by 27% in water stressed plants as compared to normal plants (Fig. 4.33). Drought stress limits P_n due to decrease in CO_2 availability, mainly through stomatal closure (Cornic and Massacci, 1996; Cornic, 2000) and/or by metabolic impairment (Flexas and Medrano, 2002). The closure of stomata decreases internal CO_2 concentration (*Ci*) and inhibits ATP synthesis and activity of

ribulose-1, 5-bisphosphate carboxylase/oxygenase that lead to a reduction in photosynthesis under drought stress (Dulai *et al.,* 2006). Literature indicated that a positive relation exists between RWC and photosynthesis (Siddique *et al.,* 2000; Moaveni, 2011).

Foliar application of Se at tillering stage gave the maximum value for g_s statistically at par with Se seed priming and fertigation under normal conditions (Fig. 4.33). Similarly in water stressed plants, foliar spray of Se at tillering stage increased g_s by 39% in respect to plants with no Se supply. Recently, Habibi (2013) reported similar results in barley and observed lower reduction in g_s of Se-supplemented water stressed plants.

Several studies confirmed decrease in *E* under limited water conditions (Egert and Tevini, 2002; Moud and Yamagishi, 2007; Rahbarian *et al.,* 2011; Bogale, 2011) that may be attributed to decrease in P_n and *gs* in water deficit plants, as observed in present study. A significant decrease of 26% was recorded in transpiration rate of plants exposed to drought stress. The maximum increase was recorded in plants supplied with Se as foliar treatment at tillering stage while Se fertigation was the second best method in improving *E* (Fig. 4.32). These results are in agreement with the findings of Kuznestov *et al.* (2003) for wheat. However, Germ *et al.* (2007) observed Se-induced increase in *E* only under well watered conditions, whereas non-significant effect of Se was observed under drought stress.

5.4.2. Biochemical Parameters

5.4.2.1. Total Soluble Sugars (TSS)

The accumulation of osmotically active molecules/ions including soluble sugars, alcohols, glycinebetaine, proline and organic acids etc. is a characteristic feature of plants to adjust the external osmotic conditions and to maintain water relations under drought stress (Mohammadkhani and Heidari, 2008; Farooq *et al.,* 2009). Drought induced accumulation of total soluble sugars (TSS) has been well reported in several crops such as wheat (Akladious, 2012), rice (Mostajeran and Rahimi-Eichi, 2009) and Soybean (Liu *et al.,* 2011). Our study confirmed these findings with an increase of 10% in TSS in water stressed wheat plants in comparison to normal plants. The plants accumulated maximum TSS at tillering stage under water deficit conditions. The plants fertigated with Se maintained the highest TSS closely

followed by Se seed priming (Fig. 4.34). Studies (Djanaguiraman *et al.,* 2004; Turakainen *et al.,* 2004; Hajiboland and Keivanfar, 2012; Zhao *et al.,* 2013) indicated an increase in TSS by Se supplementation to soybean, potato, canola and pear-jujube. Nawaz *et al.,* (2013) observed significant increase in TSS of wheat seedlings by Se seed priming under drought stress (present study). The higher concentration of TSS might be attributed to starch decomposition as a result of amylase activity under drought stress (Ghasempour *et al.,* 1998).

5.4.2.2. Total Soluble Proteins (TSP)

The plants exposed to water deficit conditions showed reduction (16%) in soluble proteins (TSP) (Fig. 4.35) which might be attributed to the reduced rate of protein biosynthesis and increase in breakdown of proteins under drought stress (Good and Zaplachinski, 1994; Rodriguez *et al.,* 2005), required for the production of low molecular weight osmolytes for osmotic adjustment or osmoregulation (Nayyar and Walia, 2003). A significant reduction in TSP was recorded by exogenous Se supply (Fig. 4.35). In contrast to above findings, Djanaguiraman *et al.,* (2004) reported an increase in TSP of Se treated Soybean plants due to enhanced nitrate reductase activity and total free amino acids (TFA) content. Similarly, Zhao *et al.* (2013) also observed an increase of 48-52% in TSP of pear-jujube tree by Se foliar fertilization. However, a decrease in TSP concentration of water stressed seedlings complies with the findings of Kochaki (1997) and Sujin (2004) who were of the view that high molecular weight soluble proteins concentration decreases while low molecular weight increases in plants under moisture deficit conditions and concluded that biochemical attributes like TSS, TSP and TFA can be used as selection tool for screening of drought tolerant cultivars (Ashraf, 1998).

5.4.2.3. Total Free Amino acids (TFA)

Amino acids contribute significantly in plant metabolism as early products of photosynthesis and nitrogen assimilation. Biosynthesis and accumulation of amino acids take place in response to environmental stresses (Hsu and Kao, 2003) such as drought that results in breakdown of structural proteins into component amino acids, which actively take part in osmotic adjustment under water deficit environment (Good and Zaplachinski, 1994). In the

present study, the limited water conditions increased total free amino acids (TFA) concentration by 45% with respect to plants growing under normal conditions. The concentration of TFA increased significantly by Se application in both water stressed and normal plants. The maximum increase (48%) was observed by Se seed treatment, statistically at par with Se fertigation as compared to plants with no Se supply under water deficit conditions. However, in normal plants, Se foliar application at tillering stage resulted in maximum value for TFA which was statistically at par with Se fertigation (Fig. 4.36). These results are in line with the findings of Hu *et al.* (2001), Khattab (2004) and Lee *et al.* (2005) who reported an increase in TFA in Se treated, green tea, rocket plants and broccoli respectively. According to Gowily *et al.* (1996) and Wu (1998), the increase in TFA concentration by Se application might be due to the disturbance in amino acid metabolism which caused increase in soluble protein content and nitrate reductase activity (Djanaguiraman *et al.,*2004). But the accumulation of TFA helps the plants in osmotic adjustment to stressed environment (Hsu and Kao, 2003) which is very true for present study.

5.4.2.4. Proline Accumulation

Proline accumulation is a typical symptom of biochemical adaptation in plants to environmental stresses (Rhodes *et al.,* 1999; Ozturk and Demir, 2002; Hsu *et al.,* 2003; Kavi Kishore *et al.,* 2005) that triggers stress signals (Maggio *et al.* 2002; Verbruggen and Hermans, 2008). It is one of the most common compatible osmolytes in water stressed plants (Sanchez *et al.,* 1998; Alexieva *et al.,* 2001) and contributes to stabilize sub-cellular structures, scavenging free radicals (Srinivas and Balasubramanian, 1995) and to maintain appropriate NADP+/NADPH ratios necessary for regular metabolic activities (Hare and Cress, 1997). It was observed in present study that proline content increased significantly in wheat plants exposed to drought stress as compared to normal plants (Table. 4.10). The high proline accumulation might be attributed to reduced protein biosynthesis in water stressed plants (Cechin *et al.,* 2008) and can be used as selection criteria for stress tolerance (Yancy *et al.,* 1982; Jaleel *et al.,* 2007a). It is well established fact that Se application results in proline accumulation under various environmental stresses such as salinity (Hawrylak-Nowak, 2009), cold stress (Abbas, 2012) and heavy metal toxicity (Akbulut and Çakir, 2010). In the present study, application of Se as foliar spray at tillering stage resulted in the highest proline

contents and caused a significant increase of 73% as compared to plants where Se was not supplied under drought stress. Similarly, an increase of 41% and 48% was recorded in plants fertigated with Se and foliarly sprayed with Se at anthesis stage, respectively (Fig. 4.37). These results are in agreement with the findings of Yao *et al.* (2009) who reported an increase in proline content in wheat seedlings by Se application under drought stress. Kuznestov *et al.* (2003) observed sharp (2 to 4 fold) increase in proline accumulation by Se application in wheat and hence improved the water status of plants facing abiotic stress. Accumulation proline due to Se application under drought stress might be the result of ROS production (Akbulut and Çakir, 2010), that reduces oxidative damage by physical quenching of singlet oxygen and hydroxyl radicals (Alia and Matysik, 2001).

5.4.2.5. Antioxidant Enzymes

Many environmental stresses such as drought (Hasanuzzaman and Fujita, 2011), heavy metals (Kumar *et al.,* 2012), UV-B (Yao *et al.,* 2010a,b), excess water (Wang, 2011), salt (Hasanuzzaman *et al.,* 2011), cold (Chu *et al.,* 2010), high temperature (Djanaguiraman *et al.,* 2010), senescence (Hartikainen *et al.,* 2000) and desiccation (Pukacka *et al.,* 2011) can result in the accumulation of ROS in plants. The increased ROS production can be detrimental to plants, but it also acts as signals for the activation of low molecular weight substances (antioxidants), such as ascorbate (AsA), glutathione (GSH) and tocopherol, and enzymes, such as, ascorbate peroxidase (APX), superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), guaiacol peroxidase (GPOX), glutathione peroxidase (GSH-Px) and glutathione reductase (GR) (Hartikainen *et al.,* 2000; Meharg and Hartley-Whitaker, 2002; Cao *et al.,* 2004; Asada, 2006) to balance the elevated ROS levels (Mittler, 2002). CAT is believed to be involved in the removal of excessive ROS, whereas APX is considered as a signaling substance for the fine modulation of ROS (Mittler, 2002) particularly H_2O_2. The results of present study showed an increase of 7% and 17% in CAT (Fig. 4.39) and APX (Fig. 4.40) activity of wheat plants respectively under drought stress. Previous studies have reported that Se stimulated significant reactivation of these two enzymes, particularly CAT (Yao *et al.,* 2009; Djanaguiraman *et al.,* 2010; Yao *et al.,* 2010a,b; Hasanuzzaman *et al.,* 2011; Yao *et al.,* 2011; Malik *et al.,* 2012). Thus, it is reasonable to speculate that the increased activities of these enzymes may indicate excessive ROS (H_2O_2). The highest CAT activity was recorded in water stressed plants foliarly applied with Se at tillering stage,

statistically at par with Se fertigation and seed treatment (Fig. 4.39). A significant effect of Se application on CAT activity was also recorded under normal conditions (Table 4.10). These results are supported by the findings of Yao *et al.* (2009) and Habibi (2013) who observed significant increase in CAT activity of wheat and barley, respectively by exogenous Se application to prevent oxidative damage and lower level of lipid peroxidation under drought stress. Sajedi *et al.* (2011) suggested that single but not the combined use of Se or micronutrients can mitigate the detrimental effects of drought stress by affecting plant metabolism including antioxidant activity.

The application of Se as foliar spray at tillering stage significantly increased APX activity (Table 4.10) which was statistically at par with Se fertigation under limited water conditions. However in normal plants, Se seed priming, fertigation and foliar application at tillering stage were statistically related to each other (Fig. 4.40). Feng and Wei (2012) reported increased activation/levels of APX and CAT in the Se accumulator *Pteris vittata* L. by Se addition. The activation of CAT and APX enzyme has been reported in several Se treated plants subjected to diverse stresses, e.g. in rapeseed seedlings under drought (Hasanuzzaman and Fujita, 2011) and Cd-stress (Hasanuzzaman *et al.,* 2012), in wheat seedlings under cold stress (Chu *et al.,* 2009) and in barley under Se phytotoxicity (Akbulut and Çakır, 2010) that might be attributed to drought induced oxidative stress directly by the production of ROS i.e. H_2O_2 and superoxide anion O^{2-} (Romero-Puertas *et al.,* 2004; Fghire *et al.,* 2013**)** or indirectly by reduction in glutathione content (Benavides *et al.,* 2005).

The non-availability of water increased POX activity by 17% as compared to normal water supply. The increase in CAT and POX activity in water stressed plants may provide an ecological adaptation for plants under stressful environments (Yao *et al.,* 2009). Plants species with low POX activity might not adapt to stress condition due to loss of membrane permeability by lipid peroxidation (Bhardwaj *et al.,* 2009). The maximum POX activity was recorded in plants foliarly applied with Se at tillering stage under water deficit conditions. The foliar spray of Se at anthesis stage and Se fertigation increased also POX activity. The lowest activity was observed in plants where no Se was supplied (Fig. 4.38). Increase in POX activity by exogenous Se supply under drought stress is in line with the findings of Yao *et al.,* (2009) who suggested that optimal Se supply is necessary to increase antioxidant activity

in water deficit plants. Chu *et al.* (2009) recorded an increase in POX activity of Se-supplemented wheat seedlings under cold stress while Nowak *et al.,* (2004) observed decrease (>20%) in POX activity at higher doses of Se. The possible mechanisms that might be responsible for enzymes activation by the addition of appropriate doses of Se include the spontaneous dismutation of O^{2-} into H_2O_2 (Hartikainen *et al.,* 2000; Cartes *et al.,* 2010) or the direct quenching of O^{2-} and OH^- by Se compounds (Xu *et al.,* 2007). Previous studies by Filek *et al.* (2009) and Kumar *et al.* (2012) reported decrease in H_2O_2 levels by the proper doses of Se, possibly due to the reactivation of antioxidants by Se especially of H_2O_2-quenchers (e.g., GSH-Px).

5.4.3. Concentration of Nutrients

The deficiency of various macronutrients and micronutrients involved in fundamental metabolic processes disturbs the growth and productivity of plants and impairs their nutritive value. The interactions between the elements in soil and in plants have been well documented (Feroci *et al.,* 1997; Pazurkiewicz-Kocot *et al.,* 2003). Literature indicated that proper doses of Se can reduce damage caused by heavy metals (HMs) in plant growth and productivity (Belzile *et al.,* 2006; Yathavakilla and Caruso, 2007; Pedrero *et al.,* 2008; Feng *et al.,* 2009a; Zembala *et al.,* 2010; Feng *et al.,* 2011; Kumar *et al.,* 2012; Malik *et al.,* 2012) that might be attributed to the reduced uptake and translocation of HMs by Se from the roots to aboveground parts (Feng *et al.,* 2013). However, improper Se dose or its abnormal assimilation may stimulate HMs uptake as reported by Bluemlein *et al.* (2009) in *Thunbergia alata*, Fargaˇsová *et al.* (2006) in *Sinapis alba* seedlings, Cartes *et al.* (2010) in ryegrass and Landberg and Greger (1994) in wheat (*Triticum aestivum* cv. Sunny). Se regulates the uptake and redistribution of some essential elements such as Iron (Fe), Zinc (Zn), Manganese (Mn) and Copper (Cu) etc. that might be involved in reactivation of associated antioxidants to reduce the ROS levels and improve stress tolerance in plants (Feng *et al.,* 2013; Yao *et al.,* 2013). Absorption of elements such as Mn, Zn, Cu and Fe is inhibited by increasing Se levels (Fargašová *et al.,* 2006). However, in some cases, the stimulating effects of Se on Cu and Zn uptake in plants have also been observed (Arvy *et al.*, 1995). Literature to explore the effects of Se on the uptake of essential elements under adverse environments is scanty.

5.4.3.1. Accumulation of Selenium (Se)

The uptake and accumulation of Se within a narrow range is beneficial for plants (Terry 2000) and is determined by the plants ability to absorb and metabolize Se, influenced by soil pH, salinity and $CaCO_3$ content (Kabata-Pendias, 2001). The soils of arid and semi-arid regions contain particularly high concentration of Se (Spadoni *et al.,* 2007). Several previous studies confirmed an increase in shoot Se concentration by Se application in various crops/plants such as tobacco (Han *et al.,* 2013), rice (Wang *et al.,* 2013; Boldrin *et al.,* 2013), maize (Chilimba *et al.,* 2012), wild barley (Yan *et al.,* 2011) and carrot (Kápolna *et al.,* 2009). However, wheat is the most efficient Se accumulator among cereal crops such as rice, maize, barley, oats etc. (Lyons *et al.,* 2003). Actively growing tissues usually contain the largest amounts of Se (Kahakachchi *et al.,* 2004) and accumulation is higher in shoot and leaf than in root tissues (Zayed *et al.,* 1998). In the present study, exogenous Se application resulted in 77% higher accumulation of Se in shoot of water stressed wheat plants (60% FC) than normal plants (100% FC). The maximum accumulation was observed in plants fertigated with Se. The plants sprayed with Se at tillering and anthesis stages accumulated more Se in shoot while negligible amount was recorded in plants applied with no Se or exogenously treated with Se as seed priming (Fig. 4.41). Higher Se accumulation in shoot of water stressed wheat plants might be the result of increased antioxidant activity by Se in plants (Hartikainen *et al.,* 2000; Lyons *et al.,* 2009) that led to the production antioxidant enzymes such as CAT, POX and APX (also observed in present study). Xu and Hu (2004) found similar results in rice and reported an increase in the antioxidant activity with the Se foliar application. However, excessive Se application might result in higher proportion of inorganic Se and poor antioxidant capacity in leaves (Han *et al.,* 2013). Increasing Se concentration was noted to reduce POX activity in wheat by Nowak *et al.* (2004).

Soil amendment with Se is an effective and safe approach to increase the Se uptake in soils with low Se (Hartikainen, 2005) and is believed to be the most important source of Se in crops (Temmerman *et al.,* 2014). Cartes *et al.* (2005) observed increased Se concentration in ryegrass seedlings by soil Se fertilization and recorded a significant positive correlation between shoot Se concentration and glutathione peroxidase (GSH-Px) activity. More recently, Ducsay *et al.* (2009) reported significant increase in Se content in the dry matter of

roots, straw and grains of wheat by soil Se application. As soil factors such as poor aeration, low pH and low organic matter etc. decrease Se availability to plants, foliar or fertigation treatment of Se could be an effective approach to improve to plant Se concentrations. Application of Se as foliar spray results in direct transfer and accumulation of Se in seed while soil applied Se is first absorbed by the root and then transferred from the root to other organs (Yang *et al.,* 2003). The addition of Se in growth medium, significantly increased the Se content in chickpea sprouts (Zhang *et al.,* 2012), and adding Se in a hydroponic solution significantly improved the Se content in *Zea mays* (Longchamp *et al.,* 2013). The Se foliar application not only enhances the Se status of plants (Kapolna *et al.,* 2009) but also increases the respiratory potential in young plants without any visible toxic effects (Germ *et al.*, 2007).

Seeds are usually a moderate source of Se, but literature indicated that cereal and legume seeds accumulate high amounts of Se (Stadlober *et al.,* 2001; Smrkolj *et al.,* 2006b). Wheat and its products are a major source of Se intake by humans, and considered as most effective crops for Se bio-fortification (Lyons *et al.,* 2003; Hawkesford & Zhao, 2007). Increase in grain Se concentration by Se application has been reported by Wang *et al.* (2013) in rice and by Chilimba *et al.* (2012) in maize. It was evident from our study that Se foliar application at anthesis stage resulted in maximum grain Se accumulation statistically at par with grain Se obtained by Se fertigation. Se foliar application at tillering was the second best method that resulted in an increase in Se concentration in grains (Fig. 4.48). Our results are consistent with the findings of Boldrin *et al.,* (2013) who observed greater proportion of Se in rice grains by foliar application of Se as selenate. The higher grain accumulation by fertigation and foliar application might be attributed to better Se transport through xylem and phloem when applied to roots and leaves respectively (Boldrin *et al.,* 2013). A great mobility of Se in the phloem was observed by its foliar application in potato (Poggi *et al.,* 2000). Poblaciones *et al.* (2014) recorded linear relationship between total Se accumulation in grain and Se dose and found selenate more efficient than selenite fertilizers.

The Se grain concentration increased by 54% in plants grown under limited water supply (60%FC) in comparison to normal plants (100% FC) (Fig. 4.48) and confirm the findings of Poblaciones *et al.,* (2014) that the growing conditions could affect the efficiency of uptake and accumulation of Se in the grain. The Se concentration in cereal grain depends

upon the roots ability to absorb and translocate Se to aboveground plant parts (Gissel-Nielsen *et al.,* 1984). In addition, chemical form of Se and time of application of Se fertilizer also affect the Se concentration in cereals (Curtin *et al.,* 2006). Kohistan-97 accumulated 45% higher Se in grain than Pasban-90 (Fig. 4.48). Variation among genotypes for Se uptake and accumulation has been reported in plants such as tomato (Pezzaroas *et al.,* 1999), barley (Yan *et al.,* 2011) and soybean (Yang *et al.,* 2003) that might be a valuable genetic resource for breeding programs (Özdemir, 2008).

5.4.3.2. Accumulation of Iron (Fe)

Iron (Fe) serves as a co-factor for various enzymes such as Superoxide Dismutase (SOD), CAT, POX and enzymes involved in the chlorophyll biosynthesis (Kabata-Pendias and Pendias, 1999). The results of the present study indicated that water stressed plants grown at 60% FC accumulated 35% less Fe in shoot and 43% in grain as compared to normal plants (100% FC). The highest shoot Fe content was recorded in plants applied with no Se while different Se application methods viz. Se fertigation, Se seed treatment, Se foliar application at tillering stage and Se foliar application at anthesis stage significantly reduced shoot Fe accumulation by 43%, 38%, 19% and 16% respectively (Fig. 4.42). The grain Fe concentration was significantly improved only in plants foliarly applied with Se at anthesis stage while other Se treatments had similar Fe contents (Fig. 4.49). These results are in accordance with the findings of Sajedi *et al.* (2009) who reported negative antagonistic interaction among Se and Fe that significantly decreased yield attributes in maize under water deficit conditions. Similarly, Zembala *et al.* (2010) observed decrease in Fe concentration by Se fertilization of rape plant (*Brassica napus*) and wheat (*Triticum* spp.) In contrast, Wang *et al.,* (2013) observed non-significant effect of Se soil and foliar application on maize grain content while Yao *et al.* (2013) found that Fe contents were significantly increased by supplemental Se supply in wheat plants exposed to ambient UV-B radiation. Se regulates Fe accumulation that might be attributed to dual effect of Se (Feng *et al.*, 2009b). Lower doses of Se reduce Fe uptake while it is significantly enhanced at higher Se doses as reported in previous studies by Feng *et al.*, (2009b) and Feng and Wei (2012) in *Pteris vittata* L. and He *et al.* (2004) in Chinese cabbage and lettuce.

5.4.3.3. Accumulation of Zinc (Zn)

Zinc (Zn) is an essential trace element that plays an important role in metabolism regulation of saccharides, nucleic acid and lipid metabolism. The Zn deficiency leads to an inhibition of cell growth and proliferation while toxicity negatively affects photosynthetic electron transport and photophosphorylation (Kabata-Pendias and Pendias, 1999). In present study, an overall increase of 48% in Zn shoot concentration of water stressed plants in comparison to normal plants was recorded. The Se induced increase in Zn in shoot is in accordance with the findings of Pazurkiewicz-Kocot *et al.* (2008) who reported significant increase in Zn concentration in leaves of corn plants grown in Se solutions. Sajedi *et al.* (2009) observed significant increase in yield and water use efficiency of water stressed maize plants by Se foliar spray in combination with other micronutrients including Zn which suggests positive interaction between Se and Zn. The grain Zn concentration decreased by 24% under water deficit conditions with respect to normal plants. Supplemental Se supply had negative effect on shoot Zn accumulation (Fig. 4.43), whereas grain Zn concentration increased by exogenous Se supply (Fig. 4.50). An evident increase in Zn grain concentration by Se supply has been reported in wheat (Yao *et al.,* 2013) and rape plants (*Brassica napus*) (Zembala *et al.,* 2010). However, Wang *et al.* (2013) observed non-significant increase in Zn grain concentration by soil and foliar fertilization of Se in maize plants.

5.4.3.4. Accumulation of Phosphorous (P)

The competitive relationships among ions during their uptake by a plant exist which affect the Se uptake (Ježek *et al.,* 2012). The relative concentration of phosphate (PO_4) and sulphate (SO_4) ions affect absorption of Se in tropical soils (Goh and Lim, 2004). The Se becomes more active after phosphorous application in soil which inhibits Se absorption. It was evident from results that exogenous Se supply showed antagonistic effect on phosphorous (P) uptake in wheat plants and inhibited its accumulation by 34% (Se fertigation), 26% (Se foliar spray at anthesis stage), 25% (Se seed priming) and 23% (foliar spray of Se at tillering stage) (Fig. 4.44). The competitive inhibition of Se in P absorption has been documented (Hopper and Parker, 1999; Li *et al.,* 2008). Increased Se content in plant tissues may decrease N, P and S content, as well as inhibit the absorption of some heavy metals, especially Mn, Zn, Cu, Fe and Cd (Ježek, *et al.,* 2012). Contradictory studies

however stated that N, P and S application can decrease Se uptake (Kabata-Pendias and Pendias, 1999). High content of available P reduces the Se availability for plants (Nakamaru *et al.,* 2008). But, Kopsell *et al.* (2007) recorded higher concentration of Se in alfalfa (*Medicago sativa* L.) by P application.

The data regarding grain P concentration showed 33% higher accumulation of P in grain of normal plants than water stressed plants. The plants grown under normal water supply maintained the highest P contents. The Se fertigation and foliar application at tillering stage caused significantly higher reduction in comparison to Se seed priming and Se foliar application at anthesis stage (Fig. 4.51). These results are in line with the findings of Boldrin *et al.* (2013) who observed that Se supplementation decreased P content of rice grains and found greater reduction in P grain accumulation by Se foliar application than application of Se in soil. This may be due to specific adsorption of Se onto Fe and aluminum (Al) oxides which reduces its availability in the soil (Boldrin *et al.,* 2013). Contradictory results showed that soil and foliar application of Se did not significantly increase P concentration in maize grains (Wang *et al.,* 2013).

5.4.3.5. Accumulation of Potassium (K)

Plants utilize K^+, Na^+ and Ca^{2+} for the regulation of turgor and cell membrane potential (Weyers and Paterson, 2001). The results of present study revealed that the plants exposed to drought stress accumulated 11% more K in shoot than normal plants (Fig. 4.45). Studies have shown an increase in K concentration in different crop species such as wheat (Ashraf *et al.,* 1998), maize (Premachandra *et al.,* 1991), sunflower (Iqbal *et al.,* 2008) and pigeon pea (Ashraf, 1994) under water deficit conditions. Plants sprayed with Se at tillering stage had significantly higher concentration of K than that of control (no Se supply). The Se fertigation also enhanced shoot K accumulation while non-significant difference was recorded between Se seed treatment and Se foliar application at anthesis stage (Fig. 4.45). However, drought stress did not significantly affect K accumulation in wheat grains (Table 4.14). Similarly, significant increase (9%) in K grain concentration was only observed by Se fertigation and Se foliar application at anthesis stage while non-significant difference was observed among other Se treatments viz. no Se supply, Se seed treatment and Se foliar application at tillering stage (Fig. 4.52). These results confirm the findings of Yao *et al.,*

(2013) and Pazurkiewicz-Kocot *et al.* (2003) who reported an increase in grain K concentration by supplemental Se in wheat and maize, respectively and were of the view that change in nutrients transport may be the first symptom of Se effects on plants. However, non-significant effect of Se foliar application was observed on K accumulation in maize grains by Wang *et al.* (2013).

5.4.3.6. Accumulation of Magnesium (Mg) and Calcium (Ca)

Magnesium (Mg) plays an essential role in the organization of protoplasmic structures in cells, plant pigment synthesis and activates many enzymes of photosynthesis and respiration systems. Magnesium ions (Mg) neutralize organic and inorganic ions and activate ribulose-1, 5-bisphosphatecarboxylase (RuBPC) in plants (Kabata-Pendias and Pendias, 1999). The exposure to drought stress limited Mg and Ca uptake in plants and reduced it by 15% and 32% respectively (Fig. 4.46; 4.47). Among different Se application methods, Se fertigation resulted in maximum accumulation of Mg in shoot and had non-significant difference with the Se foliar application at tillering stage (Fig. 4.46), while only plants foliar applied with Se at tillering stage showed significantly higher shoot Ca content and statistically at par with other treatments viz. control (no Se application), Se seed priming, Se fertigation and Se foliar application at anthesis stage (Fig. 4.46). Pazurkiewicz-Kocot *et al.* (2003) also observed an increase in Ca concentration by Se application and found higher Ca accumulation in roots and mesocotyls than the leaves of maize plants. In another study, Pazurkiewicz-Kocot *et al.* (2008) reported higher Mg content only in roots of Se treated plants while a decrease in Mg accumulation was observed in leaves of maize plants by Se application. In the present study, water stressed plants accumulated 11% less Mg in grains as compared to normal plants (Fig. 4.53). The application of Se as foliar treatment at tillering stage resulted in maximum accumulation of Mg and had non-significant difference to the plants grown under Se fertigation and Se foliar application at anthesis stage (Fig. 4.53). Contradictory results were reported by Wang *et al.* (2013) in maize who noted non-significant increase in grain Ca and Mg contents in plants treated Se as soil amendment and foliar application. The primary influence of Se on accumulation of ions in plant cells might be attributed to its interaction with the plasmalemma and metabolic cell processes. Se ions

may affect other ions transport in plant by changing the permeability coefficient of plasmatic membranes for some ions (Pazurkiewicz-Kocot *et al.,* 2003).

5.5. Yield and Yield Components

Reports regarding the effect of Se application on plants growth and yield are not consistent. It has been reported that application of Se increases the growth and yield in rice (Wang *et al.,* 2013), potato (Yassen *et al.,* 2011), lettuce seedlings (Xue *et al.,* 2001) and soybean (Djanaguiraman, 2005) etc. However, Yang *et al.* (2003) recorded non-significant effect of Se application on growth and yield of soybean, whereas a decrease in growth of potato was noted by Germ *et al,* (2007). In the present study, a significant increase in almost all yield and yield components of wheat was noted by Se application in both lysimeter and field experiments. The application of Se as foliar treatment resulted in higher grain yield as compared to other Se application methods and complies with the findings of Curtin *et al.* (2006) who found that foliar application of Se was more effective than soil fertilization in increasing the growth and yield of wheat. Likewise, Yao *et al.* (2013) recorded significant increase in spike length, weight per spike and grain yield of wheat by Se foliar application. Increase in grain yield under drought stress might be attributed to Se stimulated increase in water relations, pigments, and TSS, TFA, proline and antioxidant activity (as observed in present study). Soil texture its physic-chemical characteristics, method and time of Se application influence its relative effectiveness (Lyons *et al.,* 2003). The foliar application of Se results in the diffusion of Se ions from the surface of leaves to epidermal cells which increases its effectiveness in improving plant growth and yield (Wójcik, 2004), but high Se concentration can cause damage to leaf surface (Marschner, 1995) resulting in the reduction of metabolic activities, growth and plant productivity. Therefore, care must be taken regarding concentration of solution for Se foliar application.

Chapter-6 SUMMARY

The present study included various experiments conducted in four phases to investigate the i) effect of water stress on various physiological and biochemical attributes of wheat, ii) effect of exogenously applied selenium (Se) on growth, yield, physiological and biochemical traits of wheat grown under drought conditions, iii) appropriate rate, method and time of Se application for improving drought tolerance in wheat plants.

In the first phase (laboratory experiments), fifteen local wheat genotypes viz. Pak-81, Chakwal-86, Kohistan-97, Inqlab-91, Manthar-03, Chakwal-50, Fsd-08, Sehar-06, Pasban-90, Shafaq-06, V_0-5082, V_0-5066, V_0-4178, Ufaq-06 and Lasani-08 were evaluated for their drought tolerance potential under PEG-6000 induced water stress of -0.5 MPa at germination and seedling stage. Different physiological indices were used as screening tool. The results revealed significant variation among genotypes and on the basis of performance under water stress, genotypes were divided into three groups, i.e. tolerant, medium tolerant and sensitive ones. The genotype Kohistan-97 was observed as the most drought tolerant while Manthar-03, Chakwal-86, Pak-81 and Chakwal-50 also performed better under water deficient conditions and were characterized as medium tolerant, whereas the genotype Pasban-90 was ranked as the most sensitive genotype under water deficit conditions.

In the second phase of the study, three pot experiments were conducted to determine the appropriate rate and method (seed priming, fertigation and foliar) of Se application helpful in improving the drought tolerance potential in wheat plants subjected to water stress at seedling stage. The experiments were conducted under wire/greenhouse conditions. One drought tolerant and one sensitive genotype selected from laboratory experiments were used for these experiments. In seed priming experiment, the seeds were soaked in 0, 25, 50, 75 and 100 µM sodium selenate (Na_2SeO_4) solutions for 0.5 h and 1 h at 25 °C and later re-dried to original moisture level. The results indicated wheat genotype Kohistan-97 more responsive to Se supply than Pasban-90. Seed priming with lower concentrations of Se were not effective in improving the growth of seedlings under limited water conditions. The optimum level and duration of Se seed priming which enhanced drought tolerance potential in wheat seedlings were 75 µM Se and one hour, respectively. The data regarding fertigation

of wheat seedlings at different rates of Se supply (0, 3.68, 7.35, 11.03, 14.70 µM Se) showed that only 7.35 µM was effective in improving the physiological indices, whereas Se foliar application @ 7.06 µM maintained the highest values for PHSI, RLSI and DMSI thus improved the stress tolerance potential of wheat seedlings. The Se levels proved the best in these experiments were further tested in lysimetric and field experiments.

A lysimeter experiment was conducted during the third phase of this study. The Se levels proved optimum for priming, fertigation and foliar spray and the same wheat genotypes as used in wire house experiments were used to select the appropriate method and time of Se application. Different growth, physiological, biochemical and yield parameters were recorded for this experiment. The exposure to drought stress significantly reduced water relations, gas exchange and yield components of both wheat genotypes (Kohistan-97 and Pasban-90) whereas the accumulation of osmolytes and activity of antioxidant enzymes was significantly enhanced under water deficit conditions. The severe effect of drought stress was recorded at anthesis than tillering stage. The exogenous Se supply significantly improved Se and potassium (K) concentration in shoot and grain whereas phosphorous (P), magnesium (Mg), zinc (Zn), iron (Fe) and calcium (Ca) contents in shoot were reduced by Se supply. The grain Mg and Fe concentration increased while grain P concentrations reduced by exogenous Se supply. Non-significant effect of Se supply was recorded on grain Zn concentration. Different methods of exogenous Se supply varied significantly for improving stress tolerance potential of wheat genotypes. However, Se fertigation and Se foliar spray at tillering stage were found effective methods in mitigating the adverse effects of drought stress. A significant effect of Se seed priming and Se foliar spray at anthesis stage was also observed as compared to control (no Se supply).

Two field experiments were conducted during two consecutive years (2011-12 and 2012-13) in the fourth phase of study to estimate the effect of exogenous Se supply on yield and yield components of wheat genotypes exposed to water deficit conditions. A significant reduction in all yield attributes was noted in water stressed plants during both the years. The most effective method for Se application was Se foliar spray at tillering stage and exhibited higher values for grain yield and yield components, whereas other Se supply methods viz. Se seed priming, Se fertigation and Se foliar spray at anthesis stage were comparatively less effective.

Conclusion

- Drought stress significantly affected various growth, physiological, biochemical and yield components of both drought tolerant (Kohistan-97) and sensitive (Pasban-90) wheat genotypes. However, genotype Kohistan-97 was more successful in the maintenance of these attributes than Pasban-90, therefore, the cultivation of drought tolerant wheat varieties is suggested to obtain economical crop yield under water deficit conditions.
- The fertigation (7.35 μM) and foliar spray of Se (7.06 μM) at tillering stage was more effective than other methods (Se seed priming and Se foliar spray at anthesis stage) in alleviation of drastic effects of drought stress, however, Se may become toxic at higher levels so concentration of Se must be chosen with care.
- The exogenous Se supply significantly increased Se and K accumulation in shoot and grains, whereas it reduced Mg, Zn, P and Fe shoot concentration.

Future Prospects

- In the present study, Se was applied as fertigation treatment only at vegetative stage and all chemical and physiological parameters have been estimated after the treatment. The Se fertigation at flowering stages is suggested to understand the physiological and biochemical mechanism in a better way.
- The study of other antioxidant enzymes like glutathione peroxidase after exogenous Se supply can explain the action of Se regarding antioxidant synthesis or their involvement in stress tolerance.
- Detailed study of proteins profile is suggested to work out the synthesis of stress induced proteins.
- In the present study. limited osmoprotectants have been studied. Studies on other osmoprotectants like glycinebetaine, trehalose, organic acids etc. are suggested to understand osmotic adjustment in wheat under water deficit conditions.

Literature Cited

Abbas, S.M. 2012. Effects of low temperature and selenium application on growth and the physiological changes in sorghum seedlings. J. Stress Physiol. Biochem. 8(1):268-286.

Abdalla, M.M. 2011. Beneficial effects of diatomite on the growth, the biochemical contents and polymorphic DNA in *Lupinus albus* plants grown under water stress. Agric. Biol. J. N. Am. 2(2):207-220.

Abdel-Aal, S.M., J.C. Young, I. Rabalski, P. Hucl and J. Fregeau-Reid. 2007. Identification and quantification of seed carotenoids in selected wheat species. J. Agric. Food Chem. 55:787-794.

Abdelmalek, C. and T. Khaled. 2011. Physiological behavior of wheat genotypes from algerian semi-arid regions grown under salt stress. African J. Agri. Res. 5:636-641.

Afzal, M., S. Nasim and S. Ahmad. 2004. Operational manual seed preservation laboratory and gene bank. Plant Genetic Resources Institute, Islamabad.

Ahmad, S., R. Ahmad, M.Y. Ashraf, M. Ashraf, E.A. Waraich. 2009. Sunflower (*Helianthus annuus* L.) response to drought stress at germination and seedling growth stages. Pak. J. Bot., 41: 647-654.

Ahmad, M., F. Mohammed, K. Maqbool, A. Azamand and S. Iqbal. 2003. Genetic variability and traits correlation in wheat. Sarhad J. Agri. 19(3):347-351.

Ahmadi, A. and D.A. Baker. 2001. The effect of water stress on grain filling processes in wheat. J. Agric. Sci. 136:257–269.

Ahmed, S., E. Nawata, M. Hosokawa, Y. Domae and T. Sakuratani. 2002. Alterations in photosynthesis and some antioxidant enzymatic activities of mungbean subjected to waterlogging. Plant Sci. 163:117–123.

Akbulut, M. and S. Çakır. 2010. The effects of Se phytotoxicity on the antioxidant systems of leaf tissues in barley (*Hordeum vulgare* L.) seedlings. Plant Physiol. Biochem. 48:160-166.

Akladious, S. A. 2012. Influence of different soaking times with selenium on growth, metabolic activities of wheat seedlings under low temperature stress. African J. Biotech. 11(82):14792-14804.

Akram, H.M., N. Ahmed, A. Ali and A. Yar. 1998. Effect of stress on germination and seedling growth of wheat cultivars. J. Agric. Res. 36(3):217-222.

Akram, H.M., M.S. Iqbal, M. Saeed, A. Yar, A. Ali, K.A. Sahi and M.A. Nadeem. 2004. Drought tolerance studies of wheat genotypes. Pak. J. Biol. Sci. 7(1):90-92.

Alaei, Y. 2011. The effect of amino acids on leaf chlorophyll content in bread wheat genotypes under drought stress conditions. Middle-East J. Sci. Res. 10(1):99-101.

Alexieva, V., I. Sergiev, S. Mapelli, E. Karanov. 2001. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ. 24:1337–1344.

Alia, P.M. and J. Matysik. 2001. Effect of proline on the production of singlet oxygen. Amino Acids. 21:195–200.

Almansouri, M., J.M. Kinet and S. Lutts. 1999. Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). Plant Soil. 231:243-254.

Almeselmani, M. , A.A. Saud, K. Al-zubi, F. Hareri, M. Al-nassan ,M. A. Ammar, O.Z. Kanbar , H. Al-Naseef, A. Al-nator, A. Al-gazawy and H. Abu Al-sael. 2012. Physiological attributes associated to water deficit tolerance of syrian durum wheat varieties. Exp. Agri. Hort. Article.

Amiri, F.R. and M.T. Assad. 2005. Evaluation of three physiological traits for selecting drought resistant wheat genotypes. J. Agric. Sci. Technol. 7:81-87.

Apel, K. and H. Hirt. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol. 55:373–399.

Arjenaki, G.F., R. Jabbari and A. Morshedi. 2012. Evaluation of drought stress on relative water content, chlorophyll content and mineral elements of wheat (*Triticum aestivum* L.) varieties. Int. J. Agri. Crop Sci. 4(11):726-729.

Arner, E.S.J and A. Holmgren. 2000. Physiological functions of thioredoxin and thioredoxin reductase. FEBS J. 267(20):6102-6109.

Arnon, D.T. 1949. Copper enzymes in isolated chloroplasts polyphenol oxidase in *Beta vulgaris*. Plant Physiol. 24:1-15.

Arvy, M.P. 1993. Selenate and selenite uptake and translocation in bean plants (*Phaseolus vulgaris*). J. Exp. Bot. 44:1083–1087.

Asada, K. 2006. Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol. 141(2):391–396.

Asch, F., M. Dingkuhn, A. Sow and A. Audebert. 2005. Drought-induced changes in rooting patterns and assimilate partitioning between root and shoot in upland rice. Field Crop Res. 93:223–236.

Ashraf, M. and M.R. Foolad. 2005. Pre-sowing seed treatment—a shotgun approach to improve germination, plant growth and crop yield under saline and non-saline conditions. Adv. Agron. 88:223–271.

Ashraf, M. and P. K. Harris. 2004. Potential biochemical indicator of salinity tolerance in plants. Plant Sci. 166: 3-16.

Ashraf, M., S.H. Nawazish, and H. Athar. 2007. Are chlorophyll fluorescence and photosynthetic capacity potential physiological determinants of drought tolerance in maize (*Zea mays* L.). Pak. J. Bot. 39:1123–1131.

Ashraf, M.Y. 1998. Yield and yield components response of wheat (*Triticum aestivum* L.) genotypes grown under different soil water deficit conditions. Acta Agron. Hung. 46:45–51.

Ashraf, C.M. and S. Abu-Shakra. 1978. Wheat seed germination under low temperature and moisture stress. Agron. J. 70:135-139.

Ashraf, M. and J.W. O'Leary. 1996. Effect of drought stress on growth, water relations and gas exchange of two lines of sunflower differing in degree of salt tolerance. Int. J. Plant Sci., 157:729-732.

Ashraf, M. and S. Mehmood. 1990. Response of four Brassica species to drought stress. Environ. Expt. Bot. 30:93-100.

Ashraf, M. Y., S. A. Ala and A. Saeed Bhatti. 1998. Nutritional imbalance in wheat (*Triticum aestivum* L.) genotypes grown at soil water stress. Acta Physiol. Plant. 20:307-310.

Ashraf, M.Y. and A.H. Khan. 1993. Effect of drought stress on wheat plant in early stages. Pak. J. Agri. Res. 14(4):261-269.

Ashraf, M.Y., A. H. Khan and A. R. Azmi. 1992. Cell membrane stability and its relation with some physiological processes in wheat. Acta Agron. Hung. 41: 183-191.

Ashraf, M.Y. and S.S.M. Naqvi. 1995. Studies on water uptake, germination and seedling growth of wheat genotypes under PEG-6000 induced water stress. Pak.. J. Sci. Ind. Res. 38:130-133.

Ashraf, M.Y., A.R. Azmi, A.H. Khan and S.S.M. Naqvi. 1994. Water relations in different wheat (*Triticum aestivum* L.) genotypes under soil water deficits. Acta Physiol. Plant. 16: 231-240.

Ashraf, M.Y., F. Hussain, J. Akhtar, A. Gul, M. Ross and G. Ebert. 2008. Effect of different sources and rates of nitrogen and supra optimal level of potassium fertilization on growth, yield and nutrient. Pak. J. Bot. 40(4):1521-1531.

Aspinall, D. and L.G. Paleg. 1982. Proline accumulation physiological aspects. In: Physiology and Biochemistry of Drought Resistance in Plants. Paleg, L. G. and D. Aspinall (Eds.), Academic Press Sydney. pp. 205-241.

Association of Official Seed Analysis (AOSA). 1983. Seed Vigor Testing Handbook. Contribution No. 32 to the handbook on Seed Testing.

Austin, R.B. 1993. Augmentation yield-based selection. In: Plant Breeding Principles and Prospects (Eds. MD Hayward, NO Bosemark, I Romagosa). Chapman and Hall, London, pp. 391-405.

Azpilicueta, C.E., M.P. Benavides, M.L. Tomaro and S.M. Gallego. 2007. Mechanism of CATA3 induction by Cd in sunflower leaves. Plant Physiol. Biochem. 45: 589–595.

Azevedo R.A., R.M. Alas, R.J. Smith and P.J. Lea. 1998. Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation in the leaves and roots of wild-type and a catalase-deficient mutant of barley. Physiol. Plant. 104:280–292.

Bacelar, E.A., D.L. Santos, J.M. Moutinho-Pereira, B.C. Gonçalves, H.F. Ferreira and C.M. Correia. 2006. Immediate responses and adaptative strategies of three olive cultivars under contrasting water availability regimes: changes on structure and chemical composition of foliage and oxidative damage. Plant Sci. 170:596–605.

Bai, B.Z., J.Z. Jin, S. Bai and L.P. Huang. 1994. Improvement of TTC method determining root activity in corn. (In Chinese, with English abstract). Maize Sci. 2:44–47.

Bajji, M., S. Lutts and J. M. Kinnet. 2001. Water deficit effects on solute contribution to osmotic adjustment as a function of leaf ageing in three durum wheat (Triticum durum Desf) cultivars performing differently in arid conditions. Plant Sci. 160: 669-681.

Baker N.R. 1993. Light-use efficiency and photoinhibition of photosynthesis in plants under environmental stress. p. 221-235. *In* Smith J.A.C., Griffiths H. (ed.), Water Deficits: Plants Responses from Cell to Community, Bios Scientific Publishers, Oxford,UK.

Banon, S.J., J. Ochoa, J.A. Franco, J.J. Alarcon and M.J. Sanchez-Blanco. 2006. Hardening of oleander seedlings by deficit irrigation and low air humidity. Environ. Exp. Bot. 56:36-43.

Basal, H., C.W. Smith, P.M. Thakston and J.K. Hemphill. 2005. Seedling drought tolerance in upland cotton. Crop Sci. 45:766-771.

Bates, L.S., R.P. Waldron and I.W. Teaxe. 1973. Rapid determination of free proline for water stress studies. Plant Soil. 39:205-207.

Bayoumi, T.Y., M.H. Eid and E.M. Metwali. 2008. Application of physiological and biochemical indices as a screening technique for drought tolerance in wheat genotypes. African J. Biotech. 7(14): 2341-2352.

Belzile, N., G.J. Wu, Y.M. Chen and V.D. Appanna. 2006. Detoxification of selenite and mercury by reduction and mutual protection in the assimilation of both elements by Pseudomonas fluorescence. Sci. Total Environ. 367(2-3):704–714.

Ben Ahmed, C., B.B. Rouinab, S. Sensoyc, M. Boukhrisa and F.B. Abdallah. 2009. Changes in gas exchange, proline accumulation and antioxidative enzyme activities in three olive cultivars under contrasting water availability regimes. Environ. Exp. Bot. 67:345–352.

Benavides, M.P., S.M. Gallego and M. Tomaro. 2005. Cadmium toxicity in plants. Braz. J. Plant Physiol. 17:21–34.

Bhardwaj, P., A.K. Chaturvedi and P. Prasad. 2009. Effect of Enhanced Lead and Cadmium in soil on Physiological and Biochemical attributes of *Phaseolus vulgaris* L. Nature Sci. 7(8):63-75.

Bhutta, W.M. 2006. Role of some agronomic traits for grain yield production in wheat (*Triticum aestivum* L.) genotypes under drought conditions. Revista UDO Agricola, 6: 11-19.

Bittman, S., W.T. Buckley, K. Zaychuk, and E.A.P. Brown. 2000. Seed coating for enhancing the level of selenium in crops. USA Patent No. 6,058, 649.

Bluemlein, K., E. Klimm, A. Raab and J. Feldmann. 2009. Selenite enhances arsenate toxicity in *Thunbergia alata*. Environ. Chem. 6(6):486–494.

Blum, A. 1998. Improving wheat grain filling under stress by stem reserve mobilization. Euphytica. 100:77–83. doi: 10.1023/ A:1018303922482.

Blum, A., J. Zhang and H.T. Nguyen. 1999. Consistent differences among wheat cultivars in osmotic adjustment and their relationship to plant production. Field Crop Res. 64:287-291.

Blum, A. and C.Y. Sullivan. 1986. The comparative drought resistance of land races of sorghum and millet from dry and humid regions. Anna. Bot. 57:835-846.

Bogale, A., K. Tesfaye and T. Geleto. 2011. Morphological and physiological attributes associated to drought tolerance of Ethiopian durum wheat genotypes under water deficit condition. J. Biodiv. Environ. Sci. (JBES). 1(2):22-36.

Bohnert, H.J. and B. Shen. 1999. Transformation and compatible solutes. Sci. Hortic. 78: 237–260.

Bohnert, H.J., D.E. Nelson and R.G. Jensen. 1995. Adaptation to environmental stresses. Plant Cell 7: 1099–1111

Bojović, B. and J. Stojanović. 2005. Chlorophyll and carotenoid content in wheat cultivars as a function of mineral nutrition. Arch. Biol. Sci. Belgrade. 57(4):283-290.

Boldrin, P.F., V. Faquin, S.J. Ramos, K.V.F. Boldrin, F.W. A´vila and L.R.G. Guilherme. 2013. Soil and foliar application of selenium in rice biofortification. J. Food Compos. Anal. 31:238–244.

Borlaug, N.E. 1981. Using plants to meet World food needs in Future Dimensions of World Food and Population p. 101–82. (ed. R.G. Woods) Westview Press, Boulder, Co.

Bouslama, M. and W.T. Schapaugh. 1984. Stress tolerance in soybeans. 1. Evaluation of three screening techniques for heat and drought tolerance. Crop Sci. 24:933-937.

Bray, E., J. Bailey-Serres and E. Weretilnyk. 2000. Responses to abiotic stresses. p. 1158-1203. *In* Buchanan BB, Gruissem W, Jones RL (ed.), Biochemistry & molecular biology of plants. Am. Soc. Plant Biol. Rockville, MD, USA.

Broadley, M.R., P.J. White, J.P. Hammond, I. Zelko and A. Lux. 2007. Zinc in plants. New Phytol. 173:677–702.

Brock, M.A. 1981. Accumulation of proline in a submerged aquatic halophyte, *Ruppia* L. Oecologia 51:217–219.

Brooks, A., C.F. Jenner and D. Aspinall. 1982. Effect of water deficit on endosperm starch granule and on grain physiology of wheat and barley. Aust. J. Plant physiol. 9(4):423-436 (Soils and fertilizers 46(5):4561;1983).

Brouk, B. 1975. Plants consumed by man. Academic Press, London, 479p

Brown, T.A. and A. Shrift. 1981. Exclusion of Selenium from Proteins of Selenium-Tolerant Astragalus Species. Plant Physiol. 67(5):1051.

Brown, K.M.and J.R. Arthur. 2007. Selenium, selenoproteins and human health: a review. Public Health Nutrition, 4(2b):593-599.

Cakmak, I. 1994. Activity of ascorbate-dependent H_2O_2 -scavenging enzymes and leaf chlorosis are enhanced in magnesium- and potassium-deficient leaves, but not in phosphorus-deficient leaves. J. Exp. Bot. 45(278):1259-1266.

Cao, X.D., L.Q. Ma and C. Tu. 2004. Antioxidative responses to arsenic in the arsenic hyper accumulator Chinese brake fern (*Pteris vittata* L.). Environ Poll. 128(3):317–325.

Carlson, C.L., D.I. Kaplan and D.C. Adriano. 1989. Effects of selenium on germination and radicle emergence of selected agronomic species. Environ. Exp. Bot. 29:493–498.

Carpenter, J. F. and J. H. Growe. 1988. The mechanism of cryoprotection of proteins by solutes. Cryobiology. 25: 244-255.

Cartes, P., L. Gianfreda and M.L. Mora. 2005. Uptake of selenium and its antioxidant activity in ryegrass when applied as selenate and selenite forms. Plant and Soil. 276(1):359-367.

Cartes, P., A.A. Jara, L. Pinilla, A. Rosas and M.L. Mora. 2010. Selenium improvesthe antioxidant ability against aluminium-induced oxidative stress in ryegrass roots. Ann. Appl. Bio. 156:297–307.

Cary, E.E. and W.H. Allaway. 1969. The stability of different forms of selenium applied to low-selenium soils. Soil Sci. Soc. Am. Proc. 33:571.

Cechin, I., N. Corniani, D.F.F. Terezinha and A.C. Cataneo. 2008. Ultraviolet-B and water stress effects on growth, gas exchange and oxidative stress in sunflower plants. Rad. Environ. Biophy. 47(3):405-413.

Chaitante, C., A. Iorio Di, L. Maiuro and S.G. Scippa. 2000. Effect of water stress on root meristems in woody and herbaceous plants during the first stage of development. Form Function Physiol. 245-258.

Chance, B. and A.C. Maehly. 1955. Assay of catalase and peroxidase. Methods Enzymol. 2:764-775.

Chaves, M.M. 1991. Effects of water deficits on carbon assimilation. J. Exp. Bot. 42:1–16.

Chaves, M.M. and M.M. Oliveira. 2004. Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. J. Exp. Bot. 55:2365-2384.

Chaves, M.M., J.S. Pereira, J. Maroco, M.L. Rodrigues, C.P.P. Ricardo, M.L. Oso´rio, I. Carvalho, T. Faria and C. Pinheiro. 2002. How plants cope with water stress in the field? Photosynth. growth. Ann. Bot. 89:907–916.

Chen, C.C. and J.M. Sung. 2001. Priming bitter gourd seeds with selenium solution enhances germinability and antioxidative responses under sub optimal temperature. Physiol. Plant. 111:9–16.

Chen, H.C.H. and G.J.J. Jiang. 2010. Osmotic adjustment and plant adaptation to environmental changes related to drought and salinity. Environ. Rev. 18:309-319.

Chilimba, A.D.C., S.D. Young, C.R. Black, M.C. Meacham, J. Lammel, M.R. Broadley. 2012. Agronomic biofortification of maize with selenium (Se) in Malawi. Field Crops Res. 125:118–128.

Chu, J.Z., X.Q. Yao and Z.N. Zhang. 2009. Responses of wheat seedlings to exogenous selenium supply under cold stress. Biol. Trace Elem. Res. 136(3):355–363.

Claussen, W. 2005. Proline as a measure of stress in tomato plants. Plant Sci. 168: 241-248.

Clifford, S. C., S. K. Arndt, J. E. Corlett, S. Joshi, N. Sankhla, M. Popp and H.G. Jones. 1998. The role of solute accumulation, osmotic adjustment and changes in cell wall elasticity in drought tolerance in *Ziziphus mauritiana* (Lamk). J. Exp. Bot. 49: 967-977.

Close, T.J. 1996. Dehydrins: emergence of a biochemical role of a family of plant dehydration proteins. Physiol. Plant. 97: 795-803.

Combs, G.F. 2001. An analysis of cancer prevention by selenium. BioFactors. 14(1):153-159.

Cornic, G. and A. Massacci. 1996. Leaf photosynthesis under stress. *In* Baker RN (ed.). Photosynthesis and the Environment. The Netherlands: Kluwer Academic Publishers.

Cornic, G. 1994. Drought stress and high light effects on leaf photosynthesis. p. 297-313. *In* Baker N.R., Boyer J.R. (ed.), Photoinhibition on Photosynthesis from Molecular Mechanisms to the Field,. Bios Scientific Publishers. Oxford, UK.

Cornic, G. 2000. Drought stress inhibits photosynthesis by decreasing stomatal closure- not by affecting ATP synthesis. Trends Plant Sci. 5:187-188.

Curtin, D., R. Hanson, T.N. Lindley and R.C. Butler. 2006. Selenium concentration in wheat (*Triticum aestivum*) grain as influenced by method, rate, and timing of sodium selenate application. NZL. J. Crop Hortic. Sci. 34:329-339.

Cuvin-Aralar, M.L. and R.W. Furness. 1991. Mercury and selenium interaction: a review. Ecotoxicol. Environ. Saf. 21(3):348-64.

Davies, B. 1976. Carotenoids. p. 38-165. *In* Chemistry and biochemistry of plant pigments. (ed.) T.W. Goodwin. Academic Press, London, UK.

Delauney, A.J. and D.P.S. Verma. 1993. Proline biosynthesis and osmoregulation in plants. The Plant J. 4(2):215-223.

Dell'Aquila, A. and G. Taranto. 1986. Cell division and DNA synthesis during osmopriming treatment and following germination in aged wheat embryos. Seed Sci. Technol. 14:333–341.

Dewis, J. and F. Freitas. 1970. Physical methods of soil and water analysis. FAO Soil Bull. 10: 39-51.

Dhanda, S.S., G.S. Sethi and R.K. Behl. 2004. Indices of drought tolerance in wheat genotypes at early stages of plant growth. J. Agron. Crop Sci. 190:1-6.

Dhillon, K.S. and S.K. Dhillon. 2003. Distribution and management of seleniferous soils. Adv. Agron. 79:119–184.

Dix, P. J. and R. S. Pearce. 1981. Proline accumulation in NaCl resistance and sensitive cell lines of Nictoiana sylvestris. Z. Pflanzenphysiol. 102: 243-248.

Dixit, V., V. Pandey and R. Shyam. 2001. Differential antioxidative response to cadmium in roots and leaves of pea. J. Exp. Bot. 52:1101-1109.

Djanaguiraman M., D. A.K. Devi, A. Shanker, J. Annie Sheeba and U. Bangarusamy. 2004. Impact of selenium spray on monocarpic senescence of soybean (*Glycine max* L.). Food Agri. Environ. 2(2):44-47.

Djanaguiraman, M., A.K. Devi, A. Shanker, J.A. Sheeba and U. Bangarusamy. 2005. Selenium- an antioxidative protectant in soybean during senescence. Plant Soil. 272:77 -86.

Djanaguiraman, M., P.V.V. Prasad and M. Seppänen. 2010. Selenium protects sorghum leaves from oxidative damage under high temperature stress by enhancing antioxidant defense system. Plant Physiol. Biochem. 48(12):999–1007.

Djujic, I.S., O.N. Jozanov-Stanko, V. Djermanovic, M. Demajo and O. Bosni. 2000b. Availability of essential trace elements and their interactions in blood of humans consuming selenium enriched wheat. Selenium 2000, Venice, October 1-5 (poster). Online, acc. 29/11/2001, URL: www-tiresias.bio.unipd.it/HomeSele/postlist.htm.

Djujic, I.S., O.N. Jozanov-Stankov, M. Milovac, V. Jankovic and V. Djermanovic. 2000a. Bioavailability and possible benefits of wheat intake naturally enriched with selenium and its products. Biol. Trace Elem. Res. 77(3):273-285.

Dodd, G.L. and L.A. Donovan. 1999. Water potential and ionic effects on germination and seedling growth of two cold desert shrubs. Am. J. Bot. 86:1146-1153. doi: 10.11674/zwyf.2012.11346.

Dong, J.Z., Y. Wang, S.H. Wang, L.P. Yin, G.J. Xu, C. Zheng, C. Leia and M.Z. Zhanga. 2013. Selenium increases chlorogenic acid, chlorophyll and carotenoids of *Lycium chinense* leaves. J. Sci. Food Agri. 93:310–315.

Dowdle, P.R. and R.S. Oremland. 1998. Microbial oxidation of elemental selenium in soil slurries and bacterial cultures. Environ. Sci. Tech. 32:3749-3755.

Ducsay, L, O. Ložek and L. Varga. 2009. The influence of selenium soil application on its content in spring wheat. Plant Soil Environ. 55:80–84.

Ducsay, L., O. Ložek, L. Varga, M. Marček. 2009. Zvyšovanie obsahu selénu v obilninách. Agrochémia. 12(2):3-6.

Dulai, S., I. Molnar, J. Pronay, A. Csernak, R. Tarnai and M. Molnarlang. 2006. Effects of drought on photosynthetic parameters and heat stability of PSII in wheat and in Aegilops species originating from dry habitats. Acta Biologica Szegediensis. 50:11–17.

Egert, M. and M. Tevini. 2002. Influence of drought on some physiological parameters symptomatic for oxidative stress in leaves of chives (*Allium schoenoprasum*). Environ. Exp. Bot. 48:43-49.

Ekelund, N.G.A and R.A. Danilov. 2001. The influence of selenium on photosynthesis and "light-enhanced dark respiration" (LEDR) in the flagellate *Euglena gracilis* after exposure to ultraviolet radiation. Aquat. Sci. 63:457-465.

Ellingsen, D.G., R.I. Holland, Y. Thomassen, M. Landro-Olstad, W. Frech and H. Kjuus. 1993. Mercury and selenium in workers previously exposed to mercury vapour at a chloralkali plant. Br J Ind Med, 50(8):745-52.

El Monayeri, M.O., A.M. Hegazi, N.H. Ezzat, H.M. Saleem and S.K. tahoun. 1984. Growth and yield of some wheat and barley varieties grown under different moisture stress levels. Annls. Agri. Sci. 20(3):231-243.

El-Midaoui, M., A. Talouizte, M. Benbella, H. Serieys, Y. Griveau and A. Berville. 2001. Effect of osmotic pressure on germination of sunflower seeds (*Helianthus annuus* L.). Helia. 24:129-134.

Emmerich, W.E. and S.P. Hardegree 1991. Seed germination in polyethylene glycol solution: effect of filter paper exclusion. Crop Sci. 31:454-458.

Eurola, M. and V. Hietaniemi. 2000. Report of the Selenium Monitoring Programme 1997-1999. Publications of Agricultural Research centre of Finland, series B24. Jokoinen, Finland: Agricultural Research Centre of Finland.

Eurola, M.H., P.I. Ekholm, M.E. Ylinen, P.E. Koivistoinen and P.T. Varo PT. 2004. Selenium in Finnish foods after beginning the use of selenate-supplemented fertilizers. J. Sci. Food Agri. 56:57–70.

Fallon, K.M. and R. Phillips. 1989. Responses to water stress in adapted and unadapted carrot cell suspension cultures. J. Expt. Bot. 40:681–687.

FAO, WHO, 2001. Human vitamin and mineral requirements. Report of a Joint FAO/WHO Expert Consultation, Bangkok, Thailand, Food and Nutrition Division, FAO, Rome.

Farga˘sová, A., J. Pastierová and K. Svetková. 2006. Effect of Se-metal pair combinations (Cd, Zn, Cu, Pb) on photosynthetic pigments production and metal accumulation in *Sinapis alba* L. seedlings. Plant Soil Environ. 52:8–15.

Farooq, M., A. Wahid and D.J. Lee. 2009. Exogenously applied polyamines increase drought tolerance of rice by improving leaf water status, photosynthesis and membrane properties. Acta Physiol. Plant. 31:937–945.

Farshadfar, E., S.H. Sabaghpour and N. Khaksar . 2008. Inheritance of drought tolerance in chickpea (*Cicer arietinum* L.) J. Appl. Sci. 8(21): 3931-3937.

Feng, L.R., H.L. Du and Y.X. Wang. 2007. Influences of foliar selenium solutions spraying on selenium content, yield and quality of lettuce. J. Shanxi. Agri. Univ. (Nat. Sci Edn.). 27(3):291-294.

Feng, R., C. Weic and S. Tu. 2013. The roles of selenium in protecting plants against abiotic stresses. Environ. Exp. Bot. 87:58– 68.

Feng, R.W. and C.Y. Wei. 2012. Antioxidative mechanisms on selenium accumulation in *Pteris vittata* L., a potential selenium phytoremediation plant. Plant Soil Environ. 58(3):105–110.

Feng, R.W., C.Y. Wei, S.X. Tu and X. Sun. 2009a. Interactive effects of selenium and arsenic on their uptake by *Pteris vittata* L. under hydroponic conditions. Environ. Exp. Bot. 65(2–3):363–368.

Feng, R.W., C.Y. Wei, S.X. Tu and F.C. Wu. 2009b. Effects of Se on the essential elements uptake in *Pteris vittata* L. Plant Soil. 325(1–2):123–132.

Feng, R.W., C.Y. Wei, S.X. Tu and F.C. Wu. 2011. Detoxification of antimony by selenium and their interaction in paddy rice under hydroponic conditions. Microchem. J. 97(1):57–61.

Feroci, G., A. Fini, R. Badiello and A. Breccia. 1997. Interaction between selenium derivatives and heavy metal ions: Cu2+ and Pb2+. Microchem. J. 57:379-388. DOI: 10.1006/mchj.1997.1494.

Fghire, R., O.I. Ali, F. Anaya, O. Benlhabib, S. Jacobsen and S. Wahbi. 2013. Protective antioxidant enzyme activities are affected by drought in quinoa (*Chenopodium quinoa* wild). J. Bio. Agri. Healthcare. 3(4):62-68.

Filek, M., B. Gzyl-Malcher, M. Zembala, E. Bednarska, P. Laggner and M. Kriechbaum. 2010. Effect of selenium on characteristics of rape chloroplasts modified by cadmium. J. Plant Physiol. 167(1):28–33.

Filek, M., R. Keskinen, H. Hartikainen, I. Szarejko, A. Janiak, Z. Miszalski and A. Golda. 2008. The protective role of selenium in rape seedlings subjected to cadmium stress. J. Plant Physiol. 165(8):833–844.

Filek, M., M. Zembala, H. Hartikainen, Z. Miszalski, A. Korna´s, R. Wietecka-Posłuszny and P. Walas. 2009. Changes in wheat plastid membrane properties induced by cadmium and selenium in presence/absence of 2, 4-dichlorophenoxyacetic acid. Plant Cell Tissue Organ Cult. 96(1):19–28.

Flexas, J. and H. Medrano. 2002. Drought inhibition of photosynthesis in C3 plants: stomatal and non-stomatal limitation revisited. Ann. Bot. 89:183-189.

Fotovat, R., M. Valizadeh and M. Toorehi. 2007. Association between water-use-efficiency components and total chlorophyll content (SPAD) in wheat (*Triticum aestivum* L.) under well-watered and drought stress conditions. J. Food. Agric. Environ. 5:225-227.

Foyer, C.H., P. Descourvieres and K.J. Kunert. 1994. Photo oxidative stress in plants. Plant Physiol. 92:696-717.

Foyer, C.H. and S. Shigeoka. 2011. Understanding oxidative stress and antioxidant functions to enhance photosynthesis. Plant Physiol. 155: 93–100.

Frias, J., P. Gulewicz, C. Mart´inez-villaluenga, P. Radosław, E. Blazquez, B. Jim´enez, K. Gulewicz and C. Vidal-valverde. 2009. Influence of germination with different selenium solutions on nutritional value and cytotoxicity of lupin seeds. J. Agri. Food Chem. 57:1319–1325.

Frost, D.V. 1983. What do losses in selenium and arsenic bioavailability signify for health? Sci. Total Environ. 28:455-66.

Frugoli, J.A., H.H. Zhong, M.L. Nuccio, P. McCourt, M.A. McPeek, T.L. Thomas and C.R. McClung. 1996. Catalase is encoded by a multigene family in *Arabidopsis thaliana* (L.) Heynh. Plant Physiol. 112:327–336.

Gales, K., R.Q. Cannell and S.M. Ayling. 1983. Interaction between water logging and drought in water relations and growth of winter wheat and winter barley. J. Sci. Food Agri. 34(9):948-949, Field Crop Absts. 1984. (37):2-3.

Galle, A., J. Csiszar, M. Secenji, A. Guoth, L. Cseuz, I. Tari, J. Gyorgyey and L. Erdei. 2009. Glutathione transferase activity and expression patterns during grain filling in flag leaves of wheat genotypes differing in drought tolerance: response to water deficit. J Plant Physiol. 166(17):1878–1891.

Garg, N., and G. Manchanda. 2009. ROS generation in plants: Boon or bane? Plant Biosys. 143(1):81-96.

Germ, M., I. Kreft and J. Osvald. 2005. Influence of UV-B exclusion and selenium treatment on photochemical efficiency of photosystem II, yield and respiratory potential in pumpkins (*Cucurbita pepo* L.). Plant Physiol. Biochem. 43:445–448.

Germ, M., V. Stibilj, J. Osvald and I. Kreft. 2007. Effect of selenium foliar application on chicory (*Cichorium intybus* L.). J. Agri. Food Chem. 55:795-798.

Germ, M., I. Kreft, V. Stibilj and O. Urbanc-Berčič. 2007. Combined effects of selenium and drought on photosynthesis and mitochondrial respiration in potato. Plant Physiol. Biochem. doi:10.1016/j.plaphy.2007.01.009.

Germ, M. 2008. The response of two potato cultivars on combined effects of selenium and drought. Acta agri. Slov. 91-1:121-137.

Gharoobi, B., M. Ghorbani and M.G. Nezhad. 2012. Effects of different levels of osmotic potential on germination percentage and germination rate of barley, corn and canola. Iranian J. Plant Physiol. 2:413-417.

Ghasempour, H.R., D.F. Gaff, R.P.W. Williams and R.D. Gianello. 1998. Contents of sugars in leaves of drying desiccation tolerant flowering plants, particularly grasses. Plant Growth Reg. 24:185–191.

Ghodsi, M. 2004. Ecophysiological aspects of water deficit on growth and development of wheat cultivars, Ph.D. thesis, University of Tehran, Iran.

Giang, Y. and B. Huang. 2001. Drought and heat stress injury two cool-season turf grasses in relation to antioxidant metabolism and lipid peroxidation. Crop Sci. 41:436-442

Gill, R.K., A.D. Sharma, P. Singh and S.S. Bhullar. 2002. Osmotic stress-induced changes in germination, growth and soluble sugar content of Sorghum bicolor (L.) Moench seeds. Bulg. J. Plant. Physiol. 28:12-25.

Gill, S.S.and N. Tuteja. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48:909–930.

Giancarla, V., E. Madosă, R. Şumălan, and S. Ciulca. 2012. Evaluation of some indirect indices to identify drought tolerance in barley. J. Hort. Fort. Biotech., 16:239-241.

Gissel-Nielsen, G., U.C. Gupta, M. Lamand and T. Westermarck. 1984. Selenium in soils and plants and its importance in livestock and human nutrition. Adv. Agron. 37:397-460.

Goh, K.-H. and T. Lim. 2004. Geochemistry of inorganic arsenic and selenium in a tropical soil: effect of reaction time, pH, and competitive anions on arsenic and selenium adsorption. Chemosphere. 55(6):849-859.

González, H.H.L., E.J. González, A. Martínez, A. Pacin, S.L. Resnik. 1999. Relationship between *Fusarium graminearum* and *Alternaria alternata* contamination and deoxynivalenol occurrence on Argentinian durum wheat. Mycopathologia. 144: 97–102

Good, A.G. and S.T. Zaplachinski. 1994. The effects of drought stress on free amino acid accumulation and protein synthesis in *Brassica napus*. Physiol. Plant. 90:9-14.

GOP (Government of Pakistan). 2011-12. Economic Survey of Pakistan. p. 15-20. Islamabad, Pakistan.

Gowily, A.M., M.B. Mahmoud, M.F. Abdel-Lateef, A.A. Razak and T.E. Ramadan. 1996. Influence of selenium, TBZ and their mixture on metabolic activities of some fungi. African J. Mycol. Biotechol. 4:45–56.

Graham, R.D., R.M. Welch and H.E. Bouis. 2001. Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: principles, perspectives and knowledge gaps. Adv. Agron. 70:77-142.

Greenway, H., R. Munns. 1980. Mechanisms of salt tolerance in non halophytes. Annu. Rev. Plant Physiol. 31:149-190.

Grossman, A.R. and H. Takahashi H. 2001. Macronutrient utilization by photosynthetic eukaryotes and the fabrics of interactions. Annu. Rev. Plant Physiol. Plant Mol. Biol. 52:163–210.

Gregory, P.J. 1989. The role of root characteristics in moderating the effect of drought. p. 141-150. *In* F.W.G. Baker (ed.), Drought Resistance in Cereals. CAB International, Wallingford, UK.

Gromer, S., S. Urig and K. Becker. 2004. The thioredoxin system--from science to clinic. Med. Res. Rev. 24(1):40-89.

Guttieri, M.J., J.C. Stark, K.M.O. Brien and E. Souza. 2001. Relative sensitivity of spring wheat grain yield and quality parameters to moisture deficit. Crop Sci.. 41:327-335.

Gunes, A., N. Cicek, A. Inal, M. Alpaslan, F. Eraslan, E. Guneri and T. Guzelordu. 2006. Genotypic response of chickpea (*Cicer arietinum* L.) cultivars to drought stress implemented at pre- and post-anthesis stages and its relations with nutrient uptake and efficiency. Plant Soil Environ. 52:368-376.

Gzik., A. 1996. Accumulation of proline and pattern of alpha amino acids in sugar beet plants in response to osmotic, water and salt stress. Environ. Exp. Bot. 36: 29-38.

Habibi, G. 2013. Effect of drought stress and selenium spraying on photosynthesis and antioxidant activity of spring barley. Acta agri. Slov. 101-1:31 – 39.

Hafid, R.E., D.H. Smith, M. Karrou and K. Samir. 1998. Physical responses of spring Durum in a Mediterranean environment. Ann. Bot. 81:363-370.

Hajduch, M., R. Rakwal, G.K. Agrawal, M. Yonekura and A. Pretova. 2001. High-resolution two-dimensional electrophoresis separation of proteins from metal-stressed rice (*Oryza sativa* L.) leaves: drastic reductions/fragmentation of ribulose-1,5-bisphosphate carboxylase/oxygenase and induction of stress-related proteins. Electrophoresis. 22:824–831.

Hajiboland, R. and N. Keivanfar. 2012. Selenium supplementation stimulates vegetative and reproductive growth in canola (*Brassica napus* L.) plants. Acta agri. Slov. 99(1):13 – 19.

Hamilton, P.b and D.D. Van Slyke. 1943. Amino acids determination with ninhydrin. J. Biol. Chem. 150:231-233.

Han, D., L. Xihong, S. Xiong, S. Tua, Z. Chen, J. Li and Z. Xie. 2013. Selenium uptake, speciation and stressed response of *Nicotiana tabacum* L. Environ. Exp. Bot. 95:6–14.

Hanson, B., G.F. Garifullina, S.D. Lindblom, A. Wangeline, A. Ackley and K. Kramer *et al*. 2003. Selenium accumulation protects *Brassica juncea* from invertebrate herbivory and fungal infection. New Phytol. 159(2):461-469.

Haq, A., R. Vamil, and R.K. Agnihotri. 2010. Effect of osmotic stress (PEG) on germination and seedling survival of Lentil (*Lens culinaris* MEDIK). Res. J. Agri. Sci. 1:201-204.

Hare, P.D. and W.A. Cress. 1997. Metabolic implications of stress-induced proline accumulation in plants. Plant Growth Reg. 21:79-102.

Hartikainen, H., T. Xue and V. Piironen. 2000. Selenium as an anti-oxidant and pro-oxidant in ryegrass. Plant and Soil. 225:193-200.

Hartikainen, H. and T.L. Xue. 1999. The promotive effect of selenium on plant growth as triggered by ultraviolet irradiation. J. Environ. Quality. 28:1372-1375.

Hartikainen, H. 2005. Biogeochemistry of selenium and its impact on food chain quality and human health. J. Trace Elem. Med. Bio. 18(4):309–318.

Hasanuzzaman, M. and M. Fujita. 2011. Selenium pretreatment up-regulates the antioxidant defense and methylglyoxal detoxification system and confers enhanced tolerance to drought stress in rapeseed seedlings. Biol. Trace Elem. Res. 143:1758–1776.

Hasanuzzaman, M., M.A. Hossain and M. Fujita. 2012. Selenium-induced up-regulation of the antioxidant defense and methylglyoxal detoxification system reduces salinity-induced damage in rapeseed seedlings. Biol. Trace Elem. Res. 143:1704–1721.

Hasegawa, P.M., R.A. Bressan, J.K. Zhu and H.J. Bohnert. 2000. Plant cellular and molecular responses to high salinity. Ann. Rev. Plant Physiol. Plant Mol. Bio. 51:463–499.

Hawkesford, M.J. and F.J. Zhao. 2007. Strategies for increasing the selenium content of wheat. J. Cereal Sci. 46:282–292.

Hawrylak, B., R. Matraszek and M. Szymańska. 2007. Response of lettuce (*Lactuca sativa* L.) to selenium in nutrient solution contaminated with nickel. Veg. Crops Res. Bull. 67:63-70.

Hawrylak-Nowak, B. 2009. Beneficial effects of exogenous selenium in cucumber seedlings subjected to salt stress. Biol. Trace Elem. Res. 132(1):259–269.

He, P.P., X.Z. Lv and G.Y. Wang. 2004. Effects of Se and Zn supplementation on the antagonism against Pb and Cd in vegetables. Environ. Int. 30:167–172.

Hien, D.T., M. Jacobs, G. Angenon, C. Hermans, T.T. Thu, L. Van Son, N.H. Roosens. 2003. Proline accumulation and Δ1-pyrroline-5-carboxylate synthetase gene properties in three rice cultivars differing in salinity and drought tolerance. Plant Sci. 16:1059-1068.

Hladun, K.R., D.R. Parker, K.D. Tran and J.T. Trumble. 2013. Effects of selenium accumulation on phytotoxicity, herbivory, and pollination ecology in radish (Raphanus sativus L.). Environ. Poll. 172:70–75.

Hoekstra FA, Golovina EA, Buitink J (2001) Mechanisms of plant desiccation tolerance. Trends Plant Sci 6: 431–438

Hooda, A., A. S. Nandwal. M. S. Kuhad and D. Dutta. 1999. Plant water status and C, N and K distribution in potassium fertilized mung bean under rought and during recovery, In: Faroda, A.S., N. L. Joshi, S. Kathju and A. Karj, (Eds). Mach 1997. 207-214.

Hopper, J.L. and D.R. Parker. 1999. Plant availability of selenite and selenate as influenced by the competing ions phosphate and sulfate. Plant and Soil. 210(2):199-207.

Hsu, Y.T. and C.H. Kao. 2003. Changes in protein and amino acid contents in two cultivars of rice seedlings with different apparent tolerance to cadmium. Plant Growth Reg. 40:147–155.

Hsu, S.Y., Y.T. Hsu and C.H. Kao. 2003. The effect of polyethylene glycol on proline accumulation in rice leaves. Biol. Plant. 46:73-78.

Hu, Q., G. Pan and J. Zhu. 2001. Effect of selenium on green tea preservation quality and amino acid composition of tea protein. J. Hortic. Sci. Biotechnol. 76:344–346.

Huber, S.C., C. MacKintosh and W.M. Kaiser. 2002. Metabolic enzymes as targets for 14-3-3 proteins. Plant Mol. Biol. 50:1053±1063.

Hussain, A.I., F. Anwar, S.T.H. Sherazi and R. Przybylski. 2008. Chemical composition. Antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. Food Chem. 108:986-995.

Iglesias-Bartolomé, R., C.A. González and J.D. Kenis. 2004. Nitrate reductase dephosphorylation is induced by sugars and sugar-phosphates in corn leaf segments. Physiol. Plant. 122:62-67.

Ingram, J. and D. Bartels. 1996. The molecular basis of dehydration tolerance in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 47: 377-403.

Iqbal, S. and A. Bano. 2010. Drought and Abscisic Acid Induced Changes in Protein and Pigment Contents of Four Wheat (*Triticum aestivum* L.) Accession. J. Agric. Res. 48:1-13.

Iqbal, N., M. Ashraf and M. Y. Ashraf. 2008. Glycinebetain, an osmolyte of interest to improve water stress tolerance in sunflower (*Helianthus annuus* L.): water relation and yield. South Afr. J. Bot. 74: 274-281.

Irigoyen, J. J., D. W. Emerich and M. Sanchez-Diaz. 1992. Water stress induced changes in concentration of proline and total soluble sugars in nodulated alfalfa (Medicago sativa) plants. Physiol. Plant. 84: 55-60.

Iturbe, O.I., P.R. Escuredo, C. Arrese-Igor and M. Becana. 1998. Oxidative damage in pea plants exposed to water deficit or paraquat. Plant Physiol. 116:173–181.

Jackson, M.L. 1962. Soil chemical analysis. Constable and company, England.

Jagtap, V., S. Bhargava, P. Sterb and J. Feierabend. 1998. Comparative effect of water, heat and light stresses on photosynthetic reactions in *Sorghum bicolor* (L.) Moench. J. Exp. Bot. 49:1715-1721.

Jaleel, C.A., R. Gopi, P. Manivannan and R. Panneerselvam. 2007a. Antioxidative potentials as a protective mechanism in *Catharanthus roseus* (L.) G. Don. plants under salinity stress. Turkish J. Bot. 31, 245-251.

Jamieson, P.D, R.J. Martin and G.S. Francis. 1995. Drought influences on grain yield of barley, wheat, and maize. NZL J. Crop Hortic. 23:55–56.

Ježek P., P. Škarpa, T. Lošák, J. Hlušek, M. Jůzl and P. Elzner. 2012. Selenium – An important antioxidant in crops biofortification. p. 343-367. INTECH.

Johansson, L., G. Gafvelin and E.S.J. Arnér. 2005. Selenocysteine in proteins—properties and biotechnological use. BBA-General Subjects. 1726(1):1-13.

Kabata-Pendias A. 2001. Trace Elements in Soils and Plants. p. 241-252. (3rd ed.). Boca Raton, FL:CRC Press, USA.

Kabata-Pendias, A. and H. Pendias. 1999. Trace Elements in Soils and Plants (2nd ed.). CRC, Boca Raton.

Kahakachchi, C., H.T. Boakye, P.C. Uden and J.F. Tyson. 2004. Chromatographic speciation of anionic and neutral selenium compounds in Se-accumulating *Brassica juncea* (Indian mustard) and in selenized yeast. J. Chromatogr. A. 1054:303-312.

Kahle, H. 1988. Wirkung von blei und cadmium auf wachstum und mineralstoffhaushalt von jungbuchen (*Fagus sylvatica* L.) in sandkultur. Dissert. Bot. 127:Berlin: J. Cramer.

Kaiser, W.M. and S.C. Huber. 2001. Post-translational regulation of nitrate reductase: mechanism, physiological relevance and environmental triggers. J. Exp. Bot. 52:1981±1989.

Kaldenhoff, R., M. Ribas-Carbo and J. Flexas *et al*. 2008. Aquaporins and plant water balance. Plant Cell Environ. 31:658–666.

Kang, S.Z., L. Zhang, Y.L. Liang, X.T. Hu, H.J. Cai and B.J. Gu. 2002. Effects of limited irrigation on yield and water use efficiency of winter wheat in the Loess Plateau of China. Agri Water Manage. 55:203–216.

Kapolna, E., P.R. Hillestrom, K.H. Laursen, S. Husted and E.H. Larsen. 2009. Effect of foliar application of selenium on its uptake and speciation in carrot. Food Chem. 115:1357–1363.

Kapolna, E., K.H. Laursen, S. Husted and E.H. Larsen. 2012. Bio-fortification and isotopic labelling of Se metabolites in onions and carrots following foliar application of Se and 77Se. Food Chem. 133:650–657.

Kauser, R., A. Hur and M. Ashraf. 2006. Chlorophyll fluorescence: a potential indicator for assessment of water stress tolerance in canola (*Brassica napus* L.). Pak. J. Bot. 38(5): 1501–15.

Kavi-Kishore, P.B., S. Sangam, R.N. Amrutha, P.S. Laxmi, K.R. Naidu, K.R.S.S. Rao, S. Rao, K.J. Reddy, P. Theriappan and N. Sreenivasulu. 2005. Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. Curr. Sci. 88:424-438.

Kawakami, J., K. Iwama and Y. Jitsuyama. 2005. Soil water stress and the growth and yield of potato plants grown from microtubers and conventional seed tubers. J. Plant. Physiol. 162:903-911.

Kaya, Y., M. Akcura, R. Ayranci and S. Taner. 2006. Pattern analysis of multi-environment trials in bread wheat. Commun. Biometry Crop Sci. 1:63–71.

Keshan Disease Research Group. 1979. Epidemiologic studies on the etiologic relationship of selenium and Keshan disease. Ch. Med. J. 92(7):477-82. doi: 114372.

Khan, A.L., Z.K. Shinwari, M. Yoon-Ha Kim, M. Waqas, M. Hamayun, M. Kamran and L. In-Jung. 2012 Isolation and detection of Gibberellins and indole acetic acid from Endophyte *Chaetomium globosum* LK4 growing with drought stressed plant. Pak. J. Bot., 44(5):1601-1607.

Khattab, H. 2004. Metabolic and oxidative responses associated with exposure of *Eruca sativa* (rocket) plants to different levels of selenium. Int. J. Agric. Biol. 6:1101–1106.

Khayatnezhad, M., R. Gholamin, S. Jamaati-e-Somarin and R. Zabihi-e-Mahmoodabad. 2011. The leaf chlorophyll content and stress resistance relationship considering in Corn cultivars (*Zea mays* L.). Adv. Environ. Biol. 5(1):118-122.

Khazaei, M. 2001. Forecasting drought intensity and flow shortage.M.Sc. Thesis of Civil engineering- water. Iran Science and Industry University.166 p.

Khodarahmpour, Z. 2011. Effect of drought stress induced by polyethylene glycol (PEG) on germination indices in corn (*Zea mays* L.) hybrids. Afr. J. Biotechnol. 10:18222-18227.

Kilic, H. and T. Yagbasanlar. 2010. The Effect of Drought Stress on Grain Yield, Yield Components and some Quality Traits of Durum Wheat (*Triticum turgidum* ssp. durum) Cultivars. Not. Bot. Hort. Agrobot. Cluj. 38(1):164-170.

Kim, I.Y., M.J. Guimaraes, A. Zlotnik, J.F. Bazan and T.C. Stadtman. 1997. Fetal mouse selenophosphate synthetase 2 (SPS2): Characterization of the cysteine mutant form overproduced in a baculovirus-insect cell system. Proc. Nat. Acad. Sci. 94(2):418.

Klein, D., R. Morcuende, M. Stitt and A. Krapp. 2000. Regulation of nitrate reductase expression in leaves by nitrate and nitrogen metabolism is completely overridden when sugars fall below a critical level. Plant Cell Environ. 23: 863-871.

Kochaki, E. 1997. Agronomy and plant breeding dry farming. M.S. thesis. Ferdosi University, Mashhad, Iran.

Kopsell, D.A., D.E. Kopsella and W.M. Randle. 2003. Seed germination response of rapid-cycling *brassica oleracea* grown under increasing sodium selenate. J. Plant Nutr. 26:1355–1366.

Kopsell, D.A. and D.E. Kopsell. 2007. Selenium.p. 515-549. *In* A.W. Barker and J. Pilbeam. Handbook of plant nutrition. CRC Press.

Kumar, M.V., D. Krishnarajan, R. Manivannan and K.G. Parthiban. 2011. Formulation and evaluation of bi-layer domperidone floating tablets. IJPSR. 2(8):2217-2225.

Kumar, M., A.J. Bijo, R.S. Baghel, C.R.K. Reddy and B. Jha. 2012. Selenium and Spermine alleviates cadmium induced toxicity in the red seaweed *Gracilaria dura* by regulating antioxidant system and DNA methylation. Plant Physiol. Biochem. 51:129–138.

Kumar, P.B. and N.K. Paul. 1997. Effect of water stress on chlorophyll, proline and sugar accumulation in rape. Bangladesh J. Bot. 26:1983-85.

Kuznetsov, V.V., V.P. Kholodova, V.I.V. Kuznetsov and B.A. Yagodin. 2003. Selenium regulates the water status of plants exposed to drought. Dok. Biol. Sci. 390:266-268.

Kyparissis, A., Y. Petropoulou and Y. Manetas. 1995. Summer survival of leaves in a soft-leaved shrub (*Phlomis fruticosa* L., Labiates) under Mediterranean field conditions: Avoidance of photoinhibitory damage through decreased chlorophyll contents. J. Exp. Bot. 46:1825-1831.

Łabanowska, M., M. Filek, J. Ko´scielniak, M. Kurdziel, E. Kuli´s and H. Hartikainen. 2012. The effects of short-term selenium stress on Polish and Finnish wheat seedlings—EPR, enzymatic and fluorescence studies. J. Plant Physiol. 169:275–284.

Landberg, T. and M. Greger. 1994. Influence of selenium on uptake and toxicity of copper and cadmium in pea (*Pisum sativum*) and wheat (*Triticum aestivum*). Physiol. Plantar. 90(4): 637–644.

Larcher, W. 2003. Physiological plant ecology, (4th ed). Springer.

Larios, B., E. Agüera, P. de la Haba, R. Pérez-Vicente and J.M. Maldonado. 2001. A short-term exposure of cucumber plants to rising atmospheric CO_2 increases leaf carbohydrate content and enhances nitrate reductase expression and activity. Planta 212: 305-312.

Larson, K.L. 1992. Drought injury and resistance of crop plants. p. 147-162. *In* Physiological aspects of dry land farming (ed.): S.U. Gupta. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi.

Latif, A. and M.M. Iqbal. 2001. Fertigation techniques. p.155-159. Proc. Workshop on Technologies for Sustainable Agriculture. NIAB, Faisalabad, Pakistan.

Lawlor, D.W. and G. Cornic. 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficit in higher plants. Plant Cell Environ. 25:275-294.

Lawson, T., K. Oxborough, J.I.L. Morison and N.R. Baker. 2003. The responses of guard and mesophyll cell photosynthesis to CO_2, O_2, light, and water stress in a range of species are similar. J. Exp. Bot. 54:1743–1752.

Lee, J., J.W. Finley and J.M. Harnly. 2005. Effect of selenium fertilizer on free amino acid composition of broccoli (*Brassica oleracea* cv. majestic) determined by gas chromatography with flame ionization and mass selective detection. J. Agric. Food Chem. 53:9105-9111.

Li, H., Z. Hao, X. Wang, L. Huang and J. Li. 2009. Antioxidant activities of extracts and fractions from *Lysimachia foenum-graecum* Hance. Biores. Tech. 100:970–974.

Li, H.F., S.P. McGrath and F.J. Zhao. 2008. Selenium uptake, translocation and speciation in wheat supplied with selenate or selenite. New Phytol. 178(1):92–102.

Li, S.X. 2007. Dryland Agriculture in China. p. 630–631. Science Press, Beijing, China.

Liang, F. and M. Silburbush. 2002. Response of maize to foliar vs. soil application of nitrogen-phosphorous-potassium fertilizers. J. Plant Nutri. 11(25):2333-2342.

Liang, Z., F. Zhang, M. Shao and J. Zhang. 2002. The relations of stomatal conductance, water consumption, growth rate to leaf water potential during soil drying and rewatering cycle of wheat (*Triticum aestivum* L.). Bot. Bull. Acad. Sin. 43:187-192.

Lilley J.M., M.M. Ludlow M.M, S.R. McCouch and J.C. O'Toole. 1996. Locating QTL for osmotic adjustment and dehydration tolerance in rice. J. Exp. Bot. 47:1427-1436.

Lillo, C., C. Meyer, U.S. Lea, F. Provan and S. Oltedal. 2004. Mechanism and importance of post-translational regulation of nitrate reductase. J. Exp. Bot. 55:1275-1282.

Liu, B., Y. Li, X. Liu, C. Wang, J. Jin and S.J. Herbert. 2011. Lower total soluble sugars in vegetative parts of soybean plants are responsible for reduced pod number under shading conditions. Aus. J. Crop Sci. 5(13):1852-1857.

Longchamp, M., N. Angeli and M. Castrec-Rouelle. 2013. Selenium uptake in *Zea mays* supplied with selenate or selenite under hydroponic conditions. Plant Soil. 362:107–117.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with folin phenol reagent. J. Biol. Chem. 191:265-275.

Ludlow, M.M. and R.C. Muchow. 1990. A critical evaluation of trait for improving crop yields in water limited environments. Adv. Agron. 42:107-153.

Ludlow, M.M., M.J. Fisher and J.R. Wilson. 1985. Stomatal adjustment to water deficits in three tropical grasses and a tropical legume in controlled conditions and in the field. J. Plant Physiol. 12:131-149.

Lutts, S. 2000. Exogenous glycinebetaine reduces sodium accumulation in salt stressed rice plants. Int. Rice Res. Notes, 25:39-40.

Lyons, G., I. Ortiz-Monasterio, J. Stangoulis and R. Graham. 2005. Selenium concentration in wheat grain: Is there sufficient genotypic variation to use in breeding? Plant and Soil. 269(1):369-380.

Lyons, G., J. Stangoulis and R. Graham. 2003. High-selenium wheat: biofortification for better health. Nestle en LINK. 16(1):45.

Lyons, G.H., Y. Genc, K. Soole, J.C.R. Stangoulis, F. Liu and R.D. Graham. 2009. Selenium increases seed production in Brassica. Plant Soil. 318:73–80.

Mafakheri, A., A. Siosemardeh, B. Bahramnejad, P.C. Struik and E. Sohrabi. 2010. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Aus. J. Crop Sci. 4(8):580-585.

Maggio, A., S. Miyazaki, P. Veronese, T. Fujita, J.I. Ibeas, B. Damsz, M.L. Narasimhan, P.M. Hasegawa, R.J. Joly and R.A. Bressan. 2002. Does proline accumulation play an active role in stress-induced growth reduction. Plant J. 31:699–712.

Mahajan, S. and N. Tuteja. 2005. Cold, salinity and drought stresses: an overview. Arch. Biochem. Biophys. 444:139- 158.

Malik, J.A., S. Goel, N. Kaur, S. Sharma, I. Singh and H. Nayyar. 2012. Selenium antagonises the toxic effects of arsenic on mungbean (*Phaseolus aureus* Roxb.) plants by restricting its uptake and enhancing the antioxidative and detoxification mechanisms. Environ. Exp. Bot. 77:242–248.

Manivannan, P., C. Abdul Jaleel, B. Sankar, A. Kishorekumar, R. Somasundaram, G.M.A. Lakshmanan and R. Panneerselvam. 2007. Growth, biochemical modifications and proline metabolism in *Helianthus annuus* L. as induced by drought stress. Coll. Surf. B: Biointerfaces 59:141–149.

Marschner, H. 1995. Mineral Nutrition of Higher Plants. p. 430-433. Academic Press, London.

Masoumi, A., M. Kafi, H. Khazaei and K. Davari. 2010. Effect of drought stress on water status, elecrolyte leakage and enzymatic antioxidants of kochia (*kochia scoparia*) under saline condition. Pak. J. Bot. 42(5):3517-3524.

Miao, Y., D. Lv, P. Wang, X.C. Wang, J. Chen, C. Miao and C.P. Song. 2006. An Arabidopsis glutathione peroxidase functions as both a redox transducer and a scavenger in abscisic acid and drought stress responses. Plant Cell. 18:2749–2766.

Mikkelsen, R.L., A.L. Page and F.T. Bingham. 1989. Factors affecting selenium accumulation by agricultural crops. p. 65-94. Selenium in agriculture and the environment. (ed). Lee W. Jacobs. Soil Science Society of America & American Society of Agronomy, USA.

Meharg, A.A., J. Hartley-Whitaker. 2002. Arsenic uptake and metabolism in arsenic resistant and non-resistant plant species. New Phytol. 154 (1):29–43.

Michel, B.E. and M.R. Kaufmann. 1973. The osmotic potential of polyethylene glycol 6000. Plant Physiol. 51:914–916.

Miller, G.H., R.B. Alley, J. Brigham-Grette, J.J. Fitzpatrick, L. Polyak, M. Serreze, J.W.C. White. 2010. Arctic Amplification: can the past constrain the future? Quart. Sci. Rev. 29:1779-1790.

Ministry of Agriculture, Fisheries and Food. 1997. Dietary intake of selenium food surveillance information sheet no. 126. London, Joint Food Safety & Standards Group, UK.

Mirnoff, N. 1993. The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol. 125:27-58.

Misra, N.M. and D.P. Dwibedi. 1980. Effects of pre-sowing seed treatments on growth and dry matter accumulation of high yielding wheat under rainfed conditions. Ind J Agron 25:230–234.

Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends in Plant Sci. 7(9): 405-410.

Moaveni, P. 2011. Effect of water deficit stress on some physiological traits of wheat (*Triticum aestivum* L.). Agric. Sci. Res. J. 1:64-68.

Moayedi, A.A., A.N. Boyce, S.S. Barakbah. 2009. Study on osmotic stress tolerance in promising durum wheat genotypes using drought stress indices. Res. J. Agri. Biol. Sci. 5(5):603-607.

Mohammadi, R., E. Farshsdfar, M.A. Sarbarzeh and J. Shutka. 2003. Locating QTLs controlling drought tolerance criteria in rye using disomic addition lines. Cereal Res. Commun., 31: 257-264.

Mohammadkhani, N. and R. Heidari. 2008. Drought induced accumulation of soluble sugars and proline in two maize varieties. West Indies Appl. Sci. J. 3:448–453.

Moinuddin, J. and R. Khanna-Chopra. 2004. Osmotic adjustment in chickpea in relation to seed yield and yield parameters. Crop Sci. 44:449-455.

Moradi-Dezfuli, P., F. Sharif-zadeh and M. Janmohammadi. 2008. Influence of priming techniques on seed germination behavior of maize inbred lines (*Zea mays* L.). ARPN J. Agric. Bio. Sci. 3:22-25.

Morgan, J.M. 1984. Osmoregulation and water stress in higher plants. Annu. Rev. Plant Physiol. 35:299-319.

Mostajeran, A. and V. Rahimi-Eichi. 2009. Effects of drought stress on growth and yield of rice (*Oryza sativa* L.) cultivars and accumulation of proline and soluble sugars in

sheath and blades of their different ages leaves. American-Eurasian J. Agric. Environ. Sci. 5(2):264-272.

Moud, A.A.M. and T. Yamagishi. 2005. Application of projected pollen area response to drought stress to determine osmoregulation capability of different wheat (*Triticum aestivum* L.) cultivars. Int. J. Agri. Bio. 7(4):604-605.

Moussa, I. and S.M. Abdel-Aziz, 2008. Comparative response of drought tolerant and drought sensitive maize genotypes to water stress. Aust. J. Crop Sci. 1:31-36.

Munné-Bosch, S. and J. Peñuelas. 2003a. Photo- and antioxidative protection, and a role for salicylic acid during drought and recovery in field-grown *Phillyrea angustifolia* plants. Planta. 217:758–766.

Mushtaq, T., S. Hussain, M.A. Bukhsh, J. Iqbal and T. Khaliq. 2011. Evaluation of two wheat genotypes performance of under drought conditions at different growth stages. Crop Environ. 2 (2):20 – 27.

Nageswara, R.R.C., H.S. Talwar and G.C. Wright. 2001. Rapid assessment of specific leaf area and leaf nitrogen in peanut (*Arachis hypogaea* L.) using chlorophyll meter. J. Agron. Crop Sci. 189:175-182.

Nakamaru, Y. M. and K. Sekine. 2008. Sorption behavior of selenium and antimony in soils as a function of phosphate ion concentration. Soil Sci. Plant Nutr. 54(3):332-341.

Nawaz, F., R. Ahmad, E.A. Waraich, M.S. Naeem and R.N. Shabbir. 2012. Nutrient uptake, physiological responses and yield attributes of wheat (*Triticum aestivum* L.) exposed to early and late drought stress. J. Plant Nutr. 35:961–974.

Nawaz, F., M.Y. Ashraf, R. Ahmad and E.A. Waraich. 2013. Selenium (Se) seed priming induced growth and biochemical changes in wheat under water deficit conditions. Biol. Trace Elem. Res. 151:284-293. doi: 10.1007/s12011-012-9556-9.

Nayyar, H. and D.P. Walia. 2003. Water stress induced proline accumulation in contrasting wheat genotypes as affected by calcium and abscisic acid. Biol Plant. 46:275-279.

Nazarli, A., F. Faraji and M.R. Zardashti. 2011. Effect of drought stress and polymer on osmotic adjustment and photosynthetic pigments of sunflower. Cer. Agron. Moldova. 44(1):35-42

Nazeri, M. 2005. Study on response of triticale genotypes at water limited conditions at different developmental stages, Ph.D. thesis, University of Tehran, Iran.

Nikolaeva, M.K., S.N. Maevskaya, A.G. Shugaev and N.G. Bukhov. 2010. Effect of drought on chlorophyll content and antioxidant enzyme activities in leaves of three wheat cultivars varying in productivity. Russian J. Plant Physiol. 57: 87-95.

Nowak, J., K. Kaklewski and M. Ligocki. 2004. Influence of selenium on oxidoreductive enzymes activity in soil and plants. Soil Biol. Biochem. 36:1553–1558.

Okçu, G., M.D. Kaya and M. Atak. 2005. Effects of salt and drought stresses on germination and seedling growth of pea (*Pisum sativum* L.). Turk. J. Agric. For. 29:237–242.

Olson, O.E., E.J. Novacek, E.I. Whitehead and I.C. Palmer. 1970. Investigations on selenium in wheat. Phytochemistry 9:1181-1188.

Ommen, O.E., A. Donnelly, S. Vanhoutvin, M. Van-Oijen and R. Manderscheid. 1999. Chlorophyll content of spring wheat flag leaves grown under elevated CO_2 concentrations and other environmental stresses within the ESPACE-wheat project. Eur. J. Agron. 10:197-203.

Ottman, M.J., B.R. Tickes and S.H. Husman. 2000. Nitrogen-15 and bromide tracers of nitrogen fertilizer movement in irrigated wheat production. J. Environ. Qual. 29: 1500-1508.

Ouattar, S., R.J. Jones, R.K. Crookston and M. Kajeiou. 1987. Effect of drought on water relations of developing maize kernels. Crop Sci. 27:730-735.

Özdemir, Ö. (2008). Accumulation of selenium in different wheat genotypes and its protective role against various abiotic stress factors. M.S. thesis. Sabanci University, Turkey.

Ozturk, L. and Y. Demir. 2002. In vivo and in vitro protective role of proline. Plant Growth Regul. 38:259-264.

Padmaja, K., B.V. Somasekharaiah and A.R. Prasad. 1995. Inhibition of chlorophyll synthesis by selenium: involvement of lipoxygenase mediated lipid peroxidation and antioxidant enzymes. Photosynthetica. 31:1–7.

Pakistan Agricultural Research Council. Annual report. 2010-11.

Pan, Y., .L.J. Wu and Z.L. Yu. 2006. Effect of salt and drought stress on antioxidant enzymes activities and SOD isoenzymes of liquorice (*Glycyrrhiza uralensis* Fisch). Plant Growth Regul. 49:157-165.

Parida, A.K., V.S. Dagaonkar, M.S. Phalak, G.V. Umalkar and L.P. Aurangabadkar. 2007. Alterations in photosynthetic pigments, protein and osmotic components in cotton genotypes subjected to short-term drought stress followed by recovery. Plant Biotechnol. Rep. 1:37–48.

Passioura, J.B. and S.C. Fry. 1992. Turgor and cell expansion: beyond the Lockhart equation. Aust. J. Plant Physiol. 19:565–576.

Patakas, A., N. Nikolaou, E. Zioziou, K. Radoglou and B. Noitsakis. 2002. The role of organic solute and ion accumulation in osmotic adjustment in drought-stressed grapevines. Plant Sci. 163:361–367.

Pazurkiewicz-Kocot, K., A. Kita and M. Pietruszka. 2008. Effect of selenium on magnesium, iron, manganese, copper, and zinc accumulation in corn treated by indole-3-acetic acid. Comm. Soil Sci. Plant Anal. 39:2303–2318.

Pazurkiewicz-Kocot, K., W. Galas and A. Kita, 2003. The effect of selenium on the accumulation of some metals in *Zea mays* L. plants treated with indole-acetic acid. Cell. Mol. Biol. Lett. 8:97-103.

Pedrero, Z., Y. Madrid, H. Hartikainen and C. Cámara. 2008. Protective effect of selenium in broccoli (*Brassica oleracea*) plants subjected to cadmium exposure. J. Agric. Food Chem. 56(1):266–271.

Peng, Q. and Q. Zhou. 2009. Antioxidant capacity of flavonoid in soybean seedlings under the joint actions of rare earth element la(iii) and ultraviolet-B stress. Biol. Trace Elem. Res. 127:69–80.

Pennanen, A., T. Xue and H. Hartikainen. 2002. Protective role of selenium in plant subjected to severe UV irradiation stress. J. Appl. Bot. 76:66–76.

Pieters, A.J. and S. El-Souki. 2005. Effects of drought during grain filling on PS II activity in rice. J. Plant Physiol. 62:903-911.

Pietrini, F., M.A. Iannelli, S. Pasqualini and A. Massacci. 2003. Interaction of cadmium with glutathione and photosynthesis in developing leaves and chloroplasts of *Phragmites australis* (Cav.) Trin. ex steudel. Plant Physiol. 133(2):829–837.

Pinheiro, C., M.M. Chaves and C.P. Ricardo. 2001. Alterations in carbon and nitrogen metabolism induced bywater deficit in the stems and leaves of *Lupinus albus* L. J. Exp. Bot. 52:1063–1070.

Pirdashti, H, Sarvestani Z. Tahmasebi, G.H. Nematzadeh and A. Ismail. 2003. Effect of water stress on seed germination and seedling growth of rice (*Oryza sativa* L.) genotypes. Pak. J. Agr. 2:217-222.

Plant, J.A., D.G. Kinniburgh, P.L. Smedley, F.M. Fordyce and B.A. Klinck. 2005. Arsenic and selenium. Environ. Geochem. 9:17-66.

Plaut, Z. 2003. Plant exposure to water stress during specific growth stages. p. 673–675. Encyclopedia of Water Science, Taylor & Francis.

Poblaciones, M.J., S. Rodrigo, O. Santamaría, Y. Chen and S.P. McGrath. 2014. Agronomic selenium biofortification in *Triticum durum* under Mediterranean conditions: From grain to cooked pasta. Food Chem. 146:378–384.

Poggi, V., A. Arcioni, P. Filippini and P.G. Pifferi. 2000. Foliar application of selenite and selenate to potato (Solanum tuberosum): Effect of a ligand agent on selenium content of tubers. J. Agric. Food Chem. 48:4749–4751.

Põldma, P., T. Tõnutare, A. Viitak, A. Luik and U. Moor. 2011. Effect of selenium treatment on mineral nutrition, bulb size, and antioxidant properties of garlic (*Allium sativum* L.). J. Agric. Food Chem. 59:5498–5503.

Premchandra, G.S., H. Sameoka and S. Ogata 1990. Cell osmotic membrane-stability, an indication of drought tolerance, as affected by applied nitrogen in soil. J. Agric. Res. 115: 63-66.

Proiettia, P., L. Nasinia, D. Del Buonoa, R. D'Amatoa, E. Tedeschinib, D. Businellia. 2013. Selenium protects olive (*Olea europaea* L.) from drought stress. Scientia Hortic. 164 :165–171

Pukacka, S., E. Ratajczak and E. Kalemba. 2011. The protective role of selenium in recalcitrant *Acer saccharium* L. seeds subjected to desiccation. J. Plant Physiol. 168 (3):220–225.

Qadir, G., M. Saeed and M.A. Cheema. 1999. Effect of water stress on growth and yield performance of four wheat cultivars. Pak. J. of Biological Sci. 2(1):236-239.

Qayyum, A., A. Razzaq, M. Ahmad and M.A. Jenks. 2011. Water stress causes differential effects on germination indices, total soluble sugar and proline content in wheat (*Triticum aestivum* L.) genotypes. Afr. J. Biotechnol. 10:14038-14045.

Qiang-yun, S., M. Turakainen, M. Seppanen and P. Makela. 2008. Effects of selenium on maize ovary development at pollination stage under drought stress. Agri. Sci. China. 7:1298-1307.

Radyuk, M.S., I.N. Domaneskaya and R.A. Shecherbakove. 2009. Effect of low above zero temperature on the content of low molecular antioxidant and activities of antioxidant enzymes in green barely leaves. Bio. J. Plant Physiol. 56:175-180.

Rahbarian, R., R. Khavari-Nejad, A. Ganjeal, A. Bagheri and F. Najafi. 2011. Drought stress effects on photosynthe-sis, chlorophyll fluorescence and water relations intolerant and susceptible chickpea (*Cicer arietinum* L.)genotypes. Acta. Biol. Cracov. Ser. Bot. 53:47–56. doi:10.2478/v10182-011-0007-2.

Rahmati, H. and M. Farshadfar. 2012. Effect of osmotic stress on germination and seedling growth of *Agropyron trichophorum* genotypes. J. Basic. Appl. Sci. Res., 2: 4433-4438.

Ramanjulu, S. and C. Sudhakar. 1996. Drought tolerance is partly related to amino acid accumulation and ammonia assimilation: a comparative study in two mulberry genotypes differing in drought sensitivity. J. Plant Physiol. 150:345-350.

Ramos, S.J., V. Faquin, L.R.G. Guilherme, E.M. Castro, F.W. Ávila, G.S. Carvalho, C.E.A. Bastos and C. Oliveira. 2010. Selenium biofortification and antioxidant activity in lettuce plants fed with selenate and selenite. Plant Soil Environ. 56:584–588.

Rauf, M., M. Munir, M. Hassan, M. Ahmad and M. Afzal. 2006. Performance of wheat genotypes under osmotic stress at germination and early seedling growth stage. African J. Biotech. 6(8):971-975.

Rayman, M.P. 2002. The argument for increasing selenium intake. Proc. Nutr. Soc. 61(2): 203-215.

Raza, M.A.S., M.F. Saleem, G.M. Shah, M. Jamil and I.H. Khan. 2012. Potassium applied under drought improves physiological and nutrient uptake performances of wheat (*Triticum aestivun* L.). J. Soil Sci. Plant Nutr. 13(1):175-185.

Raza, M.A.S., M.F. Saleem, I.H. Khan, M. Jamil, M. Ijaz, M.A. Khan. 2012. Evaluating the drought stress tolerance efficiency of wheat (*Triticum aestivum* L.) cultivars. Russian J. Agric. Socio-Economic Sci., 12: 41-46.

Rebetzke, G. J. and R. A. Richards. 1999. Genetic improvement of early vigour in wheat. Aust. J. Agric. Res., 50: 291-301.

Rebetzke, G.J., R.A. Richards, N.A. Fettell, M. Long, A.G. Condon, R.I. Forrester and T.L. Botwright. 2007. Genotypic increases in coleoptile length improves stand establishment, vigour and grain yield of deep-sown wheat. Field Crops Res. 100: 10-23.

Rensburg, L.V. and G.H.J. Kruger. 1994. Evaluation of components of oxidative stress metabolism for use in selection of drought tolerant cultivars of *Nicotiana tabacum* L. J. Plant Physiol. 143:730-737.

Rhodes, D., P.E. Verslues and R.E. Sharp. 1999. Role of acids in abiotic stress resistance amino acids: Biochemi. and Bioteh. p. 39-356. Marcel. Dekker, New York, USA.

Rhodes, D. and Y. Samara. 1994. Genetics control of dsmoregulation in plants. In cellular and molecular physiology of cell volume regulation. Strange K. Boca Raton: CRC Press. pp.347-361.

Riazi, A., K. Matruda and A. Arslam. 1985. Water stress induce changes in concentration of proline and other solutes in growing regions. J. Exp. Bot. 36:1716-1725.

Rodriguez, P., A. Torrecillas, M.A. Morales, M.F. Ortuno and M.J.S. Blanco. 2005. Effects of NaCl salinity and water stress on growth and leaf water relations of *Asteriscus maritimus* plants. Environ. Exp. Bot. 53:113-123.

Rodriguez, J.D. de, J. Romero-Garcia, R. Rodriguez-Garcia and J.L.A. Sanchez. 2002. Characterization of proteins from sunflower leaves and seeds. Relationship of biomass and seed yield. In: J. Janick and A. Whipkey (eds). Trends in new crops and new uses. ASHS Press, Alexandria, VA., pp. 143-149.

Romero-Puertas, M.C., M. Rodrı´guez-Serrano, F.J. Corpas, M. Go´mez, L.A. del Rı´o and L.M. Sandalio. 2004. Cd-induced subcellular accumulation of O_2 and H_2O_2 in pea leaves. Plant Cell Environ. 27:1122–1134.

Rosyara, U.R., A.A. Ghimire, S. Subedi and R.C. Sharma. 2008. Variation in South Asian wheat germplasm for seedling drought tolerance traits. Plant Genetic Resources: Characterization and Utilization, 1-6.

Rotruck, J.T., A.L. Pope, H.E. Ganther, A.B. Swanson, D.G. Hafeman and W.G. Hoekstra. 1973. Selenium: Biochemical Role as a Component of Glutathione Peroxidase (Vol. 179, pp. 588-590). 1973 by the American Association for the Advancement of Science.

Rotte, C. and T. Leustek. 2000. Differential subcellular localization and expression of atp sulfurylase and 5'-adenylylsulfate reductase during ontogenesis of arabidopsis leaves indicates that cytosolic and plastid forms of atp sulfurylase may have specialized functions. Plant Physiol. 124(2):715.

Sadeghian, S.Y. and N. Yavari. 2004. Effect of water-deficit stress on germination and early seedling growth in sugar beet. J. Agron. Crop Sci. 190:138-144.

Saeedipour, S. 2011. Effect of drought at the postanthesis stage on remobilization of carbon reserves in two wheat cultivars differing in senescence properties. Int. J. Plant Physiol. Biochem. 3:15-24.

Sairam, R.K., S.P. Deshmukh, S.D. Shukla and S. Ram. 1990. Metabolic activity and grain yield under moisture stress in wheat genotypes. Ind. J. Plant. Physiol. 33(3):226-231 (Crop Physiology Absts; 17(11):4415; 1991).

Sajedi, N.A., M.R. Ardakani, A. Naderi, H. Madani and M.M.A. Boojar. 2009. Response of maize to nutrients foliar application under water deficit stress conditions. Am. J. Agri. Biol. Sci. 4:242-248.

Sajedi, N., H. Madani and A. Naderi, 2011. Effect of microelements and selenium on superoxide dismutase enzyme, malondialdehyde activity and grain yield maize (*Zea mays* L.) under drought stress. Not. Bot. Horti. Agrobo. 39(2):153-159.

Sajjan, A.S., V.P. Badanu and G.M. Sajjanar. 1999. Effect of external water potential on seed germination, seedling growth and vigor index in some genotypes of sunflower. p. 215-218. *In* Faroda S.A., Joshi N.L., Kathju S., Kar A. (eds.). Proc. Symp. Recent Advances in Management of arid ecosystem.

Sanchez, F.J., M. Manzanares, E.F. de Andres, J.L. Tenorio and L. Ayerbe. 1998. Turgor maintenance, osmotic adjustment and soluble sugar and praline accumulation in 49 pea cultivars in response to water stress. Field Crops Res. 59:225–235.

Sapra, V.T., E. Sarage, A.O. Anaele and C.A. Beyl. 1991. Varieties differences of wheat and triticale to water stress. J. Agron. Crop Sci. 167:23-28.

Seppanen, M., M. Turakainen and H. Hartikainen. 2003. Selenium effects on oxidative stress in potato. Plant Science, 165(2):311-319.

Schlemmer, M.R., D.D. Francis, J.F. Shanahan and J.S. Schepers. 2005. Remotely measuring chlorophyll content in corn leaves with differing nitrogen levels and relative water content. Agron. J. 97:106-112.

Schwarz, K. and C.M. Foltz. 1957. Selenium as an Integral Part of Factor 3 Against Dietary Necrotic Liver Degeneration. J. Amer. Chem. Soc. 79(12):3292-3293.

Selote, D.S., and R. Khanna-Chopra. 2004. Drought-induced spikelet sterility is associated with an inefficient antioxidant defense in rice panicles. Physiol. Plantar. 121:462-471.

Seppänen, M., M. Turakainen and H. Hartikainen. 2003. Selenium effects on oxidative stress in potato. Plant Sci. 165:311-319.

Serraj, R. and T.R. Sinclair. 2002. Osmolyte accumulation: Can it really help increase crop yield under drought conditions? Plant Cell Environ. 25:333–341.

Shangguan, Z.P., M.A. Shao and J. Dyckmans. 1999. Interaction of osmotic adjustment and photosynthesis in winter wheat under soil drought. J. Plant Physiol. 154:753–758.

Shao, H.B., L.Y. Chu, M.A. Shao, C. Abdul Jaleel and M. Hong-Mei. 2008. Higher plant antioxidants and redox signaling under environmental stresses. Comp. Rend. Biol. 331: 433–441.

Sharma, P. and R.S. Dubey. 2005. Drought induces oxidative stress and enhances the activities of antioxidant enzymes in growing rice seedlings. Plant Growth Reg. 46: 209-221.

Sharma, K.D., M.S. Kuhad and A.S. Nandwal. 1993. Influence of K nutrition on brassica genotypes in response to water stress. Plant Physiol. Biochem. 19(2):110-115.

Shewry, P.R. and H.D. Jones. 2007. Developing allergen-free foods by genetic manipulation, Managing Allergens in Food, Eds. C Mills, H Wichers & K Hoffmann-Sommergruber. CRC Press. Woodhead publishing Ltd. Cambridge UK.

Shiobara, Y., T. Yoshida and K.T. Suzuki. 1998. Effects of Dietary Selenium Species on Se Concentrations in Hair, Blood, and Urine. Toxicol. Appl. Pharmacol. 152(2):309-314.

Siddique, K.H.M., S.P. Loss and B.D. Thomson. 2003. Cool season grain legumes in dryland Mediterranean environments of Western Australia: Significance of early flowering, in: Saxena N.P. (ed.), Management of Agricultural Drought. Science Publishers, Enfield (NH), USA.

Siddique, B.M.R., A. Hamid and M.S. Islam. 2000. Drought stress effect on water relations of wheat. Bot. Bull. Acad. 41:35-39.

Simmonds, D.H. 1989. Wheat and wheat quality in Australia. p. 1-299. CSIRO Australia, Queensland.

Simova-Stoilova, L.J., Z. Stoyanova and K. Demirevska-Kepova. 2001. Ontogenic changes in leaf pigments, total soluble protein and Rubisco in two barley varieties in relation to yield. Bulg. J. Plant Physiol. 27:15-24.

Simova-Stoilova, L., K. Demirevska, T. Petrova, N. Tsenov and U. Feller. 2008. Antioxidative protection in wheat varieties under severe receverable drought at seedling stage. Plant Soil Environ. 54(12):529-536.

Sinclair, T.R. and M.M. Ludlow. 1985, Who taught plants thermodynamics? the unfulfilled potential of plant water potential. Aus. J. Plant Physiol. 12:213-217.

Singh, B.R. 1991. Selenium content of wheat as affected by selenate and selenite contained in a Cl-or SO 4-based NPK fertilizer. Nutr. Cycl. Agroecosys. 30(1):1-7.

Singh, D.V., L.M. Joshi and K.D. Srivastava. 1986. Foliar blight and spot of wheat in India, Indian J. Genet. 46:217-245.

Sivasankar, S., S. Rothstein and A. Oaks. 1997. Regulation of the accumulation and reduction of nitrate by nitrogen and carbon metabolites in maize seedlings. Plant Physiol. 114:583-589.

Sivritepe, H.O., N. Sivritepe, A. Eris and E. Turhan. 2005. The effects of NaCl pre-treatment on salt tolerance of melons grown under longterm salinity. Sci. Hort. 106:568–581.

Smirnoff, N. 1995. Antioxidant systems and plant response to the environment. *In* Smirnoff V (ed.), Environment and Plant Metabolism: Flexibility and Acclimation, BIOS Scientific Publishers, Oxford, UK.

Smrkolj, P., V. Stibilj, I. Kreft and M. Germ. 2006b. Selenium species in buckwheat cultivated with foliar addition of Se(VI) and various levels of UV-B radiation. Food Chem. 96:675-681.

Sofo, A., B. Dichio, C. Xiloyannis and A. Masia. 2005. Antioxidant defences in olive trees during drought stress: changes in activity of some antioxidant enzymes. Func. Plant Biol. 32:45–53.

Song, F.B., J.Y. Dai, W.B. Gu and H.Y. Li. 1995. The effect of water stress on leaf water status in maize. J. Jillin. Agric. Univ., 17(1):5-9.

Spadoni, M., M. Voltaggio, M. Carcea, E. Coni, A. Raggi and F. Cubadda. 2007. Bioaccessible selenium in Italian agricultural soils: Comparison of the biogeochemical approach with a regression model based on geochemical and pedoclimatic variables. Sci. Total Environ. 375:160-177.

Srinivas, V. and D. Balasubramanian. 1995. Proline is a protein-compatible hydrotrope. Langmuir 11:2830-2833.

Srivalli, B., V. Chinnusamy and R.K. Chopra. 2003. Antioxidant defense in response to abiotic stresses in plants. J. Plant Biol. 30:121-139.

Stadlober, M., M. Saner, and K.J. Irgolic. 2001. Effects of selenate supplemented fertilization on the selenium level of cereals—identification and quantification of selenium compounds by HPLC–ICP–MS. Food Chem. 73:357–366.

Steel, R.G.D., J.H. Torrie and D.A. Dickey. 1997. Principles and Procedures of Statistics, A biometrical approach. p. 178-182. McGraw Hill Co., New York, USA.

Stephen, R.C., D.J. Saville and J.H. Watkinson. 1989. The effects of sodium selenate applications on growth and selenium concentration in wheat. NZL J. Crop Hortic. Sci. 17(3):229-237.

Steyn, W.J., S.J.E. Wand, D.M. Holcroft and G. Jacobs. 2002. Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. New Phytol. 155:349–361.

Subbarao, G.V., Y.S. Chauhan and C. Johansen. 2000. Patterns of osmotic adjustment in pigeonpea - its importance as a mechanism of drought resistance. Eur. J. Agron. 12:239-249.

Sujin, R.W. and L. Ray Wu. 2004. Stress-inducible synthesis of proline in transgenic rice confers faster growth under stress conditions than that with constitutive synthesis. Plant Sci. 166:941–948.

Sullivan, C.Y. 1971. Techniques for measuring plant drought stress. Crop Sci. Soc. Am. (Special publication) 2:1-17.

Tadina, N., M. Germ, I. Kreft, B. Breznik and A. Gaberščik. 2007. Effects of water deficit and selenium on common buckwheat (*Fagopyrum esculentum* Moench.) plants. Photosynthetica. 45:472–476.

Taiz, L. and E. Zeiger. 2006. Plant physiology. 4th ed. Sinauer associates, Inc., publishers Sunderland., Massachusetts. USA.

Tatar, O. and M.N. Gevrek. 2008. Influence of water stress on proline accumulation, lipid peroxidation and water content of wheat. Asian J. Plant Sci. 7(4):409-412.

Tejada-Zarco, P. J., J.R. Miller, A. Morales, A. Berjon and J. Aguera. 2004. Hyperspectral indices and simulation models for chlorophyll estimation in open-canopy tree crops. Remote Sens. Environ. 90:463-476.

Temmerman, L.D., N. Waegeneers, C. Thiry, G.D. Laing, F. Tack and A. Ruttens. 2014. Selenium content of Belgian cultivated soils and its uptake by field crops and vegetables. Sci. Total Environ. 468–469:77–82.

Terry, N., A.M. Zayed, M.P. de Souza and A.S. Tarun. 2000. Selenium in Higher Plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 51(1):401-432.

Terzi, R. and A. Kadioglu. 2006. Drought stress tolerance and antioxidant enzyme system in Ctenanthe setosa. Acta Biol. Cracov. Ser. Bot. 48:89-96.

Teulat, B., D. Rekika, M.M. Nachit and P. Monneveux. 1997. Comparative osmotic adjustments in barley and tetraploid wheats. Plant Breeding. 116:519-523.

Tezara W., V.J. Mitchell, S.D. Driscoll and D.W. Lawlor. 1999. Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. Nature. 1401:914–917.

Thill, D.C., R.D. Schirman and A.P. Appleby. 1979. Osmotic stability of mannitol and polyethylene glycol 20000 solutions used as seed germination media. Agron. J. 71:105-108.

Thornley, J.M. 1998. Modelling shoot:root relations: the way forward. Ann. Bot. 81:165-171.

Tian, X. and Y. Lei. 2006. Nitric oxide treatment alleviates drought stress in wheat seedlings. Biol. Plant. 50:775–778.

Tian-Feng, C., Z. Wen-Jie1, L. Yong, Y. Fang, B. Yan and T. Fang. 2005. Effects of selenium stress on photosynthetic pigment contents and growth of *Chlorella vulgaris.* J. Plant Physiol. Mol. Bio. 31 (4):369-373.

Tognetti, R., R. d'Andria, G. Morelli and A. Alvino. 2005. The effect of deficit irrigation on seasonal variations of plant water use in *Olea europaea* L. Plant Soil. 273:139–155.

Trouverie, J., C. Thevenot, J.P. Rocher, B. Sotta and J.L. Prioul. 2003. The role of abscisic acid in the response of a specific vacuolar invertase to water stress in adult maize leaf. J. Exp. Bot. 54:2177-2186.

Turkan, I., Bor, M., Ozdemir, F., Koca, H., 2005. Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* Gray and drought-

sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. Plant Sci. 168, 223–231.

Turakainen, M., H. Hartikainen and M.M. Seppänen. 2004. Effects of selenium treatments on potato (*Solanum tuberosum* L.) growth and concentrations of soluble sugars and starch. J. Agri. Food Chem. 25:5378–5382.

Turner, N.C. 1986. Adaptation to water deficits: a changing perspective. Aust. J. Plant Physiol. 13:175-190.

Vaezi, H. 2005. Evaluation of molecular characters in wheat MSc Thesis, College of Agriculture Razi University, Kermanshah, Iran.

Valadabadi, S.A., A.H. Shiranirad and H.A. Farahani. 2010. Ecophysiological influences of zeolite and selenium on water deficit stress tolerance in different rapeseed cultivars. J. Ecol. Natural Environ. 2:154-159.

Valkama, E, M. Kivimäenpää, H. Hartikainen and A. Wulff. 2003. The combined effects of enhanced UV-B radiation and selenium on growth, chlorophyll fluorescence and ultrastructure in strawberry (*Fragaria × ananassa*) and barley (*Hordeum vulgare*) treated in the field. – Agri. For. Met. 120:267-278.

Vassilev, A. and I. Yordanov. 1997. Reductive analysis of factors limiting growth of Cd exposed plants: A review. Bulg. J. Plant Physiol. 23:114-133.

Verbruggen, N. and C. Hermans. 2008. Proline accumulation in plants: a review. Amino Acids. 35:753 759.

Vítová, M., K. Bi˘sová, M. Hlavová, V. Zachleder, M. Rucki and M. ˘Cí˘zková. 2011. Glutathione peroxidase activity in the selenium-treated alga *Scenedesmus quadricauda*. Aq. Toxicol. 102 (1–2):87–94.

Voetberg, G.S. and R.E. Sharp. 1991. Growth of the maize primary root at low water potentials. III. Role of increased proline deposition in osmotic adjustment. Plant Physiol. 96:1125–1130.

Wahid, A. and A. Shabbir. 2005. Induction of heat stress tolerance in barley seedlings by pre-sowing seed treatment with glycinebetaine. Plant Growth Reg. 46:133–141.

Wahid, A. and E. Rasul. 2005. Handbook of photosynthesis. p. 479-497. *In* Photosynthesis in leaf, stem, flower, and fruit, ed Pessarakli M. (Taylor and Francis Group, LLC, Boca Raton, FL).

Wang, Z. and B. Huang. 2003. Genotypic variation in abscisic acid accumulation, water relations, and gas exchange for Kentucky bluegrass exposed to drought stress. J. Amer. Soc. Hort. Sci. 128:349–355.

Wang, Yu-Dong., X. Wang, Y. Wong. 2013. Generation of selenium-enriched rice with enhanced grain yield, selenium content and bioavailability through fertilisation with selenite. Food Chem. 141:2385–2393.

Wang, C.Q., 2011. Water-stress mitigation by selenium in *Trifolium repens* L. J. Plant Nutr. Soil Sci. 174 (2):276–282.

Wang, J., Z. Wang, H. Mao, H. Zhao and D. Huang.2013. Increasing Se concentration in maize grain with soil- or foliar-appliedselenite on the Loess Plateau in China. Field Crops Res. 150:83–90.

Wang, Y.D., X. Wang, Y.S. Wong. 2012. Proteomics analysis reveals multiple regulatory mechanisms in response to selenium in rice. J. Proteom. 75:184–1866.

Waraich, E.A., R. Ahmad, Saifullah, M.Y. Ashraf, Ehsanullah. 2011. Role of mineral nutrition in alleviation of drought stress in plants. Aust. J. Crop Sci. 5:764-777.

Weyers, J.D.B. and N.W. Paterson. 2001. Plant hormones and the control of physiological processes. New Phytol. 129:375–407.

Willenborg, C.J., J.C. Wildeman, A.K. Miller, B.G. Rossnagel and S.J. Shirtliffe. 2005. Oat germination characteristics differ among genotypes, seed sizes, and osmotic potentials. Crop Sci. 45:2023-2029.

Wójcik, P. 2004. Uptake of mineral nutrients from foliar fertilization. J. Fruit Ornam. Plant Res. (special ed) 12:201–218.

Wolf, B. 1982. A comprehensive system of leaf analysis and its use for diagnosing crop nutrient status.Commun. Soil Sci. Plant Anal. 13:1035-1059.

Wolkers, J., R.F. Wikamp, S.M. Nijneijer, L.C. Burknow, E.M. de Groene, C. Lydersen, S. Dahle and X. Mmonshouwer. 1998. Phase I and phase II enzyme activities in ringed seals (phocahispida): Characterization of hepati coytochrome P450 by activity patterns inhibition studies mRNA analyses and western blotting. Aquatic toxicol. 44: 103–115.

Wright, G.C., R.C. Nageswara and G.D. Farquhar. 1994. Water use efficiency and carbon isotope discrimination in peanut under water deficit conditions. Crop Sci. 34:92-97.

Wu, L. 1998. Selenium accumulation and uptake by crop and grassland plant species. p. 657-685. *In* Willia, T, Frankenberger Jr, Engberg RA (eds.) Environmental Chemistry of Selenium. Marcel Dekker, New York, USA.

Ximénez-Embún, P., I. Alonso, Y. Madrid-Albarran and C. Camara. 2004. Establishment of selenium uptake and species distribution in lupine, Indian mustard, and sunflower plants. J. Agric. Food Chem. 52:832–838.

Xu, J. and Q. Hu. 2004. Effect of foliar application of selenium on the antioxidant activity of aqueous and ethanolic extracts of selenium-enriched rice. J. Agric. Food Chem, 52(6): 1759-1763.

Xu, H., D.K. Biswas, W.D. Li, S.B. Chen, S.B. Zhang, G.M. Jiang and Y.G. Li. 2007. Photosynthesis and yield responses of ozone-polluted winter wheat to drought. Photosynthetica. 45:582–588.

Xu, H. and R. Ihii. 1996. Wheat cultivars differences in photosynthetic response to low soil water potentials. 2. Maintenance of leaf turgor and relative water content. Jap. J. Crop Sci. 65(3):518-524.

Xue, T.L., H. Hartikainen and V. Piironen. 2001. Antioxidative and growth-promoting effects of selenium on senescing lettuce. Plant Soil. 237:55-61.

Yamur, M. and D. Kaydan 2008. Alleviation of osmotic stress of water and salt in germination and seedling growth of triticale with seed priming treatments. African J. Biotech. 7: 2156-2162.

Yan, J., F. Wang, H. Qin, G. Chen, N. Eviatar, T. Fahima and J. Cheng. 2011. Natural Variation in Grain Selenium Concentration of Wild Barley, Hordeum spontaneum, Populations from Israel. Biol. Trace Elem. Res. 142:773–786. doi: 10.1007/s12011-010-8770-6.

Yancey, P.H. 1994. Compatible and counteracting solutes. p. 81-109. *In* Strange SK (ed) Cellular and Molecular Physiology of Cell Volume Regulation. CRC Press, Boca Raton.

Yancy, P.H., M.E. Clark, S.C. Hand, R.D. Bowlus and G.N. Somero. 1982. Living with water stress: evolution of osmolyte systems. Science. 217:1214–1223.

Yang X. and C. Lu. 2006. Effects of exogenous glycinebetaine on growth, CO_2 assimilation, and photosystem IIphotochemistry of maize plants. Physiol. Plantar. 127(4):593-602.

Yang, F., L. Chen, Q. Hu and G. Pan. 2003. Effect of the application of selenium on selenium content of soybean and its products. Biol. Trace Elem. Res. 93:249–256.

Yang, Y., C. Han, Q. Liu, B. Lin and J. Wang. 2008. Effect of drought and low light on growth and enzymatic antioxidant system of Picea asperata seedlings, Acta Physiol. Plantar. 30: 433-440

Yao, X., J. Chu and G. Wang. 2009. Effects of selenium on wheat seedlings under drought stress. Biol. Trace Elem. Res. 130:283-290.

Yao, X., C. Jianzhou, H. Xueli, L. Binbin, L. Jingmin and Y. Zhaowei. 2013. Effects of selenium on agronomical characters of winter wheat exposed to enhanced ultraviolet-B. Ecotoxicol. Environ. Safety. 92:320–326.

Yao, X., J. Chu, X. He, C. Ba. 2011. Protective role of selenium in wheat seedlings subjected to enhanced UV-B radiation. Russian J. Plant Physiol. 58(2):283–289.

Yao, X.Q., J.Z. Chu and C.J. Ba. 2010a. Antioxidant responses of wheat seedlings to exogenous selenium supply under enhanced ultraviolet-B. Biol. Trace Elem. Res. 136(1):96–105.

Yao, X.Q., J.Z. Chu and C.J. Ba. 2010b. Responses of wheat roots to exogenous selenium supply under enhanced ultraviolet-B. Biol. Trace Elem. Res. 137(2):244–252.

Yassen, A.A., M.A. Safia and M.Z. Sahar. 2011. Impact of Nitrogen Fertilizer and Foliar Spray of Selenium on Growth, Yield and Chemical Constituents of Potato plants, Aus. J. Basic Appl. Sci. 5(11):1296-1303,

Yathavakilla, S. and J. Caruso. 2007. A study of Se-Hg antagonism in *Glycine max* (soybean) roots by size exclusion and reversed phase HPLC–ICPMS. Anal. Bioanal. Chem. 389:715–723. doi:10.1007/s00216-007-1458.

Yemm, E.W. and A.J.Willis. 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 57:508–514.

Ylaranta. T. 1983b. Effect of applied selenite and selenate on the selenium content of barley (*Hordeum vulgare*). Ann. Agri. Fenn. 22:164-174.

Ylaranta, T. 1984. Raising the selenium content of spring wheat and barley using selenite and selenate. Ann. Agri. Fenn. 23:75-84.

Yong-xiang, X.I.A, L.I.U. Shi-qi, L.I. He and C. Xiang-wei. 2012. Effects of selenium on physiological characteristics, selenium content and quality of garlic. Acta Metall. Sin. 18(3):733-741.

Yordanov, I., V. Velikova and T. Tsonev. 2003. Plant responses to drought and stress tolerance. Bulg. J. Plant Physiol., (Special Issue): 187-206.

Yuan, J., J. Zhang and Z. Wang. 1999. Effects of water stress at seedling growth stages on later growth and yield in wheat. Agric. Sci. China. 38(11-12):430-434.

Zahedi, H., A.H.S. Rad and H.R.T. Moghadam. 2012. Effect of zeolite and selenium foliar application on growth, production and some physiological attributes of three canola (*Brassica napus* L.) cultivars subjected to drought stress. Revista Científica UDO Agrícola 12(1):135-142.

Zarei, L., E. Farshadfar, R. Hagparast, R. Rajabi and M. S. Badieh. 2007. Evaluation of some indirect traits and indices to identify drought tolerance in bread wheat (*Triticum aestivum* L.). Asian J. Plant Sci. 6:1204-1210.

Zayed, A., C.M. Lytle and N. Terry. 1998. Accumulation and volatilization of different chemical species of selenium by plants. Planta. 206(2):284-292.

Zembala, M., M. Filek, S. Walas, H. Mrowiec, A. Korna´s, Z. Miszalski and H. Hartikainen. 2010. Effect of selenium on macro- and microelement distribution and physiological parameters of rape and wheat seedlings exposed to cadmium stress. Plant Soil. 329(1–2): 457–468.

Zhang, J., H.T. Nguyen and A. Blum. 1999. Genetic analysis of osmotic adjustment in crop plants. J. Exp. Bot. 50:292–302.

Zhang, L., Q. Li, X. Yang and Z. Xia. 2012. Effects of sodium selenite and germination on the sprouting of chickpeas (*Cicer arietinum* L.) and its content of selenium, formononetin and biochanin A in the sprouts. Biol. Trace Elem. Res. 146(3):376-80. doi: 10.1007/s12011-011-9261-0.

Zhang, Y.L., G.X. Pan, J. Chen and Q.H. Hu. 2003. Uptake and transport of selenite and selenate by soybean seedlings of two genotypes. Plant Soil. 253:437–443.

Zhang, P.Z., H. Cheng, R.L. Edwards, F.H. Chen, Y.J. Wang, X.L. Yang, J. Liu, M. Tan, X.F. Wang, J.H. Liu, C.L. An, Z.B. Dai, J. Zhou, D.Z. Zhang, J.H. Jia, L.Y. Jin and K.R. Johnson. 2008. A test of climate, sun, and culture relationships from an 1810-year Chinese cave record. Science. 322:940–942.

Zhao, F.J., F.J. Lopez-Bellido, C.W. Gray, W.R. Whalley, L.J. Clark and S.P. McGrath. 2007. Effects of soil compaction and irrigation on the concentrations of selenium and arsenic in wheat grains. Sci. Total Environ. 372(2-3):433-439.

Zhao, Y., P. Wu, Y. Wang and H. Feng. 2013. Different approaches for selenium biofortification of pear-jujube (*Zizyphus jujuba* M. cv. Lizao) and associated effects on fruit quality. J. Food, Agri. Environ. 11 (2):529-534.

Zhu, J.K. 2001. Plant salt tolerance. Trends Plant Sci. 6:66–71.

Živčák, M., J. Repková, K. Olšovská and M. Brestič. 2007. Osmotic adjustment in winter wheat varieties and its importance as a mechanism of drought tolerance. Cereal Res. Commun. 37:569-572.

Zlatev, Z. and I. Yordanov. 2004. Effects of soil drought on photosynthesis and chlorophyll fluorescence in common bean plants. Bulg. J. Plant Physiol. 30(3-4):3-18.

Zlatev, Z.S., F.C. Lidon, J.C. Ramalho and I.T. Yordanov. 2006. Comparison of resistance to drought of three bean cultivars. Plant Biol. 50:389–394.