Pak. J. Bot., 39(5): 1609-1621, 2007.

POTASSIUM-CALCIUM INTERRELATIONSHIP LINKED TO DROUGHT TOLERANCE IN WHEAT (*TRITICUM AESTIVUM* L.)

SYED ABDUL MAJID*, REHANA ASGHAR[1] AND GHULAM MURTAZA

Department of Botany, University of Azad Jammu & Kashmir, Muzaffarabad, Pakistan.
[1]Department of Botany, University of Arid Agriculture, Rawalpindi, Pakistan.
**Corresponding Author's e-mail: samajida@hotmail.com*

Abstract

Four wheat (*Triticum aestivum* L.) genotypes *viz*. Rawal-87, Inqalab-91, Potohar-93 and Chakwal-97 were grown under pre-anthesis, post-anthesis and terminal drought stress against unstressed condition in lysimeters to study the calcium and potassium interrelationship and their relation to drought tolerance at booting and milking stages of crop growth. Gypsum block method was used to monitor drought stress in the soil. Calcium and potassium contents were quantified by flame photometer from fully expanded flag leaves. The calcium and potassium contents showed compromising attitude towards drought tolerance. Results of the study suggested that Chakwal-97 cultivar had more than others tendency to adapt stressful environment. Higher ratio of K^+/Ca^{2+} in response to drought stress demonstrated the tolerance of wheat cultivars against stress. In the present study Potohar-93 showed highest ratio as compared to other cultivars at booting as well as milking stage during both of the years, showing best tolerance response against drought whereas drought imposed during different stages of growth and terminal drought affected differentially, showing tolerant abilities of different cultivars at different stages. Although Potohar-93 showed overall best tolerance, Rawal-87 responded best in pre-anthesis drought condition on the basis of K^+/Ca^{2+} ratio.

Introduction

Plants are constantly exposed to a variety of environmental stresses causing reduced crop yield. Drought as an environmental stress affects a number of physiological and developmental processes. It causes plant water deficits that reduce cell turgor, causing closure of stomata and reduction in cell enlargement, thereby reducing both the leaf surface area and the rate of photosynthesis per unit leaf area. Stomatal conductance may be influenced under drought conditions due to (1) changes in leaf-water potential and (2) metabolic changes in the leaf. There is evidence for non-hydraulic root-to-shoot communication on soil water status, which causes stomata to close without changes in the water potential and the turgor of the leaf (Gollan *et al*., 1992). The regulation of stomatal apertures by guard-cell osmotic potential was established well before the turn of the century. Early physiologists explained guard-cell osmo-regulation on the basis of starch-sugar hypothesis. However, the potassium (K^+) hypothesis dominated contemporary thinking in stomatal physiology. Numerous studies have documented K^+ uptake during stomatal opening (Talbott & Zeiger, 1996). The photosynthetic capacity of chloroplasts was inhibited upon exposure to hypertonic reaction medium (low water potential) and that inhibition was partially reversed by K^+ or NH_4^+ that facilitate stromal alkalinization (Berkowitz & Christa, 1985). Stromal alkalinization agents have also been shown to stimulate photosynthesis of dehydrated leaf slices (Berkowitz *et al*., 1983). This means that endogenous leaf K^+ content might influence the effects of dehydration on non-stomatal-mediated photosynthesis. Potassium is a major osmotically active solute of plant cells (Mengel & Arncke, 1982). Leaf K^+ is thought to facilitate reduced ψ_π (osmotic potential) leading to turgor maintenance which allows for cell expansion. Leaf K^+ is also involved in the maintenance of hydraulic conductivity gradients between leaves and soil water. Potassium has also been found to accumulate above normal levels in the leaves of

some plants under water stress conditions during osmotic adjustment and in response to lowered root medium ψ_π (Berkowitz & Christa, 1985). Kaiser (1982) has shown that species, in which photosynthesis was increasingly resistant to cell dehydration, had elevated levels of endogenous K^+. Plants respond to stress through many mechanisms, stomatal regulation being one of the most studied. A stomatal aperture is defined by two guard cells and responsible for gas exchange between plants and the atmosphere (Zeiger, 1983; Mansfield *et al.*, 1990). Changes in guard cell turgor that instigate stomatal movements are controlled by a number of ion channels and pumps (Raschke *et al.*, 1988; Hedrich & Schroeder, 1989; Blatt, 1991; Ward *et al.*, 1995). Among the ion channels in guard cells the inward K^+ channel, outward K^+ channel and anion channels in the plasma membrane are well characterized by patch-clamp studies (Schroeder *et al.*, 1984, 1987; Hedrich & Schroeder, 1989). Many environmental stress factors regulate stomatal aperture through modulation of ion channel activity in guard cells (MacRolobic, 1997). Liu *et al.*, (2000) discussed that inward potassium channel in guard cells regulated stomatal movement coupled with polyamine accumulation. As an important player in stomatal regulation the inward potassium channel is an indirect target of polyamine action. Inward potassium channel-inhibiting processes or factors often inhibit stomatal opening. Such factors include ABA, high Ca^{2+} levels and polyamines. Liu *et al.*, (2000) suggested that polyamines might serve as 'chemical messenger' for plants to respond to various stress signals. Inhibition of inward potassium channel together with other unidentified polyamine-induced cellular processes modulates stomatal aperture, which serves as one of the mechanisms for protecting plants from further stress damage.

The cell membrane plays an important role in maintaining cell integrity, being involved in signal transduction and ion homeostasis during drought stress. Osmotic shock to osmosensitive cells leads to irreversible damage of the cell membrane. On the other hand osmotolerant cells can survive in water stress only if they have inherent mechanisms involved in membrane stability. Garwe *et al.*, (2003) have suggested that a cDNA, XVSAP1, from a cDNA library from dehydrated leaves of *Xerophyta viscosa*, plays a role in membrane stability.

XVSAP1 shares high identity with the K^+ transporter family. The predicted XVSAP1 consisted of a highly hydrophobic protein with two membrane lipoprotein lipid attachment sites. The presence of these sites supports the concept that XVSAP1 associates closely with the cell membrane and could be one of the components involved in the repair of membrane damage resulting from water deficit. Wang *et al.*, (2002) correlated the increased concentration of K^+ with ABA-induced dehydration tolerance in *Spathoglottis plicata*. Wiebold & Scharf (2003) stated that potassium deficiency symptoms often appear on drought stressed corn, grain sorghum and soybeans. These symptoms may occur even if the soil tests high for potassium. Dry soil conditions limit crop root growth. Potassium moves slowly in soil, so roots must continually exploit additional soil volume for potassium. If root growth is inhibited by dry soil or compaction, potassium uptake is depressed. Nayyar & Walia (2004) observed that in response to water stress in wheat, susceptible genotype had more K^+ than tolerant genotype. Since, by reviewing the literature it would not be possible that the role / concentration of K^+ in water stressed plants be comprehensible, the importance of the study is vindicated.

Calcium has many important structural and physiological roles in plants. It is important in maintaining the stability of the cell walls, membranes and membrane bound proteins due to its ability to bridge chemical residues among these structures (Nayyar, 2003). Ca^{2+} mediates several plant processes like cytoplasmic streaming, thigmotropism, cell division, cell elongation, cell differentiation, cell polarity, photomorphogenesis, plant

defense, stress-responses and stress protection and the list is growing rapidly (Nayyar, 2003). Now it has been firmly established as an important component of a diverse array of plant signal transduction pathways (Nayyar, 2003). Among the various plant processes involving Ca^{2+}, responses of plants to their ever-changing environment draws particular attention. Intracellular Ca^{2+} concentration is kept low (100 to 200 nM) and is precisely regulated in order to save the cell from toxicity (Sanders *et al*., 1999). Various environmental stimuli affect Ca^{2+} channels located at the plasma membrane and organellar membranes to elevate its levels in the cytosol, which may serve to transduce messages and amplify signals by triggering an intracellular cascade of biochemical processes. ATP-dependent Ca^{2+}-pumps or H^+ gradient-driven Ca^{2+}/H^+ antiports located variously at the plasma and intracellular membranes maintain low cytosolic Ca^{2+} by translocating Ca^{2+} into the external space and internal pools. Opening of Ca^{2+} permeable channels in the plasma or intracellular membranes there allows Ca^{2+} to move down its concentration gradient into the cytosol and hence to generate a Ca^{2+} signal. Among the various abiotic stresses, signal transduction of water stress has invited more attention. Molecular functioning as osmosensors and abscisic acid receptors have not been identified in higher plants as yet and based upon knowledge of osmosensors in yeast and bacteria (Nayyar, 2003).

Asghari *et al.*, (2001) studied ABA, K^+, Ca^{2+} and mannitol content in two wheat lines and suggested the possibility of using the ratio of K^+ / Ca^{2+} for predicting the tolerant plants against drought. Probably it is the result of interference between the factors and involvement of them in permeability of cell membrane, such as stomatal membrane and also the membrane of chloroplasts, which are important in the regulation of guard-cell turgor and stomatal aperture. Asghari *et al.*, (2001) introduced it as a regulator or an indicator for stomatal movement. Considering the mannitol as a polyol that affects water-potential of guard-cell and scavenge hydroxyl radicals under water deficit could confirm this hypothesis. In this study they declared Boolany as non-tolerant wheat lines because of having same ratio of K^+ / Ca^{2+} in water stressed and non-stressed conditions. Whereas other one Ghods showed tolerance as the ratio of K^+ / Ca^{2+} was higher in stressed condition as compared to non-stressed condition. However, the exact role of the ratio of K^+ / Ca^{2+} on the position of stomata are not yet known which needs further investigation (Asghari *et al*., 2001).

Materials and Methods

The *In-vitro* field (lysimeter) study was carried out at experimental area of Department of Botany, University of A. J. & K., Muzaffarabad, Azad Kashmir. Wheat (*Triticum aestivum* L.) seeds of four genotypes *viz*., Rawal-87, Inqalab-91, Potohar-93 and Chakwal-97 were obtained from Barani Agricultural Research Institute (BARI), Chakwal, Pakistan. Rawal-87, Potohar-93 and Chakwal-97 were selected for their drought tolerance while Inqalab-91 as general purpose variety. Specially made for the purpose, 12 pots made of zinc sheet having size of 0.93 x 1.23 x 0.30 m^3 (3 feet x 4 feet x 1 foot) were filled with equal amount of 400 kg of previously analyzed loam-textured soil of pH 7.2. Before filling the pots, the soil was fertilized with N:P:K @ of 90:90:60 Kg ha^{-1} with urea, single super phosphate and sulphate of potash.

Each pot was considered one block, used for one replication of the treatments having four rows of all the genotypes tested on randomized basis with the distance of 20 cm according to randomized complete block design. The seeds were sown in the rows at a distance of 5 cm. For this purpose, 40 seeds were sown initially and after the germination seedlings were thinned at the required distance.

As recommended for wheat crop (Ahmad & Arain, 1999; Siddique *et al*., 1999, 2000), four irrigations for the normal water requirement of the crop were applied at: a) pre-sowing, b) tillering stage, c) pre-anthesis stage and d) post-anthesis stage to the soil saturation level.

A total of four treatments viz., a) no drought (control), b) pre-anthesis drought, c) post-anthesis drought and d) both pre- and post-anthesis drought (terminal drought) were applied to the experiment with 3 replications according to Trethowan (2000). All the replicates were applied with the first two irrigations. The stress was created by checking the third irrigation in one treatment, the fourth in the other treatment and both, the third and the fourth in the last treatment. Gypsum block method was used to monitor the water status of the soil during crop growth. Minimum level of 1.0 MPa water potential was maintained by applying a limited amount of water as and when needed. Protection from rain was provided by manually operated shelter equipped with movable sheet of transparent polythene on the fence made by iron-pipes. All agronomic practices like hoeing, weeding etc. were kept normal and uniform. Fully developed flag leaves were sampled for biochemical analysis at booting (Zadoks scale 45) and milking (Zadoks scale 70) stage (Zadoks *et al*., 1974). These samples were stored for further biochemical analysis at –50 °C in a deep freezer.

For the determination of potassium and calcium, one gram of flag leaves was boiled in 10 ml of perchloric acid for 30 minutes to get a dissolved suspension. Deionized water was added in the suspension to make the volume one liter in volumetric flask. This digested solution of leaves was used for the estimation of potassium and calcium contents with the help of JENWAY PFP 7 Flame photometer. A standard curve was prepared using KOH and CaOH respectively for the reference of K^+ and Ca^{2+} in the sample. The results were described in µg per gram of flag leaves. The experiment was conducted using randomized complete block design (RCBD). Analysis of variance was performed on the basis of factorial experiment and least significant difference test (LSD) at0.05 level of significance was used to separate the means according to Steel *et al.*, (1997). The entire statistical work was done using the computer package MSTATC. The experiment was repeated in the next year.

Results and Discussion

Drought stress affects a number of physiological and developmental processes (Mundree *et al*., 2002). Plants' strategies to cope with drought, involve a mixture of 'stress avoidance' and 'stress tolerance' (Chaves *et al*., 2002). One of the most common responses in all organisms subjected to water deficit is the production and/or accumulation of compatible osmolytes (Mundree *et al*., 2002). Osmolytes were originally thought to function mainly in osmotic adjustment by lowering cellular osmotic potential to facilitate water absorption and restore intracellular ion concentrations (Yancey *et al*., 1982). These may be important for maintaining the conformation of macromolecules (Xiong & Zhu, 2002). Many organic osmolytes are presumed to be osmoprotectants, as their levels of accumulation are insufficient to facilitate osmotic adjustment (Yokoi *et al*., 2002). Many other biological functions of osmolytes have been established after the advent of antisense transgene technology (Nanjo *et al*., 1999; Koch, 2004). But Serraj & Sinclair (2002) concluded after reviewing the studies of last 20 years that osmolyte accumulation does not really help to increase the crop yield under drought conditions. However, they discussed the beneficial response to yield that osmolyte accumulation maintains root development in order to reach the water that may be available deeper in the soil profile. So, osmolytic behavior under drought conditions is still an enigma for the stress physiologists.

Common osmolytes include sugars, amino acids and/or their derivatives, referred to as compatible metabolites because they do not interfere with the normal cellular metabolism (Sairam & Tyagi, 2004). These osmolytes can be classified into organic and inorganic osmolytes. Proline, glycine-betaine, sucrose and other derivatives of carbohydrates etc., are the example of organic osmolytes and K^+ and Ca^{2+} are the example of inorganic/ionic osmolytes (Yokoi *et al*., 2002). The K^+ contents in flag leaves were increased in response to drought stress, imposed on wheat during the different stages of crop growth (Table 1 and 2).

Table1. Effect of drought stress on Potassium contents (µg.g^{-1} FL) in flag leaves of wheat varieties at booting stage.

Stress→ varieties↓	Control	Pre-anthesis drought	Post-anthesis drought	Terminal drought	Means
Year 2002-03					
Rawal-87	94.667h	131.000ef	99.667gh	134.333e	114.917D
Inqalab-91	126.667ef	136.000e	140.000e	163.333d	141.500C
Potohar-93	115.667fg	168.667cd	172.000bcd	174.667bcd	157.750B
Chakwal-97	170.333bcd	182.333bc	185.667ab	200.000a	184.583A
Means	126.833C	154.500B	149.333B	168.083A	149.69***
Year 2003-04					
Rawal-87	100.667g	120.137ef	122.327ef	129.933ef	118.266C
Inqalab-91	130.217ef	128.087ef	150.690bc	134.703cde	135.924B
Potohar-93	117.220fg	162.147ab	152.260b	162.810ab	148.609A
Chakwal-97	161.070ab	147.070bcd	133.953de	176.767a	154.715A
Means	127.293C	139.360B	139.808B	151.053A	139.38***

Means followed by similar letters are not significant to each other at $P = 0.05$.
LSD for Varieties: 8.343
LSD for Stresses: 8.343
LSD for Varieties X Stresses: 16.69
***: Very highly significant

Table 2. Effect of drought stress on Potassium contents (µg.g^{-1} FL) in flag leaves of wheat varieties at milking stage.

Stress→ varieties↓	Control	Pre-anthesis drought	Post-anthesis drought	Terminal drought	Means
Year 2002-03					
Rawal-87	127.667def	154.667a	144.000abc	148.333abc	143.667A
Inqalab-91	117.333fg	150.333ab	137.667bcd	146.000abc	137.833A
Potohar-93	105.667g	128.000def	134.000cde	120.333efg	122.000B
Chakwal-97	155.000a	149.333ab	140.667abcd	117.000fg	140.500A
Means	126.417C	145.583A	139.083AB	132.917BC	144.25***
Year 2003-04					
Rawal-87	125.903h	147.740efg	141.923fg	163.750bcd	144.829C
Inqalab-91	187.880a	168.290b	153.353cdef	151.830def	165.338A
Potohar-93	143.277fg	133.463gh	148.923def	125.983h	137.912C
Chakwal-97	147.887efg	161.947bcde	168.230bc	145.157fg	155.805B
Means	151.237A	152.860A	153.107A	146.680 A	151.59***

Means followed by similar letters are not significant to each other at $P = 0.05$.
LSD for Varieties: 7.442
LSD for Stresses: 7.442
LSD for Varieties X Stresses: 14.88
***: Very highly significant

Potassium contents at booting stage: Very highly significantly increased K^+ contents were observed in the year 2002-03 (149.69 $\mu g.g^{-1}$ FL) as compared to that in the next year (139.38 $\mu g.g^{-1}$ FL) at booting stage (Table 1). Chakwal-97 (184.58 $\mu g.g^{-1}$ FL) in 2002-03 and Chakwal-97 (154.72 $\mu g.g^{-1}$ FL) and Potohar-93 (148.61 $\mu g.g^{-1}$ FL) in 2003-04 showed highest significant K^+ contents at booting stage, followed by Inqalab-91 and Rawal-87 during both years. A similar pattern was observed during both years where terminal drought stress induced maximum K^+ content (168.08 and 151.05 $\mu g.g^{-1}$ FL), that was significantly higher, followed by pre- and post-anthesis drought stresses which were non-significant to each other. Highest K^+ concentrations (200.00 and 176.77 $\mu g.g^{-1}$ FL) were recorded in Chakwal-97 during both the years under terminal drought stress, followed by post-anthesis stress in 2002-03 and then by pre-anthesis stress in 2003-04. Rawal-87 responded poorly as pre-anthesis and terminal drought showed a significant increase in K^+ contents as compared to control, but non significant to each other, whereas post-anthesis drought stress increased K^+ contents non-significantly during 2002-03. In the next year, although the entire drought stress conditions increased K^+ contents significantly to the control, but non-significant to each other. Potohar-93 and Inqalab-91 accumulated K^+ contents less than that of Chakwal-97, but more than that of Rawal-87.

Potassium contents at milking: At milking stage, during 2002-03 wheat varieties accumulated K^+ contents very highly significantly lower (144.25 $\mu g.g^{-1}$ FL) than that in the next year (151.59 $\mu g.g^{-1}$ FL) (Table 2). Maximum K^+ contents (143.67 $\mu g.g^{-1}$ FL) were recorded in Rawal-87, followed by Chakwal-97 (140.50 $\mu g.g^{-1}$ FL) and Inqalab-91 (137.83 $\mu g.g^{-1}$ FL) in 2002-03 at milking stage whereas in the next year, Inqalab-91 accumulated (165.34 $\mu g.g^{-1}$ FL) maximum K^+ contents, followed by Chakwal-97 (155.81 $\mu g.g^{-1}$ FL). Rawal-87 (144.83 $\mu g.g^{-1}$ FL) and Potohar-93 (137.91$\mu g.g^{-1}$ FL) accumulated significantly lower K^+ contents, non-significant to each other at milking stage. Pre-anthesis drought induced maximum K^+ contents (145.58 $\mu g.g^{-1}$ FL) during 2002-03, followed by post-anthesis drought (139.08 $\mu g.g^{-1}$ FL) which was significantly higher to K^+ contents as compared to control (126.42 $\mu g.g^{-1}$ FL). In the year 2003-04, a lower K^+ concentration (146.68 $\mu g.g^{-1}$ FL) was observed under terminal drought stress, however all stresses induced non-significant differences (152.86 and 153.11 $\mu g.g^{-1}$ FL) as compared to that of control (151.24 $\mu g.g^{-1}$ FL). Chakwal-97 showed a decreasing pattern in accumulation of K^+ contents under different drought stresses during 2002-03 at milking stage as compared to other varieties. The other three varieties accumulated K^+ contents under drought stress more than that of control. Maximum concentration (155.00 $\mu g.g^{-1}$ FL) was recorded in Chakwal-97 under control condition, followed by Rawal-87 (154.67 $\mu g.g^{-1}$ FL) under pre-anthesis drought but this was not a significant difference. Significantly higher K^+ contents were observed in Rawal-87 as a result of drought stress imposed during different periods than the control in 2002-03. In the next year, Inqalab-91 showed significantly decreased amount of K^+ contents (168.29 $\mu g.g^{-1}$ FL) under pre-anthesis drought stress as compared to that (187.88 $\mu g.g^{-1}$ FL) of control. Similarly, post-anthesis (153.35 $\mu g.g^{-1}$ FL) and terminal drought stress (151.83 $\mu g.g^{-1}$ FL) induced even significantly lower quantities of K^+ contents, which were non significant to each other (Table 2).

Potassium is most abundant cation in the cytosol of the plant cells. The cell requires this inorganic cation, compatible with protein structure at high concentration, to neutralize dissociated organic acids and anionic groups of macromolecules (nucleic acid and phospholipids) and to lower water potential (Cherel, 2004). Increased concentration

of charged elements in the cytosol may change hydration sphere of macromolecules and thus affects their conformation or charge interactions (Xiong & Zhu, 2002). Compatible osmolytes may be important for maintaining the conformation of macromolecules. Stomatal conductance may be influenced under drought conditions. This occurs due to 1) changes in leaf-water potential and 2) metabolic changes in the leaf. There is evidence for non-hydraulic root-to-shoot communication on soil water status which causes stomata to close without changes in the water potential and the turgor of the leaf (Gollan *et al*., 1992). The stomatal apertures regulated by guard cell osmotic potential, are explained on the basis of starch sugar hypothesis. However, the K^+ hypothesis dominates contemporary thinking in stomatal physiology. Leaf K^+ is thought to facilitate reduced ψ_π (osmotic potential) leading to turgor maintenance which allows for a cell expansion. Leaf K^+ is also involved in the maintenance of hydraulic conductivity gradients between leaves and soil water. When there is insufficient water, plants growing without an adequate K^+ supply, wilt quickly because of inadequate control of the stomatal opening and closing. The present study revealed that under drought stress, potassium accumulation showed its primary significance of stomatal regulation as it was one of the stress avoidance phenomena. Potassium determined the stress avoidance of the crop whenever that was imposed, by regulating the opening and closing the stomata. Nayyar & Walia (2004) concluded that susceptible genotype of wheat contained more of potassium in flag leaves under water stress than tolerant genotype. Chakwal-97 seems to be susceptible under terminal drought while Potohar-93 looks to be better in pre- and post-anthesis drought separately than Chakwal-97 at booting stage in correspond to Nayyar & Walia (2004). At milking stage not a single cultivar show a clear cut response to be tolerant against drought of any type, however Potohar-93 seems to be most tolerant one.

Calcium contents at booting stage: Drought stress influenced Ca^{2+} contents very highly significantly in wheat varieties as studied in the flag leaves at booting stage (Table 3). In the year 2003-04, significantly higher contents (28.11 $\mu g.g^{-1}$ FL) were recorded as compared to that of the previous year (24.11 $\mu g.g^{-1}$ FL). Chakwal-97 showed maximum accumulation of Ca^{2+} in 2002-03 (32.13 $\mu g.g^{-1}$ FL) as well as in 2003-04 (35.10 $\mu g.g^{-1}$ FL), followed by Inqalab-91 (27.55 and 32.73 $\mu g.g^{-1}$ FL) during both the years. Potohar-93 accumulated approximately equal amount of Ca^{2+} contents (21.42 and 21.80 $\mu g.g^{-1}$ FL) in both the years. Rawal-87 accumulated significantly lowest Ca^{2+} contents (15.36 $\mu g.g^{-1}$ FL) in 2002-03 and non-significantly higher (22.80 $\mu g.g^{-1}$ FL) than that of Potohar-93 in the next year. Terminal drought induced maximum accumulation (28.18 $\mu g.g^{-1}$ FL) of Ca^{2+}, highly significant to that of control, followed by that of induced by pre-anthesis drought (24.10 $\mu g.g^{-1}$ FL) in the year 2002-03. It was found that post-anthesis drought stress induced (21.66 $\mu g.g^{-1}$ FL) non-significantly decreased Ca^{2+} contents than that (22.10 $\mu g.g^{-1}$ FL) of control. Terminal drought, during 2003-04 too, induced significantly maximum accumulation (29.87 $\mu g.g^{-1}$FL) of Ca^{2+} contents. Post- and pre-anthesis drought increased Ca^{2+} contents non-significantly to control condition and to each other. Chakwal-97 accumulated maximum Ca^{2+} under terminal and pre-anthesis drought stress as compared to non-stressed plants in both the years. In addition, only Inqalab-91 under terminal drought stress accumulated highly significant Ca^{2+} contents. At that stage, a general trend of increased Ca^{2+} contents under terminal and pre-anthesis drought and a decreased under post-anthesis drought as compared to control was observed in both of the years.

Table 3. Effect of drought stress on Calcium contents (µg.g^{-1} FL) in flag leaves of wheat varieties at booting stage.

Stress→ varieties↓	Control	Pre-anthesis drought	Post-anthesis drought	Terminal drought	Means
Year 2002-03					
Rawal-87	15.130g	11.090h	13.877g	21.343ef	15.360D
Inqalab-91	26.653d	30.517c	23.597e	29.417c	27.546B
Potohar-93	19.913f	22.040ef	20.433f	23.283e	21.417C
Chakwal-97	26.690d	34.420b	28.747cd	38.667a	32.131A
Means	22.097C	24.517B	21.663C	28.177 A	24.114***
Year 2003-04					
Rawal-87	26.010d	18.580g	25.417de	21.183fg	22.798C
Inqalab-91	30.001c	30.588c	33.237b	37.087a	32.728B
Potohar-93	18.823g	21.700f	23.657def	23.017ef	21.799C
Chakwal-97	34.017b	38.223a	29.990c	38.183a	35.103A
Means	27.213B	27.273B	28.075B	29.867A	28.107***

Means followed by similar letters are not significant to each other at $P = 0.05$.
LSD for Varieties: 1.312
LSD for Stresses: 1.312
LSD for Varieties X Stresses: 2.625
***: Very highly significant

Table 4. Effect of drought stress on Calcium contents (µg.g^{-1} FL) in flag leaves of wheat varieties at milking stage.

Stress→ varieties↓	Control	Pre-anthesis drought	Post-anthesis drought	Terminal drought	Means
Year 2002-03					
Rawal-87	19.993f	17.040gh	18.653fg	22.930e	19.654C
Inqalab-91	40.523ab	38.537bc	42.500a	38.890bc	40.113A
Potohar-93	16.450gh	13.250i	14.637hi	13.323i	14.415D
Chakwal-97	38.243bc	30.837d	37.297c	33.310d	34.922B
Means	28.803A	24.916C	28.272AB	27.113B	27.276^{NS}
Year 2003-04					
Rawal-87	28.620c	19.367ef	21.273e	24.730d	23.498C
Inqalab-91	35.797b	37.123ab	38.600a	38.440a	37.490A
Potohar-93	17.027fg	17.367fg	15.237g	19.080ef	17.177D
Chakwal-97	29.007c	31.017c	37.850ab	35.840b	33.428B
Means	27.613B	26.218C	28.240B	29.523A	27.898^{NS}

Means followed by similar letters are not significant to each other at $P = 0.05$.
LSD for Varieties: 1.263
LSD for Stresses: 1.263
LSD for Varieties X Stresses: 2.525
^{NS}: Non significant

Calcium contents at milking stage: A non-significant increase (27.90 µg.g^{-1} FL) in Ca^{2+} contents at milking stage was recorded during 2003-04 as compared to that (27.28 µg.g^{-1} FL) in the previous year (Table 4). At milking stage, Inqalab-91 showed significantly highest Ca^{2+} contents in both of the years (40.11 and 37.49 µg.g^{-1} FL), followed by Chakwal-97 that accumulated 34.92 and 33.43 µg.g^{-1} FL during 2002-03 and 2003-04, respectively. Rawal-87 and Potohar-93 showed significantly low response in accumulating Ca^{2+} contents in both of the years at milking stage. In 2002-03, drought stress conditions decreased the Ca^{2+} contents, non-significantly under post-anthesis (28.27 µg.g^{-1} FL) and significantly under terminal (27.11 µg.g^{-1} FL) and pre-anthesis

(24.92 μg.g^{-1} FL) drought stress as compared to that (28.80 μg.g^{-1} FL) of control. In the next year, terminal drought stress induced maximum contents (29.52 μg.g^{-1} FL) of Ca^{2+}, significantly higher than that (27.61 μg.g^{-1} FL) of control and significant difference was found at post-anthesis drought stress (28.24 μg.g^{-1} FL). Minimum contents (26.22 μg.g^{-1} FL), even significantly lower than that of control, were observed under pre-anthesis drought stress. An augmented Ca^{2+} contents were observed in Inqalab-91 under post-anthesis drought stress in the year 2002-03 (42.50 μg.g^{-1} FL) and under post-anthesis and terminal drought in next year (38.60 and 38.44 μg.g^{-1} FL). Inqalab-91 accumulated Ca^{2+} contents more under post-anthesis drought (42.50 μg.g^{-1} FL) whereas less under terminal (38.89 μg.g^{-1} FL) and pre-anthesis drought (38.54 μg.g^{-1} FL) as compared to control (40.52 μg.g^{-1} FL) in 2002-03, while in next year drought stress increased the Ca^{2+} contents as compared to that of control. Similar trend of increasing and decreasing of Ca^{2+} contents was observed in Chakwal-97. However, the other two varieties, Rawal-87 and Potohar-93, did not exhibit a definite pattern in response to drought stress.

According to Matsumoto *et al*. (2002), increased cytosolic Ca^{2+} was the result of hyperosmotic stress. Stress-induced increase in cytosolic Ca^{2+}, derived from an extra-cellular pool, played a role of osmosensing in stressful environment (Matsumoto *et al*., 2002). In plant cells, the list of messengers used by signaling pathways includes Ca^{2+}, lipids, pH and cyclic GMP. However, no single messenger had been demonstrated to respond to more stimuli than had cytosolic free Ca^{2+} (Sanders *et al*., 1999). Drought stress elevated the cytosolic free Ca^{2+} in plant cells (Knight *et al*., 1997). In plants, exogenous H_2O_2 or ABA-induced H_2O_2 activated Ca^{2+} channels in guard cells and mediated stomatal closure (Pei *et al*., 2000). Increased Ca^{2+} was presumed to facilitate the stomatal closure in response to osmotic stress, by acting as secondary messenger (Shinozaki & Yamaguchi-Shinozaki, 1997). Results of present study revealed that Chakwal-97 cultivar was more adapted to stressed environment, in agreement with the conclusions of Matsumoto *et al*., (2002), followed by Inqalab-91 in the year 2002-03 where as in the next year (2003-04) Inqalab-91 remained ahead to Chakwal-97 in adaptation to drought condition.

K^+ / Ca^{2+} Ratio at booting stage: The potassium / calcium (K^+/Ca^{2+}) ratio, shown in Table 5, exhibited a very highly significantly higher value (6.58) for the year 2002-03 as compared to that (5.19) of the next year at booting stage as a result of drought stress on wheat varieties. Highest ratio was observed for Rawal-87 (7.92), followed by Potohar-93 (3.38) during 2002-03 and for Potohar-93 (6.81) and Rawal-87 (5.34) during 2003-04. Significantly enhanced ratios were observed under pre- (7.35) and post-anthesis (7.00) drought, although non-significant to each other, than that of terminal (6.13) drought and control (5.83) conditions in the year 2002-03 while in the next year, significant increase in ratios were obtained under pre-anthesis (5.50) and terminal (5.36) drought as compared to that of control (4.80). Post-anthesis drought induced K^+/Ca^{2+} ratio (5.09) non-significantly higher to that of control as well as pre-anthesis and terminal drought stress. Rawal-87 showed highest ratio under pre-anthesis drought stress, significantly different than all other ratios that were non-significant to each other in first year of study. Potohar-93 exhibited the augmented K^+/Ca^{2+} ratio under all the drought stress conditions as compared to that of control. Other two varieties did not follow any definite pattern at this stage during 2002-03. In the next year, Potohar-93 showed maximum K^+/Ca^{2+} ratio (7.49) under pre-anthesis drought stress, non-significantly followed by that (7.08) under terminal drought. Post-anthesis drought stress did not increase K^+/Ca^{2+} ratio significantly than that of control. The other three varieties did not exhibit any definite pattern; however, drought stress at different stages did differentially alter the K^+/Ca^{2+} ratio at booting stage in wheat.

Table 5. Effect of drought stress on Potassium / Calcium ratio in flag leaves of wheat varieties at booting stage.

Stress→ varieties↓	Control	Pre-anthesis drought	Post-anthesis drought	Terminal drought	Means
Year 2002-03					
Rawal-87	6.277de	11.917a	7.197cd	6.297de	7.922A
Inqalab-91	4.753gh	4.467h	5.930ef	5.553efg	5.176D
Potohar-93	5.870ef	7.693bc	8.427b	7.510bc	3.375B
Chakwal-97	6.398de	5.310fgh	6.457de	5.177fgh	5.835C
Means	5.825B	7.347A	7.003A	6.134B	6.577***
Year 2003-04					
Rawal-87	3.893ef	6.473bc	4.847d	6.137c	5.337B
Inqalab-91	4.347def	4.187def	4.607de	3.657f	4.199C
Potohar-93	6.233bc	7.493a	6.433bc	7.083ab	6.811A
Chakwal-97	4.737de	3.851ef	4.460def	4.580de	4.407C
Means	4.802B	5.501A	5.087AB	5.364A	5.189***

Means followed by similar letters are not significant to each other at $P = 0.05$.
LSD for Varieties: 0.4609
LSD for Stresses: 0.4609
LSD for Varieties X Stresses: 0.9218
***: Very highly significant

Table 6. Effect of drought stress on Potassium / Calcium ratio in flag leaves of wheat varieties at milking stage.

Stress→ varieties↓	Control	Pre-anthesis drought	Post-anthesis drought	Terminal drought	Means
Year 2002-03					
Rawal-87	6.420c	9.063a	7.730b	6.473c	7.422B
Inqalab-91	2.903f	3.913de	3.240ef	3.763ef	3.455D
Potohar-93	6.420c	9.747a	9.163a	9.112a	8.661A
Chakwal-97	4.063de	4.837d	3.767ef	3.510ef	4.044C
Means	4.952C	6.890A	5.975B	5.715B	5.883^{NS}
Year 2003-04					
Rawal-87	4.400ef	7.633bc	6.677cd	6.673cd	6.346B
Inqalab-91	5.253e	4.537ef	3.973f	3.957f	4.430C
Potohar-93	8.523b	7.703b	9.867a	6.607d	8.175A
Chakwal-97	5.100e	5.253e	4.443ef	4.077f	4.718C
Means	5.819AB	6.282A	6.240A	5.328B	5.917^{NS}

Means followed by similar letters are not significant to each other at $P = 0.05$.
LSD for Varieties: 0.4917
LSD for Stresses: 0.4917
LSD for Varieties X Stresses: 0.9834
^{NS}: Non significant

K^+ / Ca^{2+} Ratio at milking stage: At milking stage, the K^+/Ca^{2+} ratio non-significantly varied in the years 2002-03 (5.88) and 2003-04 (5.92) in wheat under drought stress (Table 6). Potohar-93 had highest ratio (8.66 and 8.18) in both the years, followed by Rawal-87 (7.42 and 6.35). Chakwal-97 and Inqalab-91 were found to be minimal, differed significantly (4.04 and 3.46) in 2002-03 and non-significantly (4.72 and 4.43) in 2003-04 to each other. Pre- and post-anthesis drought conditions exhibited augmented ratio, significant (6.89 and 5.98) in 2002-03 and non-significant (6.28 and 6.24) in 2003-04 to each other at this stage. Terminal drought stress behaved unpredictably in different years, significantly increased (5.72) in 2002-03 and non-significantly decreased (5.33) in

2003-04 as compared to control (4.95 and 5.82). In the year 2002-03, Potohar-93 exhibited highly significant increase in K^+/Ca^{2+} ratio in pre-, post-anthesis and terminal stress as compared to that of control. In addition, Rawal-87 was also found with increased ratio under pre-anthesis drought. The smallest ratio, ranges 2.90-3.91, was observed in Inqalab-91which was significantly increased only under pre-anthesis drought, although non-significantly increased under the other two stress conditions. In the next year, Potohar-93 showed maximum ratio under post-anthesis drought, followed by that under control and under pre-anthesis drought. Terminal drought did not induce the augmented ratio, rather decreased as compared to that of control. Similarly, Inqalab-91 exhibited again narrow ratio, like that of previous year and showed decreased ratios as compared to that of control. Chakwal-97 and Rawal-87 showed ambiguous pattern of widening and narrowing the ratio at milking stage in this year.

Asghari *et al*., (2001) studied ABA, K^+, Ca^{2+} and mannitol contents in two wheat lines and suggested the possibility of using K^+/Ca^{2+} ratio for predicting the tolerant plants against drought. They suggested that higher ratio of K^+/Ca^{2+} in response to drought stress demonstrated the tolerance of wheat cultivars against stress. In the present study, Potohar-93 showed highest ratio as compared to other cultivars at booting as well as milking stage during both years, showing best tolerance against drought. Whereas drought imposed during different stages of growth and terminal drought affected differentially, showing tolerant abilities of different cultivars at different stages. For example, although Potohar-93 showed overall best tolerance, Rawal-87 responded best at pre-anthesis drought stage on the basis of K^+/Ca^{2+} ratio.

Conclusions

Drought stress affects a number of physiological and developmental processes. One of the most common induced responses in all organisms undergoing water deficit is the production and/or accumulation of compatible osmolytes (Mundree *et al*., 2002). Osmolytes were originally thought to function mainly in osmotic adjustment by lowering cellular osmotic potential to facilitate water absorption and restore intracellular ion concentrations (Yancey *et al*., 1982). Many other biological functions of osmolytes like osmosensing and signaling, scavenging the free radicals, performing the role in osmotolerance and in morphogenesis have been established after the advent of antisense transegene technology (Nanjo *et al*., 1999; Koch, 2004). Many organic osmolytes are presumed to be osmoprotectants, as their levels of accumulation are insufficient to facilitate osmotic adjustment (Yokoi *et al*., 2002).

Increased concentration of charged elements in the cytosol may change hydration sphere of macromolecules and thus affects their conformation or charge interactions (Xiong & Zhu, 2002). Potassium is most abundant cation in the cytosol of the plant cells. The cell requires this inorganic cation, compatible with protein structure at higher concentration, to neutralize dissociated organic acids and anionic groups of macromolecules (nucleic acid and phospholipids) and to lower water potential (Cherel, 2004). Stress-induced increase in cytosolic Ca^{2+}, derived from an extra-cellular pool, played a role of osmosensing in stressful environment (Matsumoto *et al*., 2002). Results of present study, are in agreement with the conclusions of Matsumoto *et al*., (2002) suggested that Chakwal-97 cultivar had more than others tendency to adapt stressful environment. Asghari *et al*. (2001) suggested that higher ratio of K^+/Ca^{2+} in response to drought stress demonstrated the tolerance of wheat cultivars against stress. In the present

study, Potohar-93 showed highest ratio as compared to other cultivars at booting as well as milking stage during both of the years, showing best tolerance response against drought. Whereas drought imposed during different stages of growth and terminal drought affected differentially, showing tolerant abilities of different cultivars at different stages. For example, although Potohar-93 showed overall best tolerance, Rawal-87 responded best at pre-anthesis drought stage on the basis of K^+/Ca^{2+} ratio.

References

Ahmad, M. and M.A. Arain. 1999. Effect of drought simulation on grain weight, protein and lysine content of bread wheat (*Triticum aestivum* L.). *Pak. J. Bot*., 31: 109-114.

Asghari, R., H. Ebrahimzadeh and A.R. Khabiri. 2001. Effects of drought stress on abscisic acid, mannitol, K^+ and Ca^{2+} content in two lines of wheat (*Triticum aestivum* L.). *Pak. J. Bot*., 33: 197-202.

Berkowitz, G.A. and W. Christa. 1985. Leaf K^+ interaction with water stress inhibition of non-stomatal-controlled photosynthesis. *Plant Physiol*., 79: 189-193.

Berkowitz, G.A., C. Chen and M. Gibbs. 1983. Stromal acidification mediates *In vivo* water stress inhibition of non-stomatal-controlled photosynthesis. *Plant Physiol*., 72: 1123-1126.

Blatt, M.R. 1991. Ion channel gating in plants: physiological implications and integration for stomatal function. *J. Membr. Biol*., 124: 95-112.

Chaves, M.M., J.S. Pereira, J. Maroco, M.L. Rodrigues, C.P.P. Ricardo, M.L. Osorio, I. Carvalho, T. Faria and C. Pinheiro. 2002. How plants cope with water stress in the field: photosynthesis and growth. *An. Bot*., 89: 907-916.

Cherel, I. 2004. Regulation of K^+ channel activities in plants: from physiological to molecular aspects. *J. Exp. Bot*., 55(396): 337-351.

Garwe, D., J.A. Thomson and S.G. Mundree. 2003. Molecular characterization of XVSAP1, a stress-responsive gene isolated from *Xerophyta viscosa* Baker. *J. Exp. Bot*., 54(381): 191-201.

Gollan, T., U. Schurr and E.D. Schulze. 1992. Stomatal response to drying soil in relation to changes in the xylem sap composition of *Helianthus annuus*. I. The concentration of cations, anions, amino acids and pH in the xylem sap. *Plant Cell Environ*., 15: 551-559.

Hedrich, R. and J.I. Schroeder. 1989. The physiology of ion channels and electrogenic pumps in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol*., 40: 539-569.

Kaiser, W.M. 1982. Correlation between changes in photosynthetic activity and changes in total protoplast volume in leaf tissue from hydro-, meso- and xerophytes under osmotic stress. *Planta*, 154:538-545.

Knight, H., A.J. Trewavas and M.R. Knight. 1997. Calcium signaling in *Arabidopsis thaliana* responding to drought and salinity. *Plant J*., 12: 1067-1078.

Koch, K. 2004. Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. *Curr. Opin. Plant Biol*., 7: 235-246.

Liu, K., H. Fu, Q. Bei and S. Luan. 2000. Inward potassium channel in guard cells as a target for polyamine regulation of stomatal movements. *Plant Physiol.*, 124: 1315-1325.

MacRolobic, E.A.C. 1997. Signaling in guard cells and regulation of ion channel activity. *J. Exp. Bot*., 48: 515-528.

Mansfield, T.A., A.M. Hetherington and C.J. Atkinson. 1990. Some current aspects of stomatal physiology. *Annu. Rev. Plant Physiol. Plant Mol. Biol*., 41: 55-75.

Matsumoto, T.K., A.J. Ellsmore, S.G. Cessna, P.S. Low, J.M. Pardo, R.A. Bressan and P.M. Hasegawa. 2002. An osmotically induced cytosolic Ca^{2+} transient activates calcineurin signaling to mediate ion homeostasis and salt tolerance of *Saccharomyces cerevisiae*. *J. Biol. Chem*., 277(36): 3075-3080.

Mengel, K. and W.W. Arneke. 1982. Effect of potassium on the water potential, the pressure potential, the osmotic potential and cell elongation in leaves of *Phaseolus vulgaris*. *Physiol. Plant*., 54: 402-408.

Mundree, S.G. and J.M. Farrant. 2000. Some physiological and molecular insights into the mechanisms of desiccation tolerance in the resurrection plant *Xerophyta viscosa* Baker. In: (Eds.) Cherry *et al*., *Plant Tolerance to Abiotic Stresses in Agriculture: Role of Genetic Engineering*. Kluwer Academic Publ. NL pp. 201-222.

Nayyar, H. 2003. Calcium as environmental sensor in plants. *Curr. Sci*., 84(7): 893-902.

Nayyar, H. and D.P. Walia. 2004. Genotypic variation in wheat in response to water stress and abscisic acid-induced accumulation of osmolytes in developing grains. *J. Agron. Crop Sci*., 190(1): 39-45.

Pei, Z.M., Y. Murata, G. Benning, S. Thomine, B. Klusener, G.J. Allen, E. Grill and J.I. Schroeder. 2000. Calcium channels activated by hydrogen peroxide mediate abscisic acid signaling in guard cells. *Nature*, 406: 731-734.

Raschke, K., R. Hedrich, V. Reckmann and J.I. Schroeder. 1988. Exploring biophysical and biochemical components of osmotic motor that drives stomatal movements. *Bot. Acta*, 101: 283-294.

Sadiqov, S.T., M. Akbulut and V. Ehmedov. 2002. Role of Ca^{2+} in drought stress signaling in wheat seedlings. *Biochemistry* (Moscow), 67(4): 491-497.

Sairam, R.K. and A. Tyagi. 2004. Physiology and molecular biology of salinity stress tolerance in plants. *Curr. Sci*., 86(3): 407-421.

Sanders, D., C. Brownlee and J.F. Harper. 1999. Communicating with calcium. *Plant Cell*, 11: 691-706.

Schroeder, J.I., K. Raschke and E. Neher. 1987. Voltage dependence of potassium channels in guard-cell protoplasts. *Natl. Acad. Sci. Proc.*, USA, 84: 4108-4112.

Schroeder, J.I., R. Hedrich and J.M. Fernandez. 1984. Potassium selective single channels in guard-cell protoplast of *Vicia faba*. *Nature*, 312: 361-362.

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.

Shinozaki, K. and K. Yamaguchi-Shinozaki. 1997. Gene expression and signal transduction in water-stress response. *Plant Physiol*., 115: 327-334.

Siddique, M.R.B., A. Hamid and M.S. Islam. 1999. Drought stress effects on photosynthetic rate and leaf gas exchange of wheat. *Bot. Bull. Acad. Sin*., 40: 141-145

Steel, R.G.D., J.H. Torrie and D.A. Dickey. 1997. *Principles and Procedures of Statistics*. 3rd ed. McGraw Hill, New York.

Talbott, L.D. and E. Zeiger. 1996. Central roles for potassium and sucrose in guard-cell osmoregulation. *Plant Physiol*., 111: 1051-1057.

Trethowan, R.M. 2000. Breeding wheat for marginal environments. www.cimmyt.org /research/wheat/map/research_results/reshighlights/pdfs/resHigh_BreedWht.pdf.

Wang, X., C. Loh, H. Yeoh and W.Q. Sun. 2002. Drying rate and dehydrin synthesis associated with abscisic acid-induced dehydration tolerance in *Spathoglottis plicata* orchidaceae protocorms. *J. Exp. Bot*., 53(368): 551-558.

Ward, J.M., Z.M. Pei and J.I. Schroeder. 1995. Roles of ion channels in initiation of signal transduction in higher plants. *Plant Cell*, 7: 833-844.

Wiebold, B. and P. Scharf. 2003. Potassium deficiency symptoms in drought stressed crops. *Integrated Pest Crop Management Newsletter*, Univ. Missouri-Columbia, 13(20): 2/14.

Xiong, L. and J. K. Zhu. 2002. Molecular and genetic aspects of plant responses to osmotic stress. *Plant Cell Environ*., 25: 131-139.

Yancey, P.H., M.E. Clark, S.C. Hand, R.D. Bowlus and G.N. Somero. 1982. Living with stress: evolution of osmolyte systems. *Science*, 217: 1214-1222.

Yokoi, S., R.A. Bressan and P.M. Hasegawa. 2002. Salt stress tolerance of plants. JIRCAS Working Report, 25-33 pp.

Zadoks, J.C., T.T. Chang and C.F. Konzak. 1974. A decimal code for the growth stages of cereals. *Weed Res*., 14: 415-521.

Zeiger, E. 1983. The biology of stomatal guard cell. *Annu. Rev. Plant Physiol.*, 34: 441-475.

(Received for publication 20 July 2007)