



**Full Length Article**

## Seasonal Variations in Growth and Nutrients of two Maize Varieties under Glasshouse Generated Heat Stress

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### Abstract

This study was conducted to determine the growth, mineral nutrient responses of selected heat-resistant (Sadaf) and heat-susceptible (Agatti-2002) varieties of maize to simulated glasshouse (GH) conditions during Spring and Autumn seasons at seedling, silking and grain filling stages. Fifteen day old plants were shifted to plexiglass-fitted canopies to create GH conditions. Mean radiation intensities inside and outside the canopy during the Spring and Autumn seasons were 1085  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and 976  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively. Simulated GH conditions increased the canopy temperature 4-7°C in the Spring season and 3-5°C in the Autumn-season, but increased relative humidity by 2-3% in the Spring season and 5-9% in the Autumn-season. Such an increase in temperature, referred to as heat stress, was more damaging to the absorption of potassium ( $\text{K}^+$ ), calcium ( $\text{Ca}^{2+}$ ) and nitrate ( $\text{NO}_3^{-}$ ) during Spring season. Heat stress hampered the shoot  $\text{K}^+$ ,  $\text{Ca}^{2+}$  and  $\text{NO}_3^{-}$  in both varieties, especially in Spring-season grown maize crop at all growth stages. Although heat stress reduced the growth of both varieties, shoot dry mass and leaf area was reduced more in Spring- season grown heat-susceptible maize at all growth stages. Heat stress was less damaging to Sadaf than Agatti-2002 in both seasons. In inclusion, these results suggest that an increase in heat stress inside the canopy caused a direct effect on the root functions. Thus a decrease in growth and nutrient contents of maize at later stages of the Spring-season sown crop. © 2015 Friends Science Publishers

**Keywords:** Canopy temperature; Glasshouse; Growth; Mineral nutrients; Maize

### Introduction

Climate change is a major issue for plant distribution. Anthropogenic activities have substantially increased the emission of greenhouse gases (GHGs) such as carbon dioxide, methane and nitrous oxide, which significantly contribute to climate change. The presence of greater amount of GHGs in the atmosphere trap the heat radiated back from the earth and causes an increase in global mean temperature. This rapid increase in temperature may alter the geographical distribution as well as the growing season of crops. The crop production is hampered due to variations in threshold temperature caused by climate change (Porter, 2005). The GH increase heat stress and thereby lower the plant biomass (Hussain *et al.*, 2010). Such an increase in temperature, referred to as heat stress, alters the ion movement across plant membranes (Arbona *et al.*, 2013). In different studies, heat stress has been shown to cause a decrease in growth and photosynthesis and final economic yield (Karim *et al.*, 2000; Stone, 2001).

In annual species, there was an increase in developmental rate along with a reduced growth period by rapid carbon fixation and biomass accretion before seed set under heat stress (Morison, 1996). However, it did not

influence the glucose level in potato tubers, but there was a more accretion of starch in tubers than shoot (Lafta and Lorenzen, 1995). Heat stress also affects the meristematic activity and growth of the various parts, particularly the leaves (Salah and Tardieu, 1996). There was an increase in leaf growth up to 35°C in maize, but a decline was observed above 35°C. Above 40°C, there was a sharp decline in photosynthesis and variations in protein metabolism. For instance, enzyme inactivation, protein denaturation, aggregation, inhibited protein synthesis and its degradation was observed (Ristic *et al.*, 2008).

Heat stress may injure the membranes suddenly by denaturing the membrane proteins or increase in unsaturated fatty acids, leading to membrane rupture and loss of cellular contents (Rodríguez *et al.*, 2005). Heat stress induces oxidative stress as noted for the enhanced production of reactive oxygen species (ROS) such as superoxide radicals, singlet oxygen, hydrogen peroxides and hydroxyl radicals (Asada, 2006; Savicka and Škute, 2010). The ROS are strong oxidizing agents and harmful to all cellular compounds and negatively influence the cellular metabolic processes (Breusegem *et al.*, 2001; Hasanuzzaman *et al.*, 2013). The detoxification of these ROS is very important and plants have evolved complex strategies to deal with

them (Asthir *et al.*, 2009). The plant cells typically respond to increase in ROS levels by increasing the activities of antioxidant enzymes (SOD, GPX, APX, CAT and GR) being engaged in the oxidative defense system (Chakraborty and Pradhan, 2011).

Heat stress influences the mineral nutrition acquisition and assimilation strongly in plants (Taiz and Zeiger, 2006). Carbon (C), nitrogen (N), phosphorus (P), sulfur (S), and magnesium (Mg) are essential nutrients for primary and secondary metabolites and defense related macro- and micro molecules synthesis (Epstein and Bloom, 2005; Taiz and Zeiger, 2006). Among the mineral nutrients, nitrogen plays a major role in utilization of absorbed light energy and photosynthetic carbon metabolism (Kato *et al.*, 2003; Huang *et al.*, 2004) and Mg is structural part of chlorophyll and is also needed for photosynthesis and many other metabolic processes (Waraich *et al.*, 2011). P is needed for energy production, storage, and is a structural part of nucleic acids (Waraich *et al.*, 2012). Ca plays a key role in the maintenance of cell structure. K is needed for osmotic adjustment and activation of enzymes (Mengel and Kirkby, 2001). Nutrient absorption is dependent on rate of physiological processes during plant growth, which increases under heat stress (Tollenaar, 1989). Under heat stress, plants absorb more P through roots (Fohse *et al.*, 1988). Heat stress increases the uptake of K and P significantly, whilst they were maximally absorbed at 32 and 38°C, respectively in maize roots (Bravo and Uribe, 1981). Under heat stress, different nutrients are affected differently as N and S concentrations, while there was no effect on sodium (Muldoon *et al.*, 1984).

Variations in day-night temperatures (i.e., 30-20, 30-30 and 35-35°C) showed the pattern of diurnal uptake of nitrate and dry matter accumulation in maize seedlings, with an enhanced uptake at 30-30°C (Polisetty and Hageman, 1989). There was a considerable decrease in the shoot dry mass, relative growth rate (RGR) and net assimilation rate (NAR) under heat stress (Wahid, 2007). Heat stress also reduced the uptake of Na, Mg, S, and N but uptake of P and Ca was improved (Wahid *et al.*, 2007). In sorghum, the uptake of certain nutrients like N and P was highest at 27°C in root, stem and leaves (Ercoli, 1996).

Maize is ranked as third cereal crop after wheat and rice and is cultivated on 4.8% of the total arable area in Pakistan. About 65% of maize is cultivated in areas with proper irrigation system, while the rest is cultivated on an area that depends on the rains. Therefore, the dilemma of heat stress is often a case in certain cultivated areas of maize. In view of global warming and changing environmental conditions, the rapid and continuous changes are taking place in plant growing patterns (Wahid *et al.*, 2007). Maize shows optimum growth at 28–31°C (Medany *et al.*, 2007). Although heat stress is a fundamental constraint for maize growth, there are great intraspecific differences in maize tolerance to ambient environmental changes. We hypothesize that in GH grown maize, the changes in mineral

nutrient uptake in plants and can affect the plant growth, and that growing seasons can also distinctly change the plant behavior and performance. Therefore, this study was undertaken to determine the changes in the mineral nutrients uptake and growth attributes of selected differentially heat responsive maize cultivars grown in the GH canopy during the Spring and Autumn seasons.

## Materials and Methods

### Experimental Details

The seeds of two maize varieties, namely, Sadaf and Agatti-2002 for screening purpose were obtained from Maize and Millets Research Institute (MMRI), Yousafwala, Sahiwal, Pakistan. The experiments were performed in wire-house at the Department of Botany, University of Agriculture, Faisalabad, Pakistan during the Spring and Autumn seasons in 2007. Seeds were grown in plastic pots (30 cm high, with circumference 82 cm at top and 70 cm at the bottom) containing 13 kg of washed dry sand. A hole was made in the bottom of pots to replace the soil solution. Ten seeds of each variety were sown. After germination, the plants were fertilized with half strength nutrient solution (Hoagland and Arnon, 1950) after four days in an amount to drain the previous solution. Five healthy and equal sized, three-day old seedlings were retained in each pot for taking measurements at seedling, silking and grain filling stages. For heat stress, the pots were shifted to the canopies, placed in a wire-house at each of the above growth stages, while the control plants were kept outside the canopies in the wire-house. For control set, the top of the wire-house was covered with a polythene sheet to produce the light transmission index of 75 to 80% in and outside the canopy. The mean radiation intensities inside and outside the canopy during the Spring and Autumn seasons were 1085  $\mu\text{mol m}^{-2}\text{s}^{-1}$  and 976  $\mu\text{mol m}^{-2}\text{s}^{-1}$ , respectively. The relative humidity and temperature inside and outside the canopies were recorded in both the seasons just above the plant height (Fig. 1). The plants were kept inside the canopies at respective growth stages, and harvesting was done 20 days after treatment application.

### Morphological Attributes

Plant height and root length were recorded after harvesting carefully. After drying in an oven at 70°C for about 72 h, shoot and root dry masses were also recorded. Leaf area was taken of intact plants as maximum leaf length  $\times$  maximum leaf width  $\times$  0.68 (correction factor computed for all leaves).

### Sample Preparation for Determination of ions

The dried ground shoot material (0.15 g) was digested with  $\text{H}_2\text{SO}_4$  and 35%  $\text{H}_2\text{O}_2$ . The diluted digested mixture was used to determine the concentration of ions ( $\text{K}^+$ ,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ) in the shoot of maize plants according to the methods

described by Yoshida *et al.* (1976). Potassium ( $K^+$ ) in the digested material was determined with flame photometer (Model 410, Sherwood, Scientific Ltd, Cambridge, UK) and  $Ca^{2+}$  and  $Mg^{2+}$  were determined with the help of an atomic absorption spectrophotometer (Spectra AA-5, Varian Company) at 422.7 nm and 285.2 nm, respectively. The amount of  $K^+$ ,  $Ca^{2+}$  and  $Mg^{2+}$  were calculated from the standard curves and expressed in  $m\ mol\ g^{-1}$  dry weight.

### Ionic Estimation

**Determination of soluble phosphates ( $PO_4^{3-}$ ):** The dried ground shoot material (0.15 g) was boiled with deionized water (10 mL) and extract (2.5 mL) was mixed in Barton's reagent (2.5 mL) for the determination of  $PO_4^{3-}$  according to the method of Yoshida *et al.* (1976). The absorbance of the mixture was read at 420 nm using a spectrophotometer (Hitachi-U-2001, Japan) and water is used as blank. The amount of  $PO_4^{3-}$  were calculated from the standard curve and expressed in  $m\ mol\ g^{-1}$  dry weight.

**Determination of soluble nitrates ( $NO_3^{-}$ ):** Soluble  $NO_3^{-}$  was measured with the method of Kowalenko and Lowe (1973). The dried ground shoot material (0.5 g) was boiled with deionized water (5 mL) and the filtrate (3 mL) was dissolved in CTA working solution (7 mL) and vortexed. The absorbance of the yellow colored complex was recorded at 430 nm with a spectrophotometer (Hitachi-U-2001, Japan) over 20 min and water was used as blank. The amount of  $NO_3^{-}$  was calculated from the standard curves.

### Statistical Analysis

The experiment was laid out in completely randomized design with four replicates. The data were analyzed with the analysis of variance (ANOVA) using a COSTAT computer package (Cohort Software, Berkeley, California). The mean values were compared using LSD (least significant difference) to find the significant differences among them.

## Results

### Growth Attributes

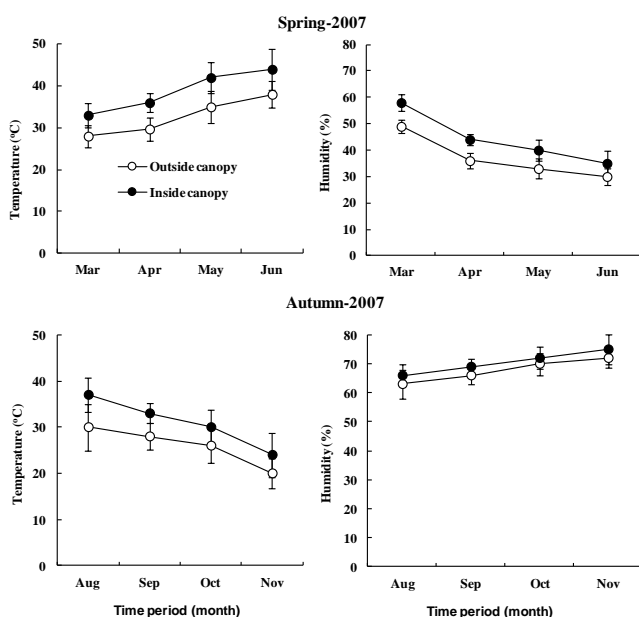
Data for changes in plant height indicated significant variations in the treatments during the Spring ( $P < 0.01$ ) and Autumn seasons ( $P < 0.05$ ). Plant height was reduced during the Spring-season at seedling stage in both varieties, although Sadaf variety showed a lower reduction (19.74%) than Agatti-2002 (30.36%) in GH grown plants. In the Autumn-season, although the heat stress enhanced more plant height in Agatti-2002 (12.94%) than Sadaf (9.49%). However, plants of both varieties were short in length during the Spring-season (Table 1). At silking stage, data showed a significant ( $P < 0.001$ ) difference in the varieties, treatments ( $P > 0.05$ ) and interaction ( $P < 0.01$ ) between these factors in the Spring-season, while in the Autumn-season,

significant ( $P < 0.05$ ) difference in the varieties, treatments ( $P < 0.01$ ) with no significant ( $P > 0.05$ ) interaction between these factors. At silking stage, Sadaf exhibited a greater (19.36%) shoot length, but in Agatti-2002, 7.65% plant height was reduced in GH grown plants under heat stress during the Spring-season. However, in the Autumn-season, both Sadaf (15.52%) and Agatti-2002 (18.68%) varieties showed greater plant height than control in GH grown plants under heat stress (Table 1). At grain filling stage, in the Autumn-season, data showed non-significant difference in the varieties ( $P > 0.05$ ), treatments ( $P < 0.05$ ) with no significant ( $P > 0.05$ ) interaction between these factors, while in the Spring-season, no significant difference in the varieties ( $P > 0.05$ ), significant in treatments ( $P < 0.01$ ) with non-significant ( $P > 0.05$ ) interaction between these factors. Plant height was increased in Sadaf (5.42%) and reduced in Agatti-2002 plants (14.14%) under heat stress (GH) in Spring season. During the Spring-season, plant height increased in Sadaf (13.55%) and reduced in Agatti-2002 (9.80%) at grain filling stage in GH grown plants under heat stress, although Spring-season grown plants had higher plant height than Autumn-season grown ones (Table 1).

For root length, both Spring-season and treatment showed a significant ( $P < 0.01$ ) variance. However, varieties exhibited only significant ( $P < 0.01$ ) difference in the Autumn-season. During the Spring-season, both varieties exhibited a similar pattern of changes in root length, although reduced in both Sadaf (26.41%) and Agatti-2002 (33.81%) under heat stress in GH grown plants. In Autumn-season, although the heat stress enhanced more root length in Agatti-2002 (11.29%) than Sadaf (3.21%). Both the varieties exhibited relatively greater root length than respective controlled plants (Table 1). At silking stage, data showed a significant ( $P < 0.01$ ) difference in varieties ( $P < 0.05$ ), treatments ( $P < 0.05$ ) with a significant ( $P < 0.01$ ) interaction of these factors during Spring-season, while non-significant difference in the variety ( $P > 0.05$ ), significant in treatments ( $P < 0.01$ ) with no significant interactions ( $P > 0.05$ ) in Autumn-season grown plants. Sadaf variety exhibited longer (20.33%) root length under heat stress and shorter in Agatti-2002 (2.64%) in GH grown plants during the Spring-season. However, in the Autumn-season, both varieties indicated a considerable rise in root length in both treated and control plants, although Agatti-2002 (26.92%) and Sadaf (~20.32%) varieties produced a longer root length in GH grown plants under heat stress than related controls (Table 1). At grain filling stage, data indicated a significant variation in treatments ( $P < 0.01$ ), non-significant ( $P > 0.05$ ) in varieties and the interaction between these factors during Spring-season, while in the Autumn-season, non-significant difference in the varieties ( $P > 0.05$ ), significant in treatments ( $P < 0.05$ ) with no significant ( $P > 0.05$ ) interaction between these factors. Sadaf variety indicated a little increased (~1%) during the Spring-season, while ~13.01% reduction occurred in this character in GH grown Agatti-2002 plants under heat stress. However, Sadaf variety indicated a small

**Table 1:** Changes in growth attributes in maize subjected to Glasshouse conditions at different growth stages during spring and autumn. Values sharing same letter in a season did not differ significantly ( $P>0.05$ )

Seasons	Growth stages	Varieties	Plant height (cm)		Root length (cm)		Shoot dry weight (g)		Root dry weight (g)		Leaf area/plant (cm <sup>2</sup> )	
			Control	GH	Control	GH	Control	GH	Control	GH	Control	GH
Spring-2007	Seedling	Sadaf	32.08 <sup>a</sup>	25.75 <sup>a</sup>	32.50 <sup>a</sup>	23.92 <sup>a</sup>	3.82 <sup>a</sup>	3.43 <sup>b</sup>	1.65 <sup>a</sup>	1.65 <sup>a</sup>	46.89 <sup>a</sup>	39.52 <sup>a</sup>
		Agatti-2002	34.58 <sup>a</sup>	24.08 <sup>a</sup>	35.25 <sup>a</sup>	23.33 <sup>a</sup>	3.02 <sup>c</sup>	1.84 <sup>d</sup>	1.89 <sup>a</sup>	1.89 <sup>a</sup>	74.42 <sup>a</sup>	61.73 <sup>a</sup>
	Silking	Sadaf	83.62 <sup>b</sup>	99.81 <sup>a</sup>	40.99 <sup>a</sup>	49.33 <sup>a</sup>	17.52 <sup>a</sup>	18.44 <sup>a</sup>	3.82 <sup>a</sup>	3.82 <sup>a</sup>	108.43 <sup>b</sup>	133.12 <sup>a</sup>
		Agatti-2002	64.56 <sup>c</sup>	59.62 <sup>c</sup>	41.08 <sup>a</sup>	39.99 <sup>b</sup>	15.85 <sup>ab</sup>	10.12 <sup>c</sup>	3.27 <sup>b</sup>	3.27 <sup>b</sup>	94.12 <sup>b</sup>	56.73 <sup>c</sup>
	Grain filling	Sadaf	121.50 <sup>a</sup>	128.08 <sup>a</sup>	35.99 <sup>a</sup>	36.33 <sup>a</sup>	60.93 <sup>a</sup>	58.89 <sup>ab</sup>	11.67 <sup>abc</sup>	11.67 <sup>abc</sup>	148.43 <sup>b</sup>	163.12 <sup>a</sup>
		Agatti-2002	96.08 <sup>a</sup>	82.50 <sup>a</sup>	39.08 <sup>a</sup>	33.99 <sup>a</sup>	49.52 <sup>bc</sup>	34.64 <sup>d</sup>	11.86 <sup>abc</sup>	11.86 <sup>abc</sup>	144.12 <sup>c</sup>	86.73 <sup>d</sup>
Autumn-2007	Seedling	Sadaf	60.58 <sup>a</sup>	66.33 <sup>a</sup>	36.33 <sup>a</sup>	37.50 <sup>a</sup>	4.90 <sup>a</sup>	4.09 <sup>a</sup>	1.80 <sup>a</sup>	1.80 <sup>a</sup>	94.62 <sup>a</sup>	90.82 <sup>a</sup>
		Agatti-2002	54.75 <sup>a</sup>	61.83 <sup>a</sup>	25.83 <sup>a</sup>	28.75 <sup>a</sup>	3.18 <sup>a</sup>	3.03 <sup>a</sup>	1.84 <sup>a</sup>	1.84 <sup>a</sup>	75.33 <sup>a</sup>	72.28 <sup>a</sup>
	Silking	Sadaf	51.69 <sup>a</sup>	59.71 <sup>a</sup>	20.92 <sup>a</sup>	25.16 <sup>a</sup>	13.84 <sup>b</sup>	15.52 <sup>ab</sup>	3.24 <sup>a</sup>	3.24 <sup>a</sup>	68.85 <sup>a</sup>	82.85 <sup>a</sup>
		Agatti-2002	47.50 <sup>b</sup>	56.37 <sup>a</sup>	21.67 <sup>a</sup>	27.50 <sup>a</sup>	13.05 <sup>bc</sup>	12.83 <sup>bc</sup>	2.88 <sup>a</sup>	2.88 <sup>a</sup>	73.53 <sup>a</sup>	68.26 <sup>bc</sup>
	Grain filling	Sadaf	78.92 <sup>a</sup>	89.61 <sup>a</sup>	25.92 <sup>a</sup>	27.17 <sup>a</sup>	50.02 <sup>b</sup>	51.40 <sup>ab</sup>	12.90 <sup>ab</sup>	12.90 <sup>ab</sup>	98.85 <sup>b</sup>	112.85 <sup>ab</sup>
		Agatti-2002	83.33 <sup>a</sup>	75.16 <sup>a</sup>	23.67 <sup>a</sup>	24.50 <sup>a</sup>	55.90 <sup>a</sup>	39.50 <sup>cd</sup>	13.78 <sup>a</sup>	13.78 <sup>a</sup>	88.53 <sup>c</sup>	78.26 <sup>d</sup>



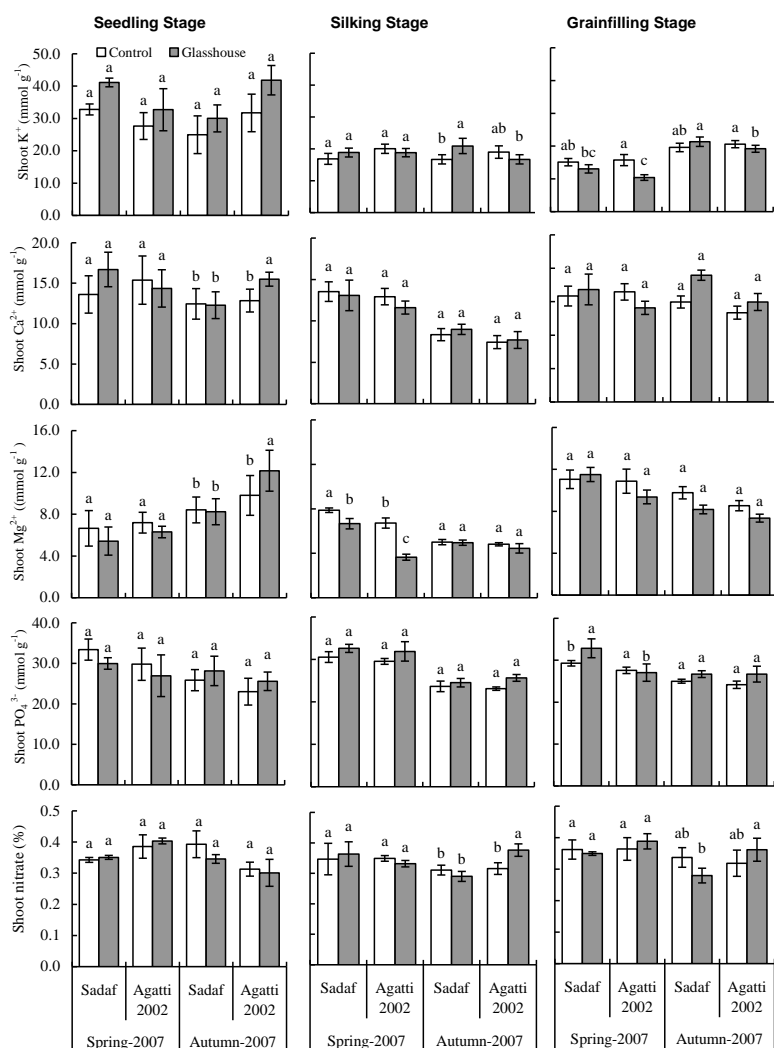
**Fig. 1:** Fluctuations in the inside and outside relative humidity and temperature in plexiglass canopy recorded during 2007 in spring and autumn

decrease (4.82%) in root length during Autumn-season, while Agatti-2002 (3.52%) showed a little bit increased in root length under heat stress in GH grown plants (Table 1).

Data for shoot dry weight indicated significant ( $P<0.01$ ) difference in varieties and growth stages during the Spring-season, but not in the Autumn-season. However, for root dry weight, such an interaction was notable at silking and grain filling stages in Spring-season only (Table 1). Shoot dry weight during the Spring-season was reduced at seedling stage in both varieties, although Sadaf (10%) indicated a lower reduction than Agatti-2002 (39%) under heat stress. At silking stage, Sadaf showed an increase (5%) in shoots dry weight and a decrease in Agatti-2002 (36%). At grain filling stage, heat stress reduced the shoot dry weight in both varieties, but this reduction was lower in

Sadaf (3%) than Agatti-2002 (30%) variety. During the Autumn-season, although the trends of changes in shoot dry weight were similar to those observed during the Spring-season except at the grain filling stage, when Sadaf variety indicated an increase in shoot dry weight, while Agatti-2002 showed reverse response in GH grown plants under heat stress. Root dry weight reduced in both varieties, although the reduction was lesser (~18%) in Sadaf than Agatti-2002 (~26%) variety during the Spring-season at seedling stage. At silking and grain filling stages, Sadaf variety showed an increase (~8 and 5%, respectively) while in Agatti-2002, a decrease (34 and 32%, respectively) in root dry weight occurred. In Autumn-season, there was a reduction of root dry weight in both varieties, but the Agatti-2002 variety suffered more under heat stress than Sadaf (~22 and 6%, respectively). At silking and grain filling stages, Sadaf variety indicated an increase (~41 and 4%, respectively) but a decrease (~20 and 34%, respectively) in root dry weight took place in Agatti-2002 variety.

Leaf area per plant exhibited a significant variation in varieties ( $P<0.01$ ) and treatments ( $P<0.05$ ) with non-significant ( $P>0.05$ ) interaction between these factors in the Spring-season. In the Autumn-season, the varieties, but not the treatment, indicated significant ( $P<0.01$ ) difference. In Spring-season, heat stress decreased the leaf area per plant in Sadaf (15.73%) variety than Agatti-2002 (17.05%) in GH grown plants. In the Autumn-season, heat stress had no more influence on the leaf area in both Sadaf (4.02%) and Agatti-2002 (4.05%). At silking stage, data showed significant ( $P<0.01$ ) difference in varieties with a significant ( $P<0.01$ ) interaction of both factors in the Spring-season. In the Autumn-season, although varieties and treatments exhibited non-significant ( $P>0.05$ ) differences, while there was a significant ( $P<0.01$ ) interaction between these factors. In Spring-season, leaf area was more increased in Sadaf (22.77%) and much reduced (39.73%) in GH grown Agatti-2002 plants. In the Autumn-season, this attribute was increased under heat stress in Sadaf (20.33%), but decreased (7.17%) in GH grown Agatti-2002 plants (Table 1). At grain filling stage, data indicated significant ( $P<0.01$ ) differences



**Fig. 2:** Alteration in nutritional and ionic relations in maize subjected to Glasshouse conditions at different growth stages during spring and autumn. The data bars having same letter in a season did not differ significantly ( $P>0.05$ )

in varieties and treatment with a significant interaction between these factors in both the seasons. Leaf area was less increased in Sadaf (9.89%) under heat stress during Spring-season, but more reduction in Agatti-2002 (39.82%) in GH grown plants. However, in Autumn-season, the trend of changes was similar to Spring-season in Sadaf (14.16%) and Agatti-2002 (11.6%) in GH grown plants (Table 1).

### Mineral Nutrients

For shoot K<sup>+</sup> in the Spring-season, there was a significant ( $P<0.01$ ) differences in the varieties and treatment with a non-significant ( $P>0.05$ ) interaction between these factors in GH grown plants at seedling stage, while significant ( $P<0.05$ ) difference in varieties, treatments and no significant ( $P>0.05$ ) interaction between these factors in the Autumn-season. At the seedling stage, K<sup>+</sup> accumulation was increased in both varieties; Sadaf (25.54%) exhibited a greater accumulation than Agatti-2002 (18.29%) under heat

stress (GH) in Spring-season, while Agatti-2002 (31.90%) variety showed a great accumulation in this character than Sadaf (20.26%) during the Autumn-season in GH grown plants (Fig. 2). At silking stage, data exhibited no significant ( $P>0.05$ ) variations in varieties, treatments and interaction between these factors during the Spring-season, but significant interactions ( $P<0.05$ ) in the varieties and treatment during the Autumn-season for K<sup>+</sup> accumulation in the shoot. Heat stress increased the level of K<sup>+</sup> accumulation in Sadaf variety during both Spring-season (11.83%) and Autumn-season (25.24%) and also the lesser accumulation in Agatti-2002 in Spring-season (6.09 %) and Autumn-season (12.03%) (Fig. 2). At grain filling stage, data indicated no significant ( $P>0.05$ ) difference in varieties, significant in treatments ( $P<0.01$ ) and interaction ( $P<0.05$ ) between these factors during the Spring-season, while no significant ( $P>0.05$ ) difference in varieties, treatments and significant interaction ( $P<0.05$ ) between these factors during the Autumn-season for shoot K<sup>+</sup>. At this stage, heat stress was

decreased the  $K^+$  in the shoot in both varieties during the Spring-season in GH grown plants, but this reduction was greater in Agatti-2002 (33.79%) variety than Sadaf (13.69%) in Spring-season grown plants. In Autumn season,  $K^+$  increased in Sadaf (8.9%), but this increase was declined in Agatti-2002 (6.69%) in GH grown plants (Fig. 2).

For shoot  $Ca^{2+}$  contents, at seedling stage, the data showed no significant ( $P>0.05$ ) in varieties, treatments ( $P<0.05$ ) and non-significant ( $P>0.05$ ) interaction during the Spring-season, while significant ( $P<0.01$ ) difference in varieties, treatments ( $P<0.05$ ) and significant ( $P<0.05$ ) interaction in the Autumn-season. At the seedling stage, increased shoot  $Ca^{2+}$  contents in Sadaf (22.77%) and decreased in Agatti-2002 (6.75%) were recorded under heat stress in GH grown Spring-season plants. The shoot  $Ca^{2+}$  contents were increased greatly in Agatti-2002 (20.66%), but Sadaf (1.4%) showed very little change in GH grown Autumn-season plants (Fig. 2). At silking stage, data exhibited no significant ( $P>0.05$ ) difference in varieties, treatments and the interaction between these factors in the Spring-season, while a significant ( $P<0.01$ ) difference in varieties, treatments ( $P>0.05$ ) and no significant interactions ( $P>0.05$ ) in Autumn-season grown plants for shoot  $Ca^{2+}$ . The contents of  $Ca^{2+}$  in the shoot were decreased more in Agatti-2002 (10.27%) variety under heat stress than Sadaf (3.32%) in GH grown plants during Spring-season. However, in the Autumn-season, Sadaf variety showed a small increased (7.69%) in  $Ca^{2+}$  contents, but did not change remarkably in Agatti-2002 (3.35%) in GH grown plants (Fig. 2). At grain filling stage, data exhibited no significant ( $P>0.05$ ) difference in varieties, treatments and interaction in the Spring-season, while a significant variation ( $P<0.05$ ) in varieties, treatments and non-significant ( $P>0.05$ ) interaction for shoot  $Ca^{2+}$  in GH grown Autumn-season plants. The contents of  $Ca^{2+}$  in the shoot were less increased in Sadaf (6.17%) variety, and decreased in Agatti-2002 (14.49%) under heat stress in GH grown Spring-season plants. However, in both varieties, the shoot  $Ca^{2+}$  contents increased in GH grown Autumn-season plants, although Agatti-2002 (12%) variety indicated more accumulation of  $Ca^{2+}$  than Sadaf (2.79%) in shoot tissue (Fig. 2).

For shoot  $Mg^{+2}$  contents, data showed significant in treatments ( $P<0.05$ ) and no significant ( $P>0.05$ ) difference in varieties and the interaction between these factors during the Spring-season at seedling stage, while in the Autumn-season, significant ( $P<0.01$ ) difference in the varieties, no significant in treatments ( $P>0.05$ ) and significant ( $P<0.05$ ) interaction of these factors. In Spring-season,  $Mg^{+2}$  contents in the shoot were reduced evenly in both Sadaf (18.44%) and Agatti-2002 (12.43%) under heat stress in GH grown plants. The  $Mg^{+2}$  contents in the shoot were increased greatly in GH grown Agatti-2002 plants (24.04%), but Sadaf variety showed no more change (2.1%) in  $Mg^{+2}$  contents during the Autumn-season (Fig. 2). At silking stage, data exhibited a significant ( $P<0.01$ ) difference in varieties, treatments and interaction during the Spring-

season, while in the Autumn-season, no significant ( $P>0.05$ ) difference in varieties, treatments and interaction between these factors for shoot  $Mg^{+2}$  contents. Heat stress decreased the shoot  $Mg^{+2}$  contents in both varieties, although a greater decreased was noted in Agatti-2002 (45.79%) than Sadaf (15.36%) during the Spring-season, while in the Autumn-season, a very little decreased in Sadaf (1.15%) variety was noted, whilst Agatti-2002 (7.73%) showed a decrease in shoot  $Mg^{+2}$  contents under heat stress in GH grown plants (Fig. 2). At grain filling stage, in Spring-season grown plants, data showed significant ( $P<0.05$ ) difference in varieties, no significant ( $P>0.05$ ) in treatments and the interaction between these factors, while Autumn-season grown plants showed a significant ( $P<0.01$ ) difference in the varieties, treatments and no significant ( $P>0.05$ ) interaction. Shoot  $Mg^{+2}$  was increased (4.17%) in Sadaf and decreased in Agatti-2002 (13.79%) in GH grown plants during Spring-season. In the Autumn-season, shoot  $Mg^{+2}$  decreased in both Sadaf (16.26%) and Agatti-2002 (13.85%) varieties in GH grown plants (Fig. 2).

For  $PO_4^{3-}$  contents, data exhibited a significant ( $P<0.05$ ) difference in varieties, treatments and no significance ( $P>0.05$ ) interaction between these factors in both the seasons. At the seedling stage,  $PO_4^{3-}$  contents in the shoot were decreased in both Agatti-2002 (9.85%) and Sadaf (10.24%) varieties in Spring-season grown plants. In Autumn-season, although the trend of changes in  $PO_4^{3-}$  contents was almost similar in both varieties. However, heat stress produced higher contents of  $PO_4^{3-}$  in shoot of Agatti-2002 (11.18%) variety than Sadaf (8.82%). At silking stage, data showed significant ( $P<0.05$ ) difference in treatments and no significant ( $P>0.05$ ) in varieties and the interaction between these factors in the Spring-season, while Autumn-season grown plants showed a significant ( $P<0.01$ ) difference in treatments only. Heat stress increased the contents of shoot  $PO_4^{3-}$  in both Agatti-2002 (7.91%) and Sadaf (6.71%) in GH grown Spring-season plants, whilst Agatti-2002 accumulated more (10.97%)  $PO_4^{3-}$  contents in shoot than Sadaf (3.73%) in GH grown Autumn-season plants (Fig. 2). At grain filling stage, data showed a significant ( $P<0.01$ ) difference in varieties, no significant in treatments ( $P>0.05$ ) and significant ( $P<0.05$ ) interaction during the Spring-season, whilst the Autumn-season grown plants showed significant ( $P<0.01$ ) difference in the treatments only for shoot  $PO_4^{3-}$ . In the Spring-season, heat stress enhanced the contents of  $PO_4^{3-}$  in the shoot of Sadaf (12.20%) variety and reduced in Agatti-2002 (~2%) in GH grown plants. However, both Sadaf (6.76%) and Agatti-2002 (10.58%) varieties showed more contents of  $PO_4^{3-}$  in the shoot of GH grown Autumn-season plants (Fig. 2).

For shoot  $NO_3^1$  contents, the data exhibited a significant ( $P<0.01$ ) difference in varieties, no significant ( $P>0.05$ ) in treatments and the interaction between these factors during the Spring-season at seedling stage, whilst no significant ( $P>0.05$ ) difference in varieties, treatments, and the interaction between these factors in Autumn-season

grown plants. In the Spring-season,  $\text{NO}_3^{-1}$  in shoot was increased in both varieties under heat stress, although Agatti-2002 (4.58%) variety accumulated more  $\text{NO}_3^{-1}$  than Sadaf (2.42%) in shoot. In the Autumn-season,  $\text{NO}_3^{-1}$  contents were declined in both varieties, but this decline was well explicit in Sadaf (11.98%) than Agatti-2002 (3.80%) in glasshouse grown plants (Fig. 2). At silking stage, the data showed no significant ( $P>0.05$ ) difference in varieties, treatments and interaction during Spring-season, while no significant in treatments ( $P>0.05$ ) and a significant ( $P<0.01$ ) difference in varieties and interaction ( $P<0.01$ ) between these factors for  $\text{NO}_3^{-1}$  contents in a shoot during the Autumn-season. The silking data indicated that Sadaf (4.78%) showed little accumulation of  $\text{NO}_3^{-1}$  contents and lesser in Agatti-2002 (4.96%) under heat stress in GH grown Spring-season plants. Heat stress decreased the  $\text{NO}_3^{-1}$  contents in Sadaf (6.56%), but increased in the shoot of Agatti-2002 (19.24%) during the Autumn-season (Fig. 2). At grain filling stage, data exhibited no significant ( $P>0.05$ ) difference in varieties, treatments and interaction between these factors during the Spring-season, while in the Autumn-season, no significant ( $P>0.05$ ) in varieties, treatments and significant ( $P<0.05$ ) interaction for  $\text{NO}_3^{-1}$  in a shoot. At this stage, heat stress enhanced the  $\text{NO}_3^{-1}$  contents in the shoot of Agatti-2002 in both Spring (6.72%) and Autumn-season (13.47%), seasons while this was decreased in Sadaf in both Spring (3.53%) and Autumn-season (17.01%) seasons under heat stress (Fig. 2).

## Discussion

Significant interactions of varieties and treatments for different attributes, appearing in one season and disappeared in another season at different phenological stages (Fig. 2; Table 1). This showed that prevailing heat stress produced an array of changes in maize growth as well as in the mineral nutrients, although the effects were relatively lesser on Sadaf (heat tolerant) than Agatti-2002 (sensitive one).

Plant growth at different phenological stages exhibits particular responses under study (Zaidi *et al.*, 2003). Regulation of gene expression under environmental hazards becomes erratic during the transition state from one to the next growth stage (Qin *et al.*, 2004; Wahid and Close, 2007). These changes in gene expression are associated with perturbations in growth and development (Srivastava, 2002; Taiz and Zeiger, 2006). In this study, both the varieties behaved in a different way at all growth stages under GH conditions (Table 1). Plants subjected to environmental stress exhibit a sufficient reduction in plant growth. However, this reduction depends on the type and duration of stress (Arbona *et al.*, 2013). Efficient photosynthetic capacity of leaves is positively associated with increased dry weight (Suárez and Medina, 2006).

The results of this experiment showed that both of maize varieties indicated many changes in  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{PO}_4^{3-}$  and  $\text{NO}_3^{-1}$  in both growing seasons and at all growth

stages. Heat stress hampered the  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{PO}_4^{3-}$  and  $\text{NO}_3^{-1}$  contents in the shoot across the seasons at all growth stages, although varietal difference was evident (Fig. 2). Sadaf accumulated more nutrients in shoot tissue than Agatti-2002. It has been revealed that all these nutrients are either specific structural or functional constituent of the plants and used as a major nutrient for plant growth (Epstein and Bloom, 2005). Moreover, these micronutrients ( $\text{P}$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ) were required by the plants for the regulation of a plethora of mechanism including protein synthesis, activities of enzymes, integrity of plasma membrane, cell wall and as a component of proteins, photosynthetic pigments and photosynthetic protein complexes (Taiz and Zeiger, 2006). Limited nutrient uptake was a common response of plants grown under heat stress (Huang *et al.*, 2004). Accumulation of nutrients, including  $\text{P}$ ,  $\text{K}^+$ , and  $\text{Ca}^{2+}$  could induce heat tolerance to plants (Waraich *et al.*, 2012). Plants generally exhibited higher endogenous level of  $\text{K}^+$  under heat stress (Marschner, 1995). In this context, increased uptake of  $\text{K}^+$  was also observed in wheat and maize plants under stress condition (Yasin *et al.*, 1993). It has been observed that Spring-season grown plants showed more evident changes at all growth stages than Autumn-season grown plants, which do not experience such a greater relative humidity and heat stress at these growth stages (Hussain *et al.*, 2010; Hussain *et al.*, 2014). Thus, it can be deduced that heat stress produced clearer trends of changes in  $\text{K}^+$ ,  $\text{Ca}^{2+}$  contents and, to some extent in shoot nitrate across the seasons, which showed a possible role of these nutrient intolerances to heat stress. Maize and many other crops grown under heat stress exhibited a greater change in the ions and nutrients, including  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{N}$  and  $\text{P}$  (Wahid *et al.*, 2007). This appears to be due to the reason that root hair production is severely impaired under heat stress (Tanaka *et al.*, 2014) and any reduction in the water and mineral uptake is closely linked to root hairs density (Taiz and Zeiger, 2010).

In this study, both the varieties responded differently under heat stress that may be linked to the prevailing environmental conditions of RH and temperature in the glass canopies (Fig. 1). In Spring-season grown plants, the relative humidity decreased and temperature sufficiently increased at the time of harvesting that significantly affect the shoot nitrate at each stage, which suggested a major role in tolerance to heat stress. However, in the Autumn-season crop, heat stress did not affect nitrate contents in the shoot as the temperature was increased more at time of sowing in each stage and relative humidity increased and the temperature inside the canopy was reduced (Fig. 2).

## Conclusion

Changes in the canopy temperature and relative humidity were responsible for alteration in plant dry masses and  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{PO}_4^{3-}$  and  $\text{NO}_3^{-1}$  in maize, across the Spring and Autumn seasons. Remarkable reduction in macronutrients

including  $K^+$ ,  $Ca^{2+}$  and  $NO_3^{-1}$  are likely due to reduction in root hair production under heat stress, but this aspect needs thorough investigation. These results have great implications in the production of such crop species, which may be well adapted to upcoming environmental changes arising due to GH induced heat stress effects.

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