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Heat Stress Effects on Forage Quality Characteristics of Maize (*Zea mays*) Cultivars

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ABSTRACT

Heat stress adversely affects the plant quality characteristics leading to altered forage quality. Purpose of this study was to determine the comparative changes in heat tolerant (Sultan) and heat sensitive (S-2002) maize (*Zea mays* L.) varieties for the nutritional quality characteristics over short intervals. Heat stress was applied by shifting the plants into an illuminated growth chamber at 40°C, while control plants were kept in another chamber at 27°C. Sultan manifested greater shoot fresh and dry weight and greater shoot water contents than S-2002 under heat stress. The varieties showed no specific trend of changes in the levels of soluble nitrate (SN), soluble phosphate (SP) and K^+ under control; while Sultan indicated no variation in SN, some reduction in SP and increased in K^+, while S-2002 indicated increase in the levels of SN and SP, while no great difference in K^+ contents under heat stress. This was attributed to heat induced reduced utilization of the available nutrients for incorporation into structural components. Although starch, oil and protein were variable in both the varieties under control condition, heat stress reduced starch contents of both the varieties, while oil and protein contents increased in Sultan but decreased in S-2002. Crude fiber increased greatly in Sultan than S-2002 under heat stress. Heat stress improved the nutritive value and metabolizable energy in Sultan but decreased in S-2002. It is concluded that tolerant maize, by virtue of better performance and nutritive value, could be a better choice for use as forage plant if cultivated in the warmer climates.

Key Words: Growth; Nutritive value; Metabolizable energy; Maize; Fodder

INTRODUCTION

Plants are primary source of nutrition for herbivores on this planet. The animals consume almost all parts of the plants but the most important of these are aerial parts and seed. What makes a plant useful for animals is its nutritional value (Sarwar *et al*., 2002). The proximate constituents determine the palatability of a plant species for animals, which depends upon both the organic (carbohydrates, proteins & fats) and inorganic (essential nutrients) constituents of nutrition (Murphy & Allen, 2003).

Heat stress is a great modifying factor for plant growth and affects both qualitative and quantitative characteristics of plants, thus affecting the primary and secondary metabolic pathways (Wahid, 2007). Prevailing stressful conditions change the metabolic phenomena eventually leading to the synthesis of novel metabolites or changing the levels of existing metabolites (Wahid, 2007). Morphologically, the heat stress effects can be noted in the form of overall leaf chlorosis, necrotic lesions and tip-burning (Wahid *et al*., 2007). Studies show that as a result of declined net photosynthesis, the production of photoassimilates is reduced and out of available amount, an impressive portion is utilized for stress tolerance (Taiz & Zeiger, 2006; Wahid *et al*., 2007).

Although some specific metabolites are crucial for plant stress tolerance (Grassmann *et al*., 2002; Vasconsuelo & Boland, 2007; Edreva *et al*., 2008), the modifications in the metabolic pathways usually lead to varied forage value (Anuraga *et al*., 1993; Mitchell *et al*., 2001; Norman *et al*., 2004). Many studies have emphatically described the enhanced synthesis of condensed tannins under high temperature stress, which add to the forage nutritional quality (Lees *et al*., 1994; Gebrehiwot *et al*., 2002). In addition, presence of proteins, carbohydrates, fatty acids and essential nutrients in the feed or forage in appropriate quantities is pivotal.

Crop growing season and plant developmental stages are other important factors that influence the forage quality (Darby & Lauer, 2002). Incidence of stress at any stage may influence the feed and forage digestibility and reduced nutrient intake (Turner *et al*., 1990). Thus, morphological development can be used to accurately predict the forage quality of any crop throughout the season (Mitchell *et al*., 2001). The palatability of any plant species at initial stages for the grazing animals might be much more than at relatively mature stages, because of the stiffer plant architecture (McArthur, 2005).

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Maize is a multipurpose crop and amongst other uses, its vegetative parts and grains are used as forage and feed for animals, respectively. Although a tropical plant species, maize shows variable response to prevailing temperatures (Hussain *et al*., 2006 & 2010), the genetic makeup of a species may play important role in reducing the deleterious effects of stress on the forage and nutritional quality. In this research, two differentially heat tolerant maize varieties were studied for changes in some nutritionally important characteristics including crude fiber, starch, oils, carbohydrates, some inorganic characters, nutritional value and in the aboveground parts of two maize varieties under heat stress at three short growth intervals.

MATERIALS AND METHODS

Experiments were conducted to determine heat stress effect on growth and nutritional quality characteristics of shoots of selected heat tolerant (Sultan) and heat sensitive (S-2002) maize (*Zea mays* L.) varieties in the Department of Botany, University of Agriculture, Faisalabad, Pakistan. Six days old seedlings were exposed to control (27^{o}C) and heat stress (42^{o}C) conditions and harvested at 2, 4 and 6 day intervals in two separate growth chambers with 450 μmol/m^{2}/s light (14 h day) and relative humidity 40/60±5% (day/night). The plants were grown in sand and supplemented with half strength Hoagland solution (Hoagland & Arnon, 1949) at three day interval during the experiment. The experiment was laid out in a completely randomized design (CRD) with three replications.

At each harvest, the shoots were cut at ground level and immediately determined for fresh weight. A part of the plants were preserved in a freezer (-40^{o}C) for fresh determination. For taking dry weight, the shoots were put in paper were and dried in an oven at 60^{o}C for seven days. The shoot water content (%) was determined as: (fresh weight-dry weight) × 100/fresh weight.

For the determination of soluble nitrate, phosphate and K, 0.5 g of the dried ground material was extracted in 5 mL of deionized water by boiling for 1 h, filtered and made the volume up to 50 mL. For soluble nitrate determination using the method of Kowalenko and Lowe (1973), three mL of the extract was dissolved in seven mL of working solution of CTA with the thrust of a pipette filler and vortexed briefly. After 20 min, the intensity of the yellow colored complex was taken at 430 nm on a spectrophotometer (U-2001, Hitachi, Japan). The value of nitrate in the test samples was determined from a standard curve prepared from nitrate standards. To measure soluble phosphates, 2.5 mL of the above extract was added to 2.5 mL of Barton's reagent (Yoshida *et al.,* 1976). The samples were vortex, kept for 20 min at room temperature and absorbance of the colored complex taken at 420 nm. The amount of soluble phosphate in unknown samples was calculated from a standard curve. The amount of K from in above extract was measured with flame photometer (Sherwood Model 410, Cambridge) and comparing with standard curve.

Among the nutritional quality characteristics, starch was determined with the method of Malik and Srivastava (1985). Dry leaf sample residue (0.5 g) after methanol extraction was oven dried and re-extracted with 5 mL of distilled water and 52% HCl (1:1 v/v). After centrifugation at 7500 × *g* for 10 min, 0.5 mL of the supernatant was mixed with anthrone reagent (1 g anthrone dissolved in 1 L of concentrated sulfuric acid). Samples covered with aluminum foil were heated at in a water bath 100^{o}C for 30 min and cooled to room temperature. Absorbance of the colored solution was taken at 625 nm and starch contents calculated. For the determination of oil, 3 g of the dried leaf samples were taken in a plastic tube (50 mL capacity, centrifuge grade) and 30 mL of n-hexane was poured. These tubes were left for 24 h in a shaker at 100 rpm. Afterwards, the samples were centrifuged at 3500 × *g* for 20 min and supernatant collected. Two successive repetitions of extraction were made on the residue by vortexing and centrifugation. Pooled but cleared extracts of all three collections were preserved for oil estimation (AOAC, 1996). For the determination of soluble proteins, 0.5 g of the frozen fresh material was extracted with phosphate buffer saline (PBS) in a pre-chilled pestle mortar (Sambrook *et al*., 2001), added with Cocktail protease inhibitors and centrifuged at 12000 × *g* for 15 min. Total soluble proteins from the supernatant were determined from the supernatant using Bradford assay (Bradford, 1976).

Crude fiber was determined as reported by Chopra and Kanwar (1991). The crude fiber estimation was based on treating the moisture and fat-free samples with 1.25% dilute H_2SO_4, followed with 1.25% NaOH, washed with hot water and then 1% HNO_3 and again with hot water. The residue was combusted in a muffle furnace (EYELA-TMF-2100, Japan) and ash weighed. Loss in weight gave the weight of crude fiber. The nutritional value was determined with method of Indrayan *et al*. (2005). Atwater system was used to determine metabolizable energy of samples, using Atwater factor of 4 kcal/g protein, 9 kcal/g fat and 4 kcal/g carbohydrates. These conversion factors were multiplied by the formulae to get energy in kJ as described by World Health Organization (1985).

Data were subjected to statistical analysis to find significant differences among different factors after carrying out analysis of variance (ANOVA). The differences among the treatments were ascertained with Duncan's Multiple Range Test (DMRT). Computer software COSTAT (CoHort software, 2003) was used for this purpose.

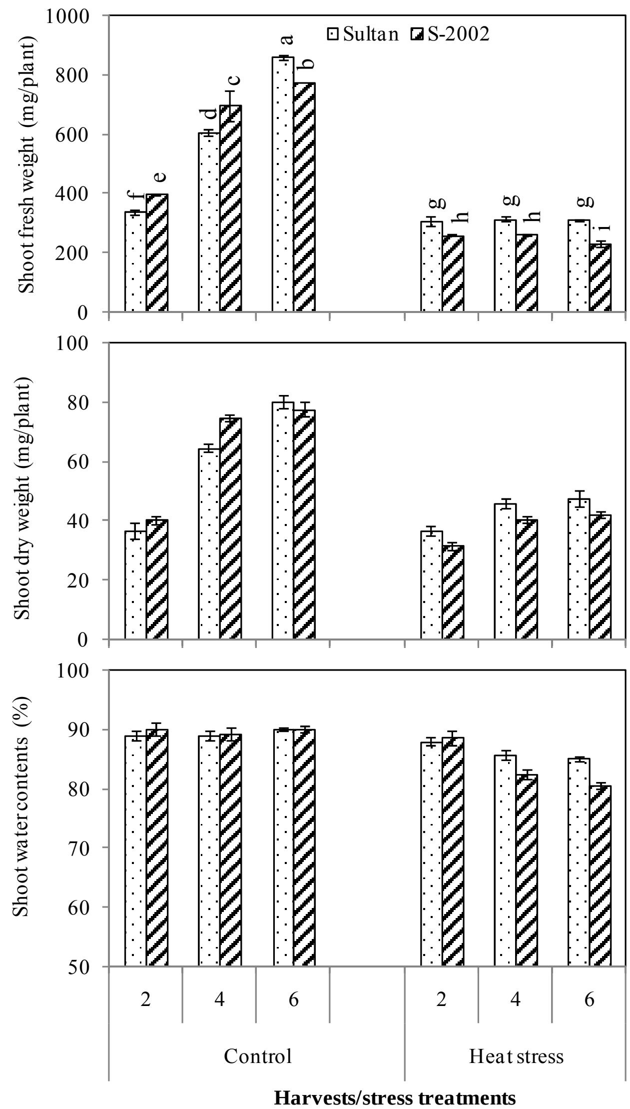
RESULTS

For aboveground fresh matter yield, varieties, harvests and heat stress treatments indicated significant ($P<0.05$) differences with significant ($P<0.01$) interactions of various factors. Although shoot fresh weight increased in both the varieties under control at all harvests in control plants, heat stress substantially lowered fresh weight in both the varieties at all harvests, Sultan showed no decline, while S-2002 indicated a decline (Fig. 1). Aboveground dry weight indicated non-significant (P>0.05) difference in the varieties but significant (P<0.01) for harvest times and heat stress treatments, with significant varieties × temperatures (P<0.05) and temperatures × harvests (P<0.01) interactions. Shoot dry weight increased in both the varieties at all harvests under control, but a decline in this attribute was evident under heat stress. However, under heat stress, shoot dry weight although was lesser than control plants, Sultan showed a steady increase, while S-2002 a reduction on day-2, improved but equal on day-4 and day-6 (Fig. 1). Data for shoot water contents indicated significant (P>0.01) difference in the varieties, heat stress treatments and harvests with significant (P<0.01) interaction of these factor except a non-significant (P>0.05) interaction of these factors. Under control condition, the shoot water contents were similar in both the varieties at all harvests. However, under heat stress Sultan exhibited a lesser but S-2002 a greater decline over harvests (Fig. 1).

Results regarding nitrate contents revealed significant (P<0.01) differences in the varieties, temperature treatments and harvests with significant (P<0.05) interactions of these factors. Varieties, harvests and treatments showing no differences except a reduced nitrate at day-6 in S-2002 under control, heat stress did not affect the nitrate contents in Sultan, while it increased steadily in S-2002 (Fig. 2). Phosphate contents indicated significant (P<0.01) differences in the heat treatments, but non-significant difference in the varieties and harvests, while there was significant (P<0.01) interaction of the three factors. Under control condition (27°C), Sultan indicated similar phosphate at all harvests, although S-2002 indicated a slightly increased phosphate at day-4 and then reduced at day-6. However, under heat stress Sultan indicated the highest phosphate contents at day-2, which reduced but were steady subsequently, whilst S-2002 indicated increased but similar value of this attribute at day-4 and day-6 (Fig. 2). The K contents data revealed significant (P<0.01) differences in the heat treatments and harvests but not the varieties (P>0.05), while interaction of these factors was significant (P<0.01). Under control condition, Sultan indicated similar K at day-2 and day-6 but an increased one at day-4, while in S-2002 this value was fairly constant at day-2 and day-4 but decreased at day-6. However, under heat stress K was similar in both the varieties at day-2 and day-4 but increased greatly in Sultan at day-6 (Fig. 2).

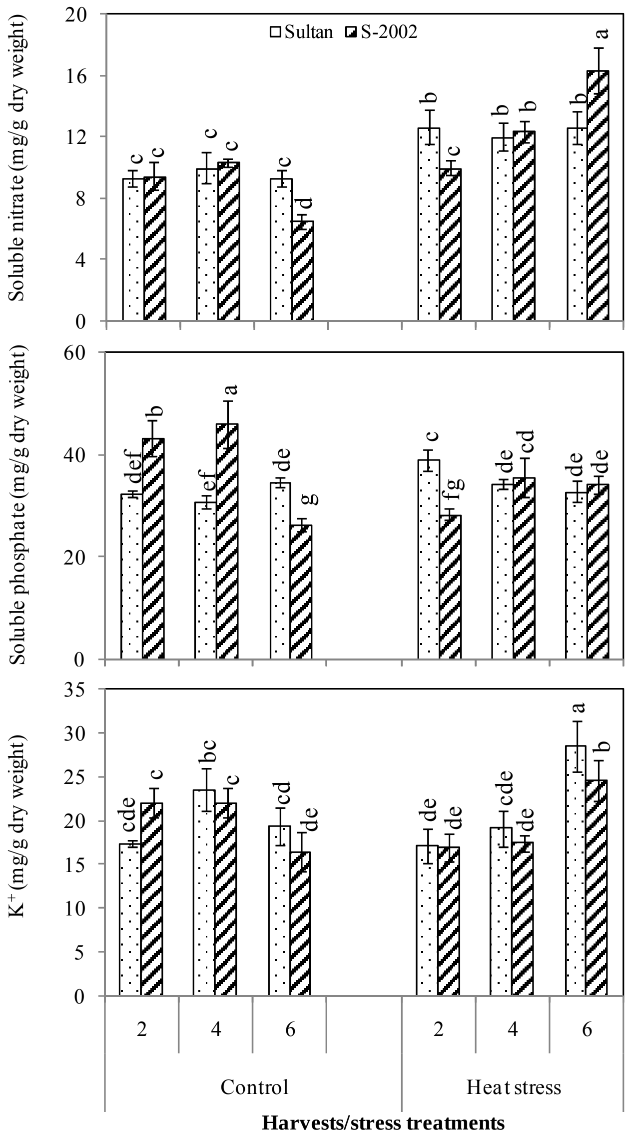
Data for starch contents revealed no-significant (P>0.05) difference in the varieties, but significant (P<0.01) ones in the heat stress treatments, with significant (P<0.01) interactions of these factors. Sultan, under control condition, although indicated much greater starch contents at day-2, got reduced at day-4 and day-6, while they increased at day-4 and then severely declined at day-6. However, under heat stress although starch contents increased in both the

Fig. 1: Time course changes in the shoot fresh and dry weight and water contents of differentially heat tolerant maize varieties under heat stress at three harvests. In this and subsequent figures, alphabets on the columns indicate significant (P<0.05) interaction of varieties, harvests and stress treatments



varieties, Sultan manifested a much lower increase as compared to S-2002 (Fig. 3). In case of root starch, statistical analysis of data exhibited significant (P<0.01) difference in the varieties, heat stress treatments and harvests, while all possible interactions of these factors were significant (P<0.01). Under control condition, the starch contents were lower than under heat stress. Sultan indicated a decreased, while S-2002 an increased starch contents at (control). On the other hand under heat stress, Sultan displayed an increased, while S-2002 a consistently decreased root starch contents (Fig. 3). Results regarding oil contents revealed significant (P<0.01) differences in the varieties, temperature treatments and harvests with

Fig. 2: Time course changes in the shoot soluble nitrate, soluble phosphate and potassium contents of differentially heat tolerant maize varieties under heat stress at three harvests



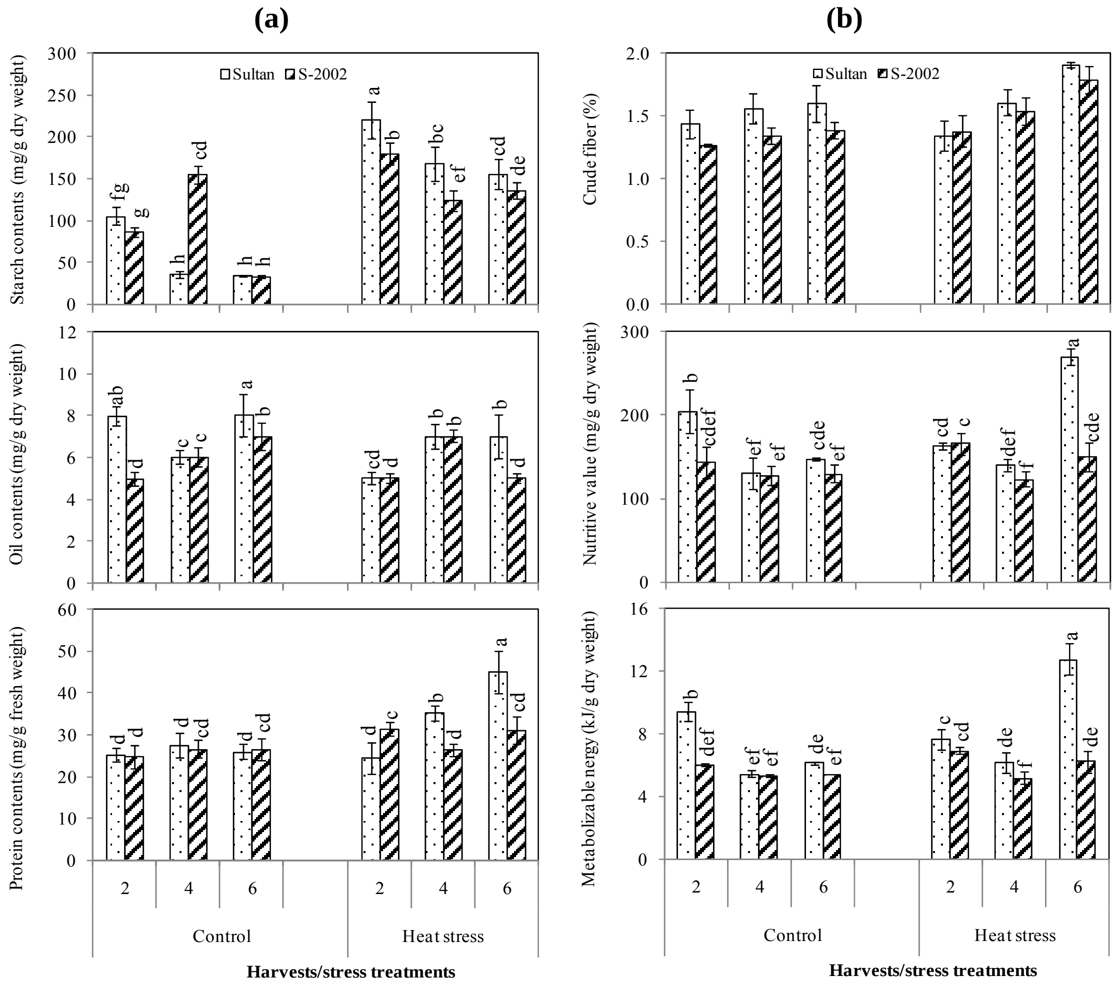
significant (P<0.01) interactions of these factors, except the only non-significant (P>0.05) varieties × heat stress treatments interaction. Sultan indicated similar oil contents at day-2 and day-6, but decreased at day-4 under control, which consistently increased in S-2002. However, under heat stress Sultan displayed consistently increased oil contents at all harvest, while S-2002 indicated similar oil contents at day-2 and day-6 but increased at day-4 (Fig. 3). In case of root oil contents, data exhibited significant difference in the varieties (P<0.01), heat stress treatments (P<0.05) and harvests (P<0.01), while all possible interactions of these factors were significant (P<0.01) except a non-significant interaction of varieties × harvests. Under control condition, both the varieties indicated much increased root oil contents at day-2, which declined substantially at day-4 and then increased at day-6. Under heat stress although both the varieties indicated a declining root oil contents, Sultan excelled the S-2002 and displayed greater root oil contents (Fig. 3). Statistical analysis of data for proteins contents revealed significant (P<0.01) differences in the varieties heat stress treatments and harvests. All possible interactions of these factors were also significant (P<0.01). Under control condition, both the varieties showed similar protein contents at all harvests. However, under heat stress the proteins contents increased in Sultan but decreased in S-2002 at day-4 and then increased at day-6 (Fig. 3). For root proteins contents, there was significant (P<0.01) difference in the varieties, heat stress treatments and harvests, whereas all interactions of these factors were significant (P<0.01) except the only non-significant (P>0.05) varieties × interaction. Under control condition, S-2002 indicated relatively greater root protein contents than Sultan. However, root protein contents increased in both the varieties at all harvests, while S-2002 showed greater value of this attribute was noted in S-2002 at day-2 and day-66 (Fig. 3).

For crude fiber, there were significant (P<0.01) differences in the varieties, temperature treatments and harvests with significant interactions of varieties × treatments (P<0.05) and temperature × harvests (P<0.01). Under control condition, the crude fiber increased in both the varieties, although it was greater in Sultan at all harvests. Heat stress increased the crude fiber in both the varieties at all harvests but was smaller in Sultan at day-2 and greater at day-4 and day-6 than S-2002 (Fig. 3). Varieties, heat treatments and harvests indicated significant (P<0.01) differences, with significant (P<0.01) interactions of these factors. Under control condition, the nutritive value declined in both the varieties at all harvests, although Sultan indicated a lesser decline. However, under heat stress this character was similar at day-2, reduced at day-4 and again increased at day-6, although this increase was much greater (~50%) in Sultan (Fig. 3). The metabolizable energy revealed significant (P<0.01) differences in the varieties, heat stress treatments and harvests with significant (P<0.01) interactions of these factors. Under control, Sultan indicated a reduction, while S-2002 indicated a fairly steady state level of metabolizable energy. However, under heat stress although Sultan manifested a pronounced increase in metabolizable energy at all harvests, while S-2002 indicated no consistent trend (Fig. 3).

DISCUSSION

This study revealed that heat stress was deterrent to the growth of both the maize varieties as determined in terms of changes in fresh and dry weight and water contents of shoot over three time intervals (Fig. 1). However, the responses of the varieties were quite variable, since Sultan (tolerant maize) performed better than the S-2002 (sensitive maize).

Fig. 3: Time course changes in the shoot (a) starch, oil and protein contents and (b) crude fiber, nutritional value and metabolizable energy differentially heat tolerant maize varieties under heat stress at three harvest periods



This can be implicated in the better ability of the former variety (Sultan) to harness and utilize the available light water and nutrient than the latter (S-2002). Heat stress, by acting as a dehydrative force, leads to disturbed cell water relations (Wahid & Close, 2007). Since heat stress has a direct effect on the above ground plant parts, the crop varieties capable of exhibiting greater aboveground biomass are on an advantage (Gawronska *et al*., 1992; Cruz-Aguado *et al*., 2000). In this study, since the water supply to the roots was not limiting, the inability of the S-2002 to show reduced shoot water contents appeared due to disturbed conduction of water and minerals to the shoot; thus implying the hampered xylem activity.

Studies show that stress deteriorates both qualitative and quantitative attributes of plant. This is because the plant metabolic pathways are diverted to invest more energy for stress tolerance (Buxton, 1996; Wahid *et al*., 2007). Thus, the plants capable of having high stress tolerance ability also tend to show better nutritional quality (Cherney *et al*., 1993; Suyama *et al*., 2007), although reports are scant on this aspect under high temperature. Results with forage standpoint showed that heat tolerant maize (Sultan) grown under heat stress compared with sensitive maize (S-2002) indicated a greater crude fiber, starch and oils, increased proteins, steady state level of nitrate, greater and root phosphate and greater K (Fig. 2), showing no-great adverse effect of heat stress. Heat stress, as a dehydrative force (Wahid & Close, 2007), influences the physiological phenomena of cells and reduces the utilization of available essential nutrients (Wahid *et al*., 2007). Greater quantities of all these constituents are crucial for superior nutritional quality of forage (Redfearn, 2010). It is can be further noted from the above that tolerant maize provided a more balanced forage quality than sensitive maize.

Results with forage standpoint showed that heat tolerant maize compared with sensitive maize (S-2002) indicated a steady state level of SN, SP and K (Fig. 2), as well as greater crude fiber, starch and oil contents and proteins (Fig. 3) under heat stress. Better heat tolerance ability of the tolerant maize, in this study, was linked to proportionately greater tissue concentrations of nitrate, phosphate and K, since heat stress generally hampers the uptake of nutrients. It is imperative to mention that these nutrients act as structural constituents and functional activators of starch, proteins and oils (Epstein & Bloom, 2005), which are of imperative as forage. The nutritional value of a plant can be further assessed from the metabolizable energy, which presents true status of the forage value for ruminant animals (Russell *et al*., 1992). The metabolizable energy, measured mainly from the starch, fatty acids and proteins, increased in the tolerant maize under heat stress especially at later stages (Fig. 3). Sultan in comparison with S-2002 provided a more balanced diet and greater metabolizable energy without much deterioration of the forage quality (Figs. 2 & 3). This implied that maintenance of higher tissue nutrients is a likely determinant of heat stress tolerance and better forage value of tolerant maize (Redfearn, 2010).

To conclude, heat stress reduced the growth and altered the forage quality more of the sensitive than tolerant maize. Maintenance of greater nutritional properties of the tolerant maize may offer better opportunities to provide as forage for animals in the relatively hot environments. Further studies are imperative using large number of samples and at more harvests.

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