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Role of Copper on Physiological Parameters and Salt Tolerance in Sweet Sorghum (*Sorghum bicolor* L.) Cultivars

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ABSTRACT

This research was carried out to determine effect of sorghum cultivars and copper foliar spray on physiological traits under saline water situation via split plot experiment based on randomized complete blocks design with four replications during 2015 and 2016 year. The main plot consisted sorghum cultivars at three level (Kimia, KGS10, KGS4) and Copper foliar spray (C_1: 0 or control, C_2: 0.20, C_3: 0.30 and C_4: 0.40 kg Cu ha^{-1}) by source of $CuSO_4$ at stem elongation stage (55 days after planting date) belonged to sub plot. Result of combined analysis of variance revealed effect of different sorghum cultivar, Cu foliar application and interaction effect of treatment on all measured traits was significant at 1% probability level. Foliar application of copper had effective role on salt tolerant and physiological parameters of the studied sorghum cultivars were significantly affected by the exposure to well water and copper. The cultivar Kimia was observed as more salt tolerant and cultivar KGS4 was more salt sensitive on the basis of starch, amylose and amylopectin rates. Cultivar Kimia was also observed to produce high rates of amylose and amylopectin compared with the other cultivars. Results of this experiment showed that effect of copper on physiological contents is a useful tool for measuring the salt tolerance among sorghum cultivars to identify possible donors for future sorghum quality enhancement and breeding and be useful to the local sorghum growing farmers under salt stress. According to result of current research Kimia cultivar with foliar application of 0.4 kg Cu ha^{-1} it can suggested to farmers to decrease negative effect of salinity situation.

Keywords*:* *Amylose, Genotype, Protein, Starch, Stress.*

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INTRODUCTION

Sorghum is the fourth most important cereal crop grown in the world. Sorghum is grown on approximately 44 million hectares in 99 countries. In Bangladesh, 254 tons of sorghum grains are produced annually from about 187 ha of land with an average of 1.36 tons per hectare (FAOSTAT, 2013). Sorghum has potential uses such as: food (grain), feed (grain and biomass), fuel (ethanol production), fiber (paper), fermentation (methane production) and fertilizer (utilization of organic byproducts) (Roy *et al*., 2018). Sorghum production in Iran has spanned almost 120 yr. The crop has served producers and end users well, as advancements in cultivar development have produced the high-performing, well adapted, premium quality cultivars. For example, screening of seven salinity tolerant and ten salinity sensitive sorghum genotypes was reported (Chuck and Donnelly, 2014). Grain sorghum as a staple food grain in several developing countries (Buah and Mwinkaara, 2009) is an important crop in arid and semiarid regions, because of its environmental adaptability. Also, sorghum is one of the most widely adapted forage crops to the arid and semi-arid tropics and dry-temperate areas of the world (Kidambi *et al*., 1990; Blum, 2004). The productivity of grain sorghum could be increased by improving the cultural practices, such as irrigation regime, nitrogen fertilizer and plant density. Sorghum is an important cereal grain due to its drought resistance and relatively low input costs. Worldwide, sorghum is ranked fifth among cereal grains in terms of quantity and importance (Rooney and Awika, 2005). Agricultural crops exhibit a spectrum of responses under salt stress. Salinity not only decreases the agricultural production of most crops, but also, effects soil physicochemical properties, and ecological balance of the area. The impacts of salinity include-low agricultural productivity, low economic returns and soil erosions, (Hu and Schmidhalter, 2002). Salinity effects are the results of complex interactions among morphological, physiological, and biochemical processes including seed germination, plant growth, and water and nutrient uptake (Akbarimoghaddam *et al*., 2011). Salinity affects almost all aspects of plant development including: germination, vegetative growth and reproductive development. Soil salinity imposes ion toxicity, osmotic stress, nutrient (N, Ca, K, P, Fe, Zn) deficiency and oxidative stress on plants, and thus limits water uptake from soil (Bano and Fatima, 2009). Salinity is common in arid regions due to an inability to flush accumulated salts from the soil (Bahizire, 2007). Saline soils are defined as those that contain sufficient soluble salts in the root zone to impair plant growth (Omami, 2005). Approach like combination of physiological and metabolic aspects of salt tolerance is essential to overcome the effects of salinity stress and develop salt-tolerant of the plant varieties (Roychoudhury and Chakraborty, 2013). In general, sorghum is known as moderately tolerant to salinity (Metwali, 2015). Salt tolerance is usually assessed as the percent biomass production in sorghum in saline control conditions affected with foliar of copper over a prolonged period of time or in terms of survival (Chao *et al*., 2015). Nutrients play a very important role in chemical, biochemical, physiological, metabolic, geochemical, biogeochemical, and enzymatic processes. Magnesium has major physiological and molecular roles in plants, such as being a component of the chlorophyll molecule, a cofactor for the many enzymatic processes associated with the phosphorylation, de-phosphorylation, and the hydrolysis of various compounds, and as a structural stabilizer for various nucleotides (EL-Metwally *et al*., 2010). In one such study, advanced breeding in salt tolerant sorghum lines, germplasm accessions showed higher values for Cu than popular cultivars (Lavenson *et al*., 2014). The range of Cu content was 3.05 to 4.12 mg kg^{-1} of soil. Most of these approaches suffer from salt stress problems by physiological parameters, but apply of Cu foliar has been found to be the best way of decreasing salinity effects in sorghum (Liu *et al*., 2013). Main cause of trace element deficiency is the introduction of high yielding crop varieties and more use of fertilizers. Micronutrient deficiencies in crop plants become important worldwide because over growing population of world is affected by lower level of micronutrient in human food (Welch and Gramham, 1999) and poor content of essential nutrients and micronutrients in grains of modern high yielding wheat cultivars are mostly recognized (Fan *et al*., 2008). Copper (Cu) is one of eight essential plant micronutrients. Copper is required for many enzymatic activities in plants and for chlorophyll and seed production. Deficiency of copper can lead to increased plant susceptibility to disease, one example being ergot which can cause significant yield loss in small grains. Most Minnesota soils supply adequate amount of copper for crop production. However, copper deficiency can occur in high organic matter and sandy soils (Sutradhar *et al*., 2017). The amount of copper available to plants varies widely among soils. Copper in the soil is held with clay minerals as a cation (Cu^{2+}) and in association with organic matter. Some silicate minerals and carbonate contain copper as impurities (Sutradhar *et al*., 2017). Copper is not mobile in organic soils as it is attracted to soil organic matter and clay minerals. Copper deficiencies often occur in soils with peaty soils with greater concentrations of organic matter. Copper binds with organic matter more tightly than any other of the crop micronutrients. Crops sensitive to copper deficiency grown on peat soils with organic matter content more than 8% are likely to show copper deficiency symptoms (Sutradhar *et al*., 2017). Copper is an essential plant nutrient that plays an efficient role in chlorophyll development, and protein formation from amino acids and gives rigidity to plant because copper strengthens plant cell wall. In all plants Cu is essential for more than 30 enzymes which acts as redox catalysts like nitrate reductase, cytochrome oxidase or act as dioxygen carrier like heamocynin (Mohamed and Taha, 2003). Copper also has an influence on the metabolic processes of plant like photosynthesis and reduction of respiration in pollen capability and its deficiency increases infertility of spikelet in lot of unfilled grains (Dobermann and Fairhurst, 2000). Copper use efficiency is improved if the fertilizer is water soluble and the particle size of the fertilizer is small. A single application of copper can last for many years. Foliar application of copper can also be an effective way to correct copper deficiency in small grains and vegetable crops. The growth stage and application time has a major influence on the effectiveness of the treatment (Sutradhar *et al*., 2017). Copper mobilizes from old leaves to younger parts of the plant to some extent with the degree of mobilization greater when Cu is more available to the plant and movement is related to leaf senesce (Loneragan, 1981). Mature plants deficient in Cu have delaying heading, and empty or partially-filled heads due to lack of viable pollen and also senesce (Graham, 1975). Copper is naturally present in soil in several soluble (hydroxy and carbonate) and insoluble (oxide and sulphide) forms and with the soluble form differing in its availability to plants dependent on other soil properties predominantly soil pH, clay content and the presence of organic matter (Fernandes and Henriques, 1991). Plant roots take up Cu from soil solution as water soluble Cu^{2+} in the soil or from fertiliser. Uptake of Cu from the soil and into the plant depends on (Fernandes and Henriques, 1991): a. limited movement of the nutrient via mass flow or diffusion (from the soil to the root) b. the chemical availability of the nutrient c. growth of roots through the soil (root interception) d. active and passive uptake of the nutrient at the root surface itself. Foliar fertilization with micronutrients has been intensively used in the late years because this practice allows the application of minerals at the appropriate time during plant development (according to plant needs), it allows uniformity in nutrient distribution and increase in the nutrient absorption, and consequently it avoids losses in the environment (Ruiz-Garcia and Gomez-Plaza, 2013). Foliar application of Cu is an alternative to soil-applied Cu fertilizer. Foliar application has the advantage of allowing Cu to be applied strategically based on seasonal progress and the occurrence of visual symptoms. The most common form of foliar fertilizer is $CuSO_4$ (25% Cu). Alternative forms are copper oxychloride (52% Cu) and chelated-Cu (15% Cu) (Brennan, 1990). The tolerant genotypes could be recommended for cultivation in moderately salt affected areas (Roy *et al*., 2014). Previously, experiments on the selection of salt tolerance cultivars of sorghum were conducted using copper foliar application improved physiological factors included amylose, amylopectin, starch and proteins (Faraco, 2015). However, physiological parameters (proteins, starch, tannin, amylose and amylopectin) at the mid of flowering stage and foliar of copper at the stem elongation stage may provide an easy and inexpensive natural ways to select salt tolerant sorghum cultivars to be used for cultivation or breeding programs (Jaros *et al*., 2015). Starch is the major component of grain sorghum, constituting 70% of dry grain weight (Hoseney *et al*., 1981). Many important physicochemical, thermal, and rheological properties of starch are influenced by the ratio of amylose and amylopectin, the two major polymers in the starch granule, and by the structure of amylopectin. The amylose content strongly affects starch gelatinization and retro gradation (Fredriksson *et al*., 1998), paste viscosity (Yanagisawa *et al*., 2006), gelation (Biliaderis and Zawistowski, 1990), and R-amylase digestibility (Skrabanja *et al*., 1999). The fine structure of amylopectin (chain-length distribution) also was found to influence starch gelatinization and retro gradation properties (Jane *et al*., 1999). Amylose content of sorghum grain depends on the dose of a recessive gene (Sang *et al*., 2008). Starch has 2 major molecular components, amylose and amylopectin. Amylose is linear with few branches. It plays a critical role in the properties and uses of starch (Zhu and others, 2011). Amylopectin is the major component of starch and larger than amylose. (Craig and Stark, 1984). The protein content of sorghum (11.3%) is nearly equal and is comparable to that of wheat and maize. Average starch content of the seeds range from 56 to 73% and is relatively rich in iron, phosphorous and vitamin B-complex (ICRISAT, 2013). Protein content is controlled by genetic, environment and soil fertility.

Positive correlations between environmental factors and wheat protein content have been reported during grain filling (Huebner *et al.*, 1997). Thus the objective of the study was to ascertain the extent of physiological traits that included starch, amylose, amylopectin, proteins, and tannin in response to foliar application of copper on different sorghum cultivar under saline water. These traits are important because they have a direct effect on salt resistant in sorghum cultivars for planting in saline lands.

MATERIALS AND METHODS

Field and Treatment Information

This research was carried out to assessment effect of sorghum cultivars and copper foliar spray on physiological traits under saline water situation via split plot experiment based on randomized complete blocks design with four replications during 2015 and 2016 year. The main plot consisted sorghum cultivars at three level (Kimia, KGS10, KGS4) and Copper foliar spray (C_1: 0 or control, C_2: 0.20, C_3: 0.30 and C_4: 0.40 kg Cu ha^{-1}) by source of $CuSO_4$ at stem elongation stage (55 days after planting date) belonged to sub plot. Also average of irrigated water was 23 mm (each time) for each plot. Place of research was located in Islamic Azad University Research Field in Ahvaz City at longitude 48°40'E and latitude 31°20'N with 18 m above the sea level in Khuzestan province (Southwest of Iran). Details of the experimented soil and irrigated water are shown in table 1 and 2. The row spacing was 70 cm and density was 150,000 seed.ha^{-1} during the May to September season. Before the cultivation, 240 kg of phosphorus (based on 120 kg ha^{-1} of triple super phosphate source) and 300 kg of nitrogen from urea source (360 kg ha^{-1}) were mixed with soil by disc and plants were irrigated by saline water from the well which stated in experimented field.

Table 1. The physical and chemical soil characteristics

Soil depth	Soil texture	pH	EC (dS.m^{-1})	N (%)	P (mg.kg^{-1})	K (mg.kg^{-1})	CO_3 (mg.kg^{-1})	HCO_3 (mg.kg^{-1})
0-30	Silty loam	7.69	2.74	0.072	2.47	139	0.31	3.89

Table 2. The properties of saline irrigated water in experimented field

Na (meq.lit^{-1})	K (meq.lit^{-1})	Mg (meq.lit^{-1})	EC (dS.m^{-1})	Cl (meq.lit^{-1})	CO_3 (meq.lit^{-1})	HCO_3 (meq.lit^{-1})	SO_4 (meq.lit^{-1})
39.3	0.15	3.7	4.6	3.52	0.75	9.6	1.5

Measured Traits

The grain from two middle rows in each four row of the experimental unit was harvested, dried, threshed and used for subsequent laboratory starch (%), amylose (%), amylopectin (%), proteins (%) and tannin (mg.100 ml^{-1}) tests.

Determination of protein content: Total nitrogen and protein was determined using Kjeldahl method (AOAC, 1999). Sorghum grain was finely milled and 0.1 g was transferred into a digestion tube. Selenium catalyst mixture weighing 1g was mixed with the sample and 5 ml of sulphuric acid (96%) was added into the tube. The tubes were then heated slowly in the digestion apparatus, until the digest was clear. The sample was transferred to a 100 ml volumetric flask, and distilled water was added into a 100 ml graduated flask. A boric acid indicator solution (5 ml) was then transferred to 100 ml conical flask containing 5 drops of mixed indicator and was placed under the condenser of the distillation apparatus. The clear supernatant (10 ml) was then transferred into the apparatus and 10 ml of 46% sodium hydroxide was added and then rinsed again with distilled water. Color changed from pink to green when the first distillation drops mixed with the boric acid indicator solution. A total of 150 ml of the distillate was collected and titrated with 0.0174N sulphuric acids until the color changed from green to pink. The titer volume was then read. Total nitrogen (N) was then determined as follows:

Equ. 1. $\% N = |a \times N \times Mw \times 100/ b \times c| \times 100\%$.

Where, a= ml of sulphuric acid used for titration of the sample, N = Normality of sulphuric acid (0.0174), Mw = Molecular weight of N_2 (0.014), c = ml digest taken for distillation (10 ml), b = g sample taken for analysis (0.1 g), % crude protein = 6.25 × % N.

Determination of starch content: Percent starch content was estimated by the Anthrone method (Almodares *et al*., 2007). A powdered sample (0.25 g) was homogenized in hot 80% ethanol to remove sugars. The residue was then centrifuged and dried well over a water bath. To the residue, 5.0 ml of distilled water and 6.5 ml of 52% perchloric acid was added, and then extracted at 0°C for 20 min. The supernatants were centrifuged, pooled and made up to 100 ml. of this supernatant; 0.1 ml was pipetted out and made up to 1 ml with distilled water. The standards were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of the working solution and the volume made up to 1 ml in each tube with water. To these standards, 4 ml of anthrone reagent was added to each tube and the sample heated for eight minutes in a boiling water bath. The sample was cooled rapidly and the intensity of green to dark green color was read using a spectrophotometer at 630 nm. The glucose content in the sample was determined using the standard calibration graph, and then the value was multiplied by a factor of 0.9 to determine starch content.

Determination of Amylose and amylopectin content: Amylose was determined using the Davis *et al.* (2006) method where a sample (0.1 g) of the powdered flour was weighed, and 1 ml of distilled ethanol added followed by 10 ml of 1 N NaOH. The sample was heated for 10 min in a boiling water bath. The volume was made up to 100 ml. To a 2.5 ml extract, 20 ml of distilled water was added followed by three drops of 0.1% phenolphthalein. Drop wise HCl 0.1N was then added until the pink color disappeared. To this solution, 1 ml iodine reagent was added till the volume was 50 ml and the color read at 590 nm using a spectrophotometer. Standard amylose working solution 0.2, 0.4, 0.6, 0.8 and 1 ml was taken and the color developed as in the case of the test samples. The amount of amylose present in the sample was calculated using the drawn standard graph. The blank was obtained by diluting 1 ml of iodine reagent to 50 ml with distilled water. Amylose content was obtained thus:

Equ. 2. Amylose (%) = $|X/2.5| \times 100$ mg amylose, Where X is the absorbance obtained.

The amylopectin content was obtained thus: % Starch-% Amylose

Determination of tannin content: A sample of 0.5 g of the milled flour was weighed and transferred to a 250 ml conical flask, and then 75 ml of water added. The flask was heated gently and boiled for 30 min, then centrifuged at

2000 rpm for 20 min. The supernatant was collected in a 100 ml volumetric flask. A measure of 1 ml of the sample extract was transferred to a 100 ml volumetric flask containing 75 ml water. Five ml of folin reagent and 10 ml of 35% sodium carbonate solution were added, and then diluted to 100 ml with water. The sample was shaken and the absorbance read at 700 nm after 30 min. A graph was prepared using 0-100 mg tannic acid, where 1 ml contained 100 mg tannic acid. The tannin content of sample was calculated as tannic acid equivalent from standard curve. Tannins content was determined by Folin-Denis method (Serna-Saldivar *et al*., 2012).

Statistical Analysis

Data were analyzed by combine analysis of variance via SPSS (Statistical Product and Service Solutions) software and mean comparison was done with Duncan's multiple-range tests at 5% probability level.

RESULTS

Protein

Result of combined analysis of variance revealed effect of different sorghum cultivar, Cu foliar application and interaction effect of treatment on protein content was significant at 1% probability level (Table 3). Mean comparison result of interaction effect of different level of sorghum cultivar and Cu foliar application indicated Kimia cultivar with 0.40 $kg.ha^{-1}$ copper application had the highest amount of protein with 14.69% to 14.91% along 2014 and 2015, respectively (Table 4).

Table 3. Combine analysis of variance of measured traits affected sorghum cultivars and copper

S.O.V	df	Protein	Starch	Amylose	Amylopectin	Tannin
Year (Y)	1	0.518^{ns}	0.545^{ns}	0.671^{ns}	0.895^{ns}	0.599^{ns}
Replication (R)	3	0.759^{ns}	0.837^{ns}	0.912^{ns}	0.744^{ns}	0.848^{ns}
R×Y	3	0.611^{ns}	0.802^{ns}	0.645^{ns}	0.681^{ns}	0.974^{ns}
Cultivar (C)	3	823.54^{**}	991.35^{**}	909.46^{**}	931.25^{**}	890.55^{**}
C×Y	2	0.745^{ns}	0.638^{ns}	0.901^{ns}	0.896^{ns}	0.845^{ns}
C×R	6	704.26^{**}	645.37^{**}	518.79^{**}	740.31^{**}	702.79^{**}
C×R×Y	6	0.805^{ns}	0.749^{ns}	0.698^{ns}	0.705^{ns}	0.715^{ns}
Cu	3	513.29^{**}	641.18^{**}	503.79^{**}	606.92^{**}	639.91^{**}
Cu × Y	3	0.825^{ns}	0.710^{ns}	0.598^{ns}	0.441^{ns}	0.752^{ns}
Cu × R	9	456.32^{**}	533.45^{**}	418.92^{**}	479.85^{**}	477.68^{**}
Cu × R × Y	9	0.947^{ns}	0.876^{ns}	0.809^{ns}	0.744^{ns}	0.711^{ns}
Cu × C	6	374.88^{**}	336.71^{**}	401.98^{**}	403.60^{**}	369.84^{**}
Cu × R × Y × C	18	0.774^{ns}	0.611^{ns}	0.707^{ns}	0.813^{ns}	0.655^{ns}
CV (%)	-	5.26	5.71	6.49	8.11	7.58

^{ns}, * and ** are non-significant and significant at 5 and 1% probability levels, respectively.

These values are suitable to bio ethanol production, since protein content is a major factor to ensure the feasibility of the process. After Kimia, the highest amount of protein content (13.62 and 13.66%) was achieved by KGS10 cultivar with use 0.40 $kg.ha^{-1}$ copper foliar application and the lowest one (5.74, 5.79%) belonged to the KGS4 cultivar with non-application of copper treatment (Table 4).

Starch

According result of combined analysis of variance effect of different sorghum cultivar, Cu foliar application and interaction effect of treatment on starch content was significant at 1% probability level (Table 3). Assessment mean comparison result indicated in different level of sorghum cultivar and Cu foliar application the maximum starch content (70.62, 69.88%) was noted for Kimia cultivar with 0.40 kg.ha^{-1} copper application and minimum of that (32.11, 32.33%) belonged to KGS4 cultivar with non-application of copper treatment along 2014 and 2015 (Table 4).

Amylose

Result of combined analysis of variance showed effect of different sorghum cultivar, Cu foliar application and interaction effect of treatment on amylose content was significant at 1% probability level (Table 3). Between different levels of sorghum cultivar and Cu foliar application the maximum amylose content (19.92, 19.42%) was observed in Kimia cultivar with use of 0.40 kg.ha^{-1} copper and the lowest one (9.26, 9.42%) was found in KGS4 cultivar with non-application of copper treatment along 2014 and 2015 (Table 4).

Table 4. Mean comparison interaction effect of sorghum cultivars and Cu foliar application on measured traits

Treatment			Protein	Starch	Amylose	Amylopectin	Tannin
Year	Cultivar	Cu	(%)	(%)	(%)	(%)	(mg.100 ml^{-1})
2014	Kimia	C_1	8.44*^{d}	49.83^{d}	16.01^{d}	33.82^{d}	20.36^{d}
		C_2	9.96^{cd}	54.09^{cd}	17.24^{cd}	36.85^{cd}	25.57^{cd}
		C_3	11.75^{bc}	60.57^{bc}	18.69^{bc}	41.88^{bc}	32.81^{bc}
		C_4	14.69^{a}	70.62^{a}	19.92^{a}	50.70^{a}	43.12^{a}
	KGS10	C_1	7.53^{ef}	39.66^{ef}	12.97^{ef}	26.69^{ef}	16.48^{e}
		C_2	8.41^{e}	44.15^{e}	14.06^{de}	30.09^{de}	20.76^{d}
		C_3	10.33^{cd}	52.57^{cd}	15.84^{d}	36.73^{d}	28.61^{c}
		C_4	13.62^{b}	59.98^{bc}	17.01^{cd}	42.97^{bc}	38.59^{b}
	KGS4	C_1	5.74^{f}	32.11^{f}	9.26^{g}	22.85^{fg}	14.87^{ef}
		C_2	6.96^{ef}	37.56^{ef}	11.59^{f}	25.97^{ef}	18.73d^{e}
		C_3	8.89^{e}	44.72^{e}	13.94^{ef}	30.78^{de}	23.46^{cd}
		C_4	10.85^{cd}	51.43^{cd}	15.62^{d}	35.81^{d}	34.11^{bc}
2015	Kimia	C_1	8.57^{d}	49.52^{d}	15.97^{d}	33.55^{d}	20.41^{d}
		C_2	10.01^{cd}	54.03^{cd}	17.12^{cd}	36.91^{cd}	25.42^{cd}
		C_3	11.59^{bc}	60.67^{bc}	18.75^{bc}	41.92^{bc}	32.45^{bc}
		C_4	14.91^{a}	69.88^{a}	19.49^{a}	50.39^{a}	43.29^{a}
	KGS10	C_1	7.71^{ef}	39.60^{ef}	12.83^{ef}	26.77^{ef}	16.52^{e}
		C_2	8.55^{e}	44.59^{e}	14.20^{de}	30.39^{de}	20.89^{d}
		C_3	10.8^{cd}	52.72^{cd}	15.91^{d}	36.81^{d}	28.75^{c}
		C_4	13.66^{b}	59.45^{bc}	16.94^{cd}	42.51^{bc}	38.91^{b}
	KGS4	C_1	5.79^{f}	32.33^{f}	9.42^{g}	22.91^{fg}	14.65^{ef}
		C_2	7.02^{ef}	37.30^{ef}	11.78^{f}	25.52^{ef}	18.75^{de}
		C_3	8.71^{e}	44.44^{e}	13.60^{ef}	30.84^{de}	23.72^{cd}
		C_4	10.54^{cd}	51.41^{cd}	15.93^{d}	35.48^{d}	34.25^{bc}

*Similar letters in each column show non-significant difference at 5% probability level in Duncan test.
(Copper foliar spray; $\mathbf{C_1}$: 0 or control, $\mathbf{C_2}$: 0.20, $\mathbf{C_3}$: 0.30 and $\mathbf{C_4}$: 0.40 kg Cu ha^{-1})

Amylopectin

According result of combined analysis of variance effect of different sorghum cultivar, Cu foliar application and interaction effect of treatment on amylopectin content was significant at 1% probability level (Table 3). Evaluation mean comparison result revealed in different level of sorghum cultivar and Cu foliar application the maximum amylopectin content (50.70, 50.39%) was noted for the Kimia cultivar with 0.40 kg.ha^{-1} copper application and minimum of that (22.85, 22.91%) belonged to KGS4 cultivar with non-application of copper treatment along 2014 and 2015 (Table 4). Similar result was reported by another researchers (Hypla, 2016; Pamal, 2017).

Tannin

Result of combined analysis of variance showed effect of different sorghum cultivar, Cu foliar application and interaction effect of treatment on tannin content was significant at 1% probability level (Table 3). Among different levels of sorghum cultivar and Cu foliar application the maximum amount of tannin (43.12, 43.29%) was belonged to Kimia cultivar with use of 0.40 $kg.ha^{-1}$ copper and the lowest one (14.87, 14.65%) was found in KGS4 cultivar with non-application of copper treatment along 2014 and 2015 (Table 4).

DISCUSSION

Data presented in Table 3 indicated that all cultivars significantly affected due to Cu foliar application in protein, starch, amylose, amylopectin and tannin in two cropping year. These values are suitable to bio ethanol production, since protein content is a major factor to ensure the feasibility of the process. Data of Table 3 illustrates total amylose and amylopectin for the three cultivars of sweet sorghum showed significant differences exist among cultivars in different copper level. Kimia contained the highest values of total amylose, and amylopectin compared to the other two cultivars in C_4 treatment. The data indicated that C3 and C4 treatments could enhance the investigated traits under salt stress (Table 4). Table 4 showed in KGS4 cultivar reduction of starch was 26.81% and 13.89% compared with Kimia and KGS10 cultivars, respectively. From all the tested cultivars, C_4 treatment gave the best values conversion efficiency on physiological parameters. The outcomes for protein, relative copper rates are shown in Table 3, where there were significant differences between the four copper treatments. Overall, the average protein was 11.24% for Kimia, 10.07% for KGS10 and 8.06% for KGS4 (Table 4). The average protein in cultivars in 2015 was higher than 2014 but this difference was not significant and related to copper treatment. However, it is interesting that variation in tannin was observed when sorghum treated with Cu foliar application. Overall impact of saline water and copper was significant on tannin of different cultivars. Tannin is a polyphenolic that sorghum crops do presently contain condensed tannin (El-Naim *et al*., 2015). Nevertheless, sorghum crops contain a diverse variety of polyphenolic compounds and phenolic acids, which are usually at substantially higher concentrations than other feed grains (Hefny *et al*., 2015). It is our contention is that the copper properties can induce phenolic compounds in grain sorghum (Liu *et al*., 2013). In the present study, protein and starch was affected to concentrations of copper which stimulate 3-deoxyanthocyanins in amino acids conjugation and convert them to protein. Moreover, Metwali (2015) suggested that phenolic compounds, including tannin, contain hydroxyl groups and may interact with and form complexes with proteins at salt stress. It has been reported that Cu spray offered sorghum based saline water had better performance than plants offered based saline water (Zhu, 2015). In the present study, high contents of tannin, starch and protein in sorghum and is a good evident for positive effect of copper on salt tolerant performance. As shown in Table 4, amylose, amylopectin and starch rates were positively influenced by copper concentrations and saline water. Duncan test was conducted to compare the relative importance of copper and cultivar on salt tolerant and they could not be considered separately; therefore, they may be equally important (Table 4). Copper is present in sorghum endosperm as discrete protein bodies and embedded in the glutelin protein matrix in association with starch granules (Hung *et al*., 2015). Rani *et al*. (2015) reported that copper could inhibit sodium uptakes transporters. This was attributed to the dissipation of the Na^+ which drives the active absorption of copper. The influence of concentrations of copper in sorghum in Table 4 suggested that basis of salinity tolerance is more complex and probably results in the expression of physiological parameters and salt concentration in irrigation water (Khoddami *et al*., 2015). Plant response to salinity includes increasing salt concentration in rhizosphere to a threshold level in this study and copper may enhanced roots probe deeper into soil for search of more water, since salt stress ionic toxicity (Shergo *et al*., 2013). We recommend producers analyze higher year of fields for micro elements to evaluate Na management and identify sites where a Cu foliar spray can be omitted yield quality without drainage or leaching requirement.

CONCLUSION

Physiological parameters of sorghum cultivars were significantly affected by exposure to saline water and copper spray. Kimia was observed as more salt tolerant and KGS4 was more salt sensitive on the basis of copper, starch, protein and tannin content. Kimia were also observed to produce better seeds compared with the other studied local cultivars. Results of this experiment can be useful to the local farmers as copper is proper for the development of sorghum cultivars under salt stress.

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