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Copper Strategy in Response to Saline Water in Sweet Sorghum (*Sorghum bicolor L.*)

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RESEARCH ARTICLE

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ABSTRACT

BACKGROUND: Balanced supply of essential nutrients is one of the most important factors in increasing crop yields. Copper (Cu) is one of eight essential plant micronutrients and is required for many enzymatic activities in plants and for chlorophyll and seed production.

OBJECTIVES: These field results were used to estimate salt stress direct effects and improved methods for high production in saline soils with affected by micro nutrients.

METHODS: Current study was done according split plot design during 2020 and 2021 in sweet sorghum. Plants were Irrigated by saline water with different Electrical Conductivity |EC = 2 (T_1), 3 (T_2), 4 (T_3) milimohs cm^{-1} and control (T_4)| in main plots and copper foliar spray |0 (C_0), 0.20 (C_1) and 0.40 (C_2) kg net Cu ha^{-1}| belonged to sub plots at stem elongation stage.

RESULT: The response of sweet sorghum to salt stress was statistically evaluated with affected by Cu and showed a significant difference in all traits but no differ in either year. Results from this analysis indicated that adding 0.40 kg net Cu ha^{-1} did significantly increase forage yield, sugar yield and ethanol yield; however, the increase in these traits did impact the overall silage quality expressed in estimated ethanol per litter per hectare. Compared forage and sugar yield exhibited significantly that C_2 could enhance crop ability to Na in which decrease of sugar yield and plant weight was only 19.2% and 24.29% in 2013 and 17.7% and 25.52% in 2014 respectively, in T_3 compared to T_4.

CONCLUSION: The key findings of this study can support the hypothesis that Cu can stimulate acclimation process in crops in saline lands which cause high ethanol fermentation and biomass production in sweet sorghum.

KEYWORDS: *Ethanol, Forage, Salinity, Stress, Sugar yield.*

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1. BACKGROUND

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 high-performing, well-adapted, premium quality cultivars. Reports to date have assessed sorghum yield and ethanol production across to saline lands need modern fermentation technologies (Sonoda *et al.*, 2019). 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. 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). Many studies have attributed the Cu benefit in crops to decrease salt levels in soil (Ballabio *et al.*, 2018). In total, the direct Cu contribution in sorghum to salt tolerance coupled with factors such as improved soil physical, chemical, and biological properties may synergistically high production (Pena *et al.*, 2018). Plant roots take up Cu from soil solution as water soluble Cu^{2+} in the soil or from fertilizer. Uptake of Cu from the soil and into the plant depends on: 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). Tziros *et al.* (2021) reported that total sugar concentration was related to environmental stresses such as salt stress. The sucrose concentration in juice was 9% less but sugar yield was 15% when EC of irrigated water was 3 to 3.5 milimohs cm^{-1} (Zambito *et al.*, 2020), and forage yield was 0.298 kg m^{-2} and 0.179 kg m^{-2}, due to Cu foliar spray at stem elongation stage (Wang *et al.*, 2017). Sugar yield dry matter (Sharma et al., 2018) and ethanol yield (Pensini *et al.*, 2021) can be affected by Cu at harvest time when EC of irrigated water was increased. In the same study, total sugar yield was decreased about 81.2% by saline water applying when EC was 4.8 milimohs cm^{-1} but applied of 0.40 kg net Cu ha^{-1} could enhance sugar yield about 31.4% in this condition .This was indicated that Cu rate in stalk juice was decreased with high EC rates in irrigation water (Ma *et al.*, 2016). The salt rates in irrigation water in crop production and converting it to sugar is an important factor to determine sweet sorghum life cycle (Liu *et al*., 2019). Sorghum can become relatively slow growth with affected by salt stress and then initiate rapid growth after use of sweet water (Copaja *et al*., 2021). Therefore, sweet sorghum may be adapted to saline water during the growing season. Also, Cu foliar application may be a method for induced sugar content in sweet sorghum production (Adrees *et al*., 2015).This is the main reason for ethanol production in forage crops in saline soils that may convert to solonetz soils in dry regions (Tarla *et al*., 2020). The objective of this study was to determine Cu foliar spray in Sweet sorghum where the EC of irrigated water was more than 3.2 milimohs cm^{-1} that had a 10-yr history of using forage crops.

2. OBJECTIVES

These field results were used to estimate salt stress direct effects and improved the methods for high production in saline soils with affected by micro nutrients.

3. MATERIALS AND METHODS

3.1. Field and Treatments Information

Research was conducted in Karaj Research Field during 2013 and 2014. The soil was deep silty clay loam. The previous crop was winter wheat. P and N fertilizer was broadcast applied without incorporation as triple superphosphate and urea. N and P were applied about 180 kg N ha^{-1} and 150 kg P_2O_5 ha^{-1}. The experiment consisted with split plot arrangement based on saline water Electrical Conductivity |EC = 2 milimohs cm^{-1} (T_1), EC = 3 milimohs cm^{-1} (T_2), EC = 4 milimohs cm^{-1} and (T_3) and control (T_4)| was in main plot and copper foliar spray |0 (C_0), 0.20 (C_1) and 0.40 (C_2) kg net Cu ha-^{1}| by CuSO4 source with 50% purity at stem elongation stage was in sub plot, sorghum cultivar was 6826 and plant population was 125,000 seed ha^{-1}. The row spacing and plot length for the five-row main plots was 80 cm and 15 m, respectively with four replications for each treatment. The salt concentration in irrigation water was 2500, 7500 and 12500 ppm (2.5, 7.5 and 12.5 gr lit^{-1}) in T1 to T_3, respectively. The irrigated water was averaged 23 mm (each time) for each plot.

3.2. Measured Traits

Fresh biomass weight was obtained at harvest time. Leaves and panicles were removed from four stalks per treatment in one block. These four stalks were weighed with and without leaves and panicles to determine the mean conversion factor for converting all fresh biomass weights to fresh stalk weight. Brix is a measure of dissolved sugar to water mass a liquid ratio. Brix was determined for four stalk segments per plot, with segments taken along the length of the stalks. Juice and sugar yield were calculated by 70% of Brix expressed in mg ha^{-1} sugar juice. Ethanol yield from sugar were based on industry survey data and theoretical yields. Sugar yield of sweet sorghum was estimated as 80% of Brix value, with 89% of the sugar extracted from stalks and 90% of sugar extracted that converted to ethanol.

3.3. Statistical Analysis

Combine variance analysis was performed with the SAS-STAT package (SAS Institute, 2018). Means were compared by Duncan's test where *P* = 0.05.

4. RESULT

The response of sweet sorghum to salt stress was statistically evaluated with affected by Cu and showed a significant difference in all traits but no differ in either year (Table 1).

Table 1. Combine variance analysis in mention traits.

S.O.V	df	Forage Yield	Sugar Yield	Ethanol Yield
Year (Y)	1	0.518^{ns}	0.545^{ns}	0.671^{ns}
Replication (R)	3	0.759^{ns}	0.837^{ns}	0.912^{ns}
R×Y	3	0.611^{ns}	0.802^{ns}	0.645^{ns}
Cu	2	823.54**	991.35**	909.46**
Cu × Y	2	0.745^{ns}	0.638^{ns}	0.901^{ns}
Cu × R	6	704.26**	645.37**	518.79**
Cu × R × Y	6	0.805^{ns}	0.749^{ns}	0.698^{ns}
Saline Water (S)	3	513.29**	641.18**	503.79**
S × Y	3	0.825^{ns}	0.710^{ns}	0.598^{ns}
S × R	9	456.32**	533.45**	418.92**
S × R × Y	9	0.947^{ns}	0.876^{ns}	0.809^{ns}
S × Cu	6	374.88**	336.71**	401.98**
S × R × Y × Cu	18	0.774^{ns}	0.611^{ns}	0.707^{ns}
CV (%)	-	5.26	5.71	6.49

** Significant differences at P=0.01 level. ^{ns}: Non-Significant.

Results from this analysis indicated that adding 0.40 kg net Cu ha^{-1} did significantly increase forage yield, sugar yield and ethanol yield (Table 2); however, the increase in these traits did impact the overall silage quality expressed in estimated ethanol per litter per hectare (Table 2). Forage yield was affected to salt stress and Cu foliar spray and this trait was decreased in T2 and T3 about 31.2% (0.781 to 0.538 kg m^{-2}) to 45.2% (0.781 to 0.428 kg m^{-2}) in 2013 and 29.7% (0.795 to 0.559 kg m^{-2}) to 45.8% (0.795 to 0.431 kg m^{-2}) in 2014 in C0 and 21.4% (0.897 to 0.706 kg m^{-2}) to 30.3% (0.897 to 0.626 kg m^{-2}) in 2013 and 21.7% (0.899 to 0.704 kg m^{-2}) to 30.4% (0.899 to 0.626 kg m^{-2}) in 2014 in C1 and 16.6% (0.984 to 0.821 kg m^{-2}) to 25.7% (0.984 to 0.745 kg m^{-2}) in 2013 and 16.8% (0.972 to 0.809 kg m^{-2}) to 25.6% (0.972 to 0.724 kg m^{-2}) in 2014 in C2 compared to T4 (Table 2). The data indicated that T_2 and T_3 could decrease the forage yield, through decrease in sugar and ethanol rates (Table 2). Under salt stress, plant growth requires a drainage system for high production but copper can eliminated sodium ions in soil horizons (Yost *et al.*, 2013). A difference in total sugar concentration was found at harvest time, indicated that saline water could decreased sugar yield in sweet sorghum, or salt stress had direct effect in our study to decrease sugar yield in T_2 and T_3 about 21.3% (2.57 to 2.02 mg ha^{-1}) to 34.9% (2.57 to 1.67 mg ha^{-1}) in 2013 and 21.6% (2.55 to 2.00 mg ha^{-1}) to 34.5% (2.55 to 1.67 mg ha^{-1}) in 2014 in C0 plants and 11.7% (3.91 to 3.45 mg ha^{-1}) to 22.6 (3.91 to 3.02 mg ha^{-1}) in

2013 and 11.4 (3.91 to 3.47 mg ha^{-1}) % to 22.8% (3.91 to 3.02 mg ha^{-1}) in C1 in 2014 and 8.6% (5.01 to 4.58 mg ha^{-1}) to 17.9% (5.01 to 4.05 mg ha^{-1}) in 2013 and 8.9% (5.15 to 4.70 mg ha^{-1}) to 17.8% (5.15 to 4.24 mg ha^{-1}) in C2 in 2014 compared to T4 (Table 2).

Table 2. Mean comparison in mention traits.

Year	**EC (milimohs cm^{-1})**	**Cu (kg.ha^{-1})**	**Forage yield (kg.m^{-2})**	**Sugar yield (mg.ha^{-1})**	**Ethanol yield (L.ha^{-1})**
2013	T1	(C0)	0.625^{*b}	2.18^{a}	1276.05^{b}
	T2		0.538^{c}	2.02^{ab}	1091.58^{c}
	T3		0.428^{d}	1.67^{b}	942.73^{d}
	T4		0.781^{a}	2.57^{a}	1341.12^{a}
	T1	(C1)	0.748^{b}	3.69^{a}	1412.3^{b}
	T2		0.706^{bc}	3.45^{ab}	1396.9^{bc}
	T3		0.626^{c}	3.02^{b}	1234.62^{cd}
	T4		0.897^{a}	3.91^{a}	1591.30^{a}
	T1	(C2)	0.855^{b}	4.76^{a}	1649.05^{b}
	T2		0.821^{bc}	4.58^{ab}	1598.59^{bc}
	T3		0.745^{cd}	4.05^{bc}	1464.94^{d}
	T4		0.984^{a}	5.01^{a}	1758.62^{a}
2014	T1	(C0)	0.627^{b}	2.12^{a}	1259.05^{b}
	T2		0.559^{c}	2.00^{ab}	1102.79^{c}
	T3		0.431^{d}	1.67^{b}	960.39^{d}
	T4		0.795^{a}	2.55^{a}	1356.44^{a}
	T1	(C1)	0.753^{b}	3.69^{a}	1403.6^{b}
	T2		0.704^{bc}	3.47^{ab}	1388.34^{bc}
	T3		0.626^{c}	3.02^{b}	1199.55^{cd}
	T4		0.899^{a}	3.91^{a}	1579.45^{a}
	T1	(C2)	0.847^{b}	4.85^{a}	1649.05^{b}
	T2		0.809^{bc}	4.70^{ab}	1593.13^{bc}
	T3		0.724^{cd}	4.24^{bc}	1466.11^{d}
	T4		0.972^{a}	5.15^{a}	1764.26^{a}

*Letters means significant differences in each column by Duncan's test at 5% level.

Ethanol yield in T1 to T4 which ranged from 1276 to 1758 L ha^{-1} in 2013 and 1259 to 1764 L ha^{-1} in 2014, showed positive correlation between EC and Cu foliar spray (Table 2). Ethanol yield was decreased in T2 and T3 about 18.6% (1341.12 to 1091.58 L ha^{-1}) to 29.7% (1341.12 to 942.73 L ha^{-1}) in 2013 and 18.7% (1356.44 to 1102.79 L ha^{-1}) to 29.2% (1356.44 to 960.39 L ha^{-1}) in 2014 in C0 plants and 12.2% (1591.30 to 1396.9 L ha^{-1}) to 23.4% (1591.30 to 1234.62 L ha^{-1}) in 2013 and 12.1% (1579.45 to 1388.34 L ha^{-1}) to 23.8% (1579.45 to 1199.55 L ha^{-1}) in 2014 in C1 and 9.1% (1758.62 to 1598.59 L ha^{-1}) to 16.7% (1758.62 to 1464.94 L ha^{-1}) in 2013 and 9.7% (1764.26 to 1593.13 L ha^{-1}) to 16.9% (1764.26 to 1466.11 L ha^{-1}) in 2014 in C2 compared to T$_4$ treatments (Table 2). We recommend that producers analyze second or higher year of fields for micro elements to evaluate sodium management and identify sites where a Cu foliar spray can be omitted the yield quality without drainage or leaching requirement.

5. DISCUSSION

Exceed of Cu levels and low decrease of forage yield in response to high EC, suggested a sufficient Cu supply and salt decomposition through the foliar application (Table 2). The levels of salt in soil were classified as optimal and marginal, suggesting that Cu can replace to sodium in soil depth (Delpuech *et al*., 2018) and perform stalk NO3 translocation in plant (Chapagain *et al*., 2020). These results suggested that Cu foliar application can be eliminated high sodium levels in fields. An explanation for more significant relation in sugar and ethanol yield is provided by Gil *et al*. (2021), who showed that sweet sorghum is sensitive to salt stress at the initiation of reproductive growth that could reduced the ethanol yield in cultivars. However, these findings can explain forage yield differences in response to copper. Our observation showed that non-Cu plants were sensitive to salt stress, while Cu-plants were only moderately sensitive. Cu-plants had high growth and reached to harvesting by early September, while non-Cu plants were in initiation of vegetative growth in October. The performance of sweet sorghum, was important across saline water for sugar content and forage yield (Table 2). Because of later maturity, Cu-plants were harvested after seasonal rainfall, and high sugar and ethanol concentration may be related to this physiological trait. This greater Cu-plants response to salt stress was attributed to more water and nutrient uptake in compared with non-Cu plants. This was showed that sweet sorghum was able to rapidly use of water after the temporary salt-stress may be occurred in soil (Islam *et al*., 2021). *Kraus et al*. (2021) reported that, ethanol yield in sweet sorghum, was related to amount of sugar in saline soils where the ranged of EC was 2 to 4 milimohs cm^{-1} (Table 2). Although this suggests that 20 to 25% of sodium could remain in soil system, this is the evidence in this study that long-term of Cu foliar application has increased plant acclimation to sodium stress. Although Cu can temporarily mobilize of N, the lack of this element in soil may suggest that N has been lost from the system via NO_3 leaching (Healy *et al*., 2016). These regulations considered the performance efficiency for forage yield based on saline soils systems (Ni *et al*., 2018).This suggests that these Cu traits have not only association with water and minerals uptake but also as a tool for accessing a high potential in forage yield production in sweet sorghum plants in high sodium lands (*Novara et al*., 2020). So, resistance to salt stress will be needed for ethanol production in cultivars.

6. CONCLUSION

Compared forage and sugar yield exhibited significantly that C_2 could enhance crop ability to Na in which decrease of sugar yield and plant weight was only 19.2% and 24.29% in 2013 and 17.7% and 25.52% in 2014 respectively, in T_3 compared to T_4. The key findings of this study can support the hypothesis that Cu can stimulate acclimation process in crops in saline lands which cause high ethanol fermentation and biomass production in sweet sorghum.

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FOOTNOTES

CONFLICT OF INTEREST: Author declared no conflict of interest.

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