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EFFECT OF EXCESSIVE SOIL MOISTURE STRESS ON SWEET SORGHUM: PHYSIOLOGICAL CHANGES AND PRODUCTIVITY

FEI ZHANG[Φ], YANQIU WANG[Φ], HUILIN YU, KAI ZHU[*], ZHIPENG ZHANG AND FENG LUAND JIANQIU ZOU[*]

Innovation Center, Liaoning Academy of Agricultural Sciences, Shenyang, Liaoning, China
**Corresponding author˙ e-mail: jianqiuzou@126.com, zhukai72@163.com*
[Φ]Authors contributed equally to this work

Abstract

Sweet sorghum [*Sorghum bicolor* (L.) Moench] is a potential bioenergy feedstock. Research explaining the response of sweet sorghum to excessive soil moisture (EM) stress at different growth stage is limited. To investigate the effect of EM stress on sweet sorghum antioxidant enzymes, osmotic regulation, biomass, quality, and ethanol production, an experiment was conducted in a glasshouse at the National Sorghum Improvement Center, Shenyang, China. Sweet sorghum (cv. LiaoTian1) was studied in four irrigation treatments with a randomized block design method. The results showed that the protective enzyme, particularly the SOD, CAT and APX in it, was significantly affected by EM stress. EM stress deleteriously affected sweet sorghum growth, resulting in a remarkable reduction of aboveground biomass, stalk juice quality, stalk juice yield, and thus, decreased ethanol yield. EM stress also caused significant reduction in plant relative water content, which further decreased stalk juice extraction rate. Sweet sorghum grown under light, medium, and heavy EM treatments displayed 5, 19, and 30% fresh stalk yield reduction, which showed a significant difference compared to control. The estimated juice ethanol yield significantly declined from 1407 ha^{-1} (under optimum soil moisture) to 1272, 970, and 734 L ha^{-1} respectively.

Key words: Excessive soil moisture, Sweet sorghum, Agronomic, Physiological, Productivity.

Abbreviations: EM: excessive soil moisture; FSY: fermentable sugar yield; RWC: relative water content; SJY: stem juice yield; SMC: soil moisture content; WAE: week after excessive moisture treatment.

Introduction

The increasingly demand for clean energy have led to considerable interests in developing eco-friendly renewable biofuels (Fischer & Schrattenholzer, 2001). Bioenergy crops are estimated to be cultivated on 1500 million hectares world wide by 2050 (Field *et al.,* 2008). This area will comprise a wide range of environments, demanding the bioenergy crops to be cultivated on marginal lands with recurrently stressful conditions (Chapotin & Wolt, 2007).

Sweet sorghum [*Sorghum bicolor* (L.) Moench], a C_4graminaceous crop, has potential to be used as a feedstock for biofuel production (Reddy *et al*., 2005). Sweet sorghum is capable of producing 10 to 20% fermentable sugars in stalks and is valuable for manufacturing ethanol (Reddy *et al*., 2005; Smith *et al*., 1987). Sweet sorghum growsrapidlyand thus, can be grown in a short season (Yu H.L *et al.,* 2015). Sweet sorghum adapts to low fertility soils (Yu *et al*., 2015), tolerates drought conditions (Mastrorilli *et al*., 1999; Song *et al*., 2015), and produces high biomass yields (Rao *et al*., 2013). Compared to other bioenergy crops of woody perennial species such as switch grass (*Panicum virgatum* L.) and miscanthus (*Miscanthus* × *giganteus*), sweet sorghum is easier and cheaper to establish and is more flexible to grow because it does not lock into the commitments of multiple years of production (Houx & Fritschi, 2013). For abiotic stresses, the effect of drought stress on sweet sorghum has been adequately studied (Massacci *et al*., 1996; Mastrorilli *et al*., 1999) but the information explaining the relationship between excessive soil moisture (EM) stress and sweet sorghum productivity is still limited.

In tropical and subtropical regions of the world, most of the dry crops grown during the summer-rainy season commonly encounter the sporadic or prolonged period of EM stress due to storm, poor soil drainage, river overflowing/flooding, or high water table (Promkhambut *et al*., 2010; Zaidi *et al*., 2003; Shamim *et al*., 2014)). It has been reported that proline, soluble sugars, soluble proteins and free amino acids were the keyosmotic regulation substances contributing towards osmotic adjustment (Kavikishore *et al*., 2005). It is also hypothesized that modulation of the activity of these antioxidant enzymes （SOD, POD, MDA, CAT, O^{2-}, APX） at different growth stages may be important in imparting resistance to a plant against EM stress (Ashraf, 2009). EM may induce a detrimental effect on sweet sorghum agronomic performance, reducing ethanol production, especially when planted in paddy fields, which are predominant in the tropical or subtropical regions (Promkhambut *et al*., 2010). Therefore, investigation is essential to clarify the responses of sweet sorghum to EM stress.

The diffusion rate of gases, particularly oxygen, was noted to be 10,000 times slower in water than in air. Thus, dryland crops grown in soils that are excessively saturated with water often display reduced gas exchange between the plant tissues and the atmosphere, exposing the plant to anaerobic conditions (Armstrong *et al*., 1994). Unlike rice (*Oryza sativa* L.), sorghum has no aerenchyma, which is the channel for transporting gases between inundated roots and aerial portions of the plant. Therefore, sorghum roots may suffer oxygen deficit when it is exposed to prolonged period ofEM stress, regarding the oxidative respiratory activities (Pardales *et al.*, 1991).

Long-term EM stress or temporarily waterlogging was documented to influence the anatomical and morphological features of plant roots and decrease leaf elongation rate, leaf area, and plant height (Henshaw *et al.,* 2007). Moreover, EM was noted to reduce stomatal conductance, transpiration and photosynthetic rate, among other physiological activities of dry land crops such as corn (*Zea mays* L.) (Zaidi *et al*., 2003) and winter wheat (*Triticum aestivum* L.) (Musgrave, 1994).

Sweet sorghum antioxidant enzyme activity modulation, osmotic adjustment and productivity at different growth stages under EM has not yet been well studied. Investigation of how sweet sorghum responds to EM is essential to evaluate the potential of its cultivation in lands subject to this type of environmental stress. The primary objectives of this research were (1) to investigate the morphological and photosynthetic responses of sweet sorghum under prolonged period of EM stress; and (2) to evaluate the effect of continuous EM stress on the antioxidant enzyme activity modulation, osmotic adjustment reaction and productivity including juice quality traits, stalk, biomass, and ethanol yields.

Materials and Methods

experiment description: This research was conducted in a glasshouse at Agricultural Research Center, Liaoning Academy of Agricultural Sciences in Shenyang, China (38.47°N, 120.28°W). The experiments were performed from May to October in 2011, 2012, and 2013. Sweet sorghum (cv. LiaoTian1) seeds were planted in pots (32 cm diameter and 28 cm deep) with drainage holes. The pots were placed 36 cm between plants (pot center to center) to attain a population density of 77000 plants ha^{-1}.Pots were filled with 7 kg sieved air-dried fine loam soil which was taken from the 0 to 20 cm depth of soil surface before planting. The soil contains 15% organic matter, 0.113% total N, 0.170% total P, 2.229% total K, 74 mg kg^{-1} available N, 16.0 mg kg^{-1} available P, 143 mg kg^{-1} available K, and a pHof6.7.

Irrigation was given to pots to obtain a field capacity of 25to 30% soil moisture content (SMC). This SMC was maintained by weighing the pots using an electronic platform balance (gravimetric method). Sweet sorghum seeds were planted on 6 May in 2011 and 2012 and 8 May in 2013. Five seeds were planted per pot. Seedlings were thinned to one per pot after emergence. Seedlings were then subjected to the EM treatments. Drainage holes were plugged and pots were watered to achieve SMC of 40, 60, and 80%, which regarded as light, medium, and heavy EM treatments, respectively. Control pots were well drained and regularly adjusted to the field capacity with approximately 25 to 30% SMC. In addition, approximately three pots with plugged drainage were randomly assigned in each replication and regularly adjusted to the field capacity with approximately 25 to 30% SMC, which used for comparing the differences of plugged or unplugged pots. The SMC in pots were monitored daily and maintained constantly throughout the experimentation.

Fertilizer of 50 kg $ha^{-1}(NH_4)_2HPO_4$ was applied when sweet sorghum was planted and50 kg ha^{-1} $CO(NH_2)_2$ was applied 8 weeks after emergence. Plants were grown in the glasshouse with natural lighting. The glasshouse was well ventilated throughout the experimentation by opening the side windows. Local weather data including averaged daily temperature and total monthly photoperiod were presented in Table 1. Insects and diseases is prevented and controlled by spraying pesticide periodically, and weeds were controlled by hand-weeding throughout the experimental period.

Table 1. Averaged daily temperature and monthly total photoperiod during the study period in 2011–2013 at Agricultural Research Center, Liaoning Academy of Agricultural Sciences, Shenyang, China (38.47°N, 120.28°W).

Year	Month	Temperature	Photoperiod
		---------°C---------	--------h--------
2011	May	19.0	266.8
	June	22.5	213.6
	July	25.1	231.1
	Aug.	24.4	168.2
	Sept.	17.6	207.7
2012			
	May	19.5	307.6
	June	22.7	139.8
	July	26.0	229.1
	Aug.	23.9	146.7
	Sept.	18.0	189.2
2013			
	May	20.0	318.4
	June	23.2	114.8
	July	26.1	253.4
	Aug.	24.6	126.0
	Sept.	18.8	138.6

Antioxidant system: Antioxidant system of leaves of sweet sorghum were determined at 3, 9 and 15 weeks (WAE) after the EM treatments when plants of unstressed control reached seeding, heading, or grain filling stage using the method described as follows. POD activity was determined by following the method of Upadhyaya *et al*. (1985). SOD activity, MDA content, CAT activity, O^{2-}, APX activity were measured using the method by Nakano (1980).

Osmotic regulation system: Osmotic regulation system of leaves of sweet sorghum were determined at 3, 9 and 15 weeks (WAE) after the EM treatment when plants of unstressed control reached seeding, heading ,or grain filling stage using the method described as follows. Proline content was measured as described by Bates (Bates *et al.,* 1973). Protein, soluble sugars, reducing sugars and free amino acids were measured using the method of Zhang *et al*. (2015).

Relative water content and root volume: Relative water content (RWC) of leaves and roots of sweet sorghum were determined at 3, 9 and 15 weeks (WAE) after the EM treatment when plants of unstressed control reached

seeding, heading, or grain filling stage using the method described by Zhang *et al*. (2015). Roots were removed from soils by washing away soils using a gentle stream of water. During the treatments, three replicates uppermost, fully expanded leaves and roots were sampled from each treatment and their fresh weight (FW) was measured. Samples were placed in deionized water for 7 h to determine turgid mass (TM). Total twelve samples were taken from the three replicates for four EM treatments dried in an oven at 80°C for 48 h and weighed (DW). RWC was calculated using a formula of RWC (%) = [(FW – DW)/(TM – DW)] × 100.

Photosynthetic performance: Net photosynthesis (Pn), Stomatal conductance (Gs) and transpiration rate (Tr) were determined at 3, 9 and 15 WAE during seeding, heading, or grain filling stage of control plants using a LI-6400 photosynthesis system (Li-Cor, Inc., Superior St., Lincoln, NE 68504). These measurements were carried out at local time from 9:30 AM to 11:00 AM. The measurements were repeated three times on flag leaves. Means of three readings were used for statistical analysis.

Plant height, total biomass, stalk and grain yield: Plants were harvested at 18 WAE at 26 September in 2011 and 2012 and 29 September in 2013 when control plants reached physiological maturity which typically featured the emergence of maximum dry seed weight. Each plant (A pot) represented an experimental unit. Total 12 samples taken from the three replicates for four EM treatments to measure plant weight, total biomass, stalk and grain yield. Plant height was determined prior to harvesting by measuring the distance from base level to the tallest point of the plants by using a ruler. Plants were cut at ground level by using a knife. Total fresh aboveground biomass was determined. Plants were subsequently sectioned to three segments including stalks (panicles and stalks were separated at peduncle), grain, and leaves along with sheathes. Fresh aboveground biomass, stalk and grain yields, as well as leaves and sheath yield were estimated by multiplying weight per plant and by planting density.

Stalk juice trait and calculated ethanol yields: Stalk juice was extracted by pressing the fresh stalks in a sugarcane crusher for three times (ET-ZZJ83, No. 42 Dabu Rd, Xinhua St., Guangzhou, China 510280). The juice was weighed immediately after extraction. Extracted stalk juice was filtered with filter papers to remove large solids. 100 mL of the fresh filtered juice was transferred to test tubes and measured for Brix values with a PAL-1 digital refractometer (Atago USA Inc., 11811 NE, 1st Street, Bellevue, WA 98005). Each plant (A pot) represented an experimental unit. Total twelve samples were taken from the three replications for four EM treatments. The fresh weight of the bagasse was measured and then the bagasse was oven dried at 105°C for 30 min followed by 80°C until weights stabilized to determine bagasse dry matter yield. Juice extraction rate was calculated using a formula of juice extraction rate (%) = (fresh juice weight/fresh stalk weight) × 100. Stalk juice yield (SJY) (Mg ha^{-1}) was calculated by multiplying juice weight per plant by planting density.

Fermentable sugar yield (Mg ha^{-1})(FSY), and juice ethanol yield (L ethanol ha^{-1}) (JEY) were estimated based on the reports of Wortmann *et al*. (2010) using the equations of FSY (Mg ha^{-1}) = SJY× [Brix (kg Mg^{-1}) × 0.75] and JEY (L ethanol ha^{-1}) = FSY × 665.Further, the bagasse ethanol yield was estimated using a conversion factor of 202 L ethanol Mg^{-1} dry bagasse (Li *et al*., 2010). Houx & Fritschi (2013) noted that the conversion factors for ethanol production from bagasse varied widely. Nevertheless, the use of conversion factor in this research could provide helpful comparisons for the treatments of EM on sweet sorghum bagasse ethanol production.

Experimental design and statistical analyses: Experiments were conducted in randomized complete block design with 3 replications. Total 12 samples from 4 EM treatments were studied.

Data was subjected to analysis of variance (ANOVA) using PROC MIXED model in SAS (version 9.2, SAS Institute Inc., 101 SAS Campus Dr., Cary, NC 27513), specifying years, replications, and year by replication interactions as random and EM treatments and WAE as fixed effects. Statistical procedure of orthogonal contrasts was used to compare treatment effect at $p<0.05$.

Results and Discussion

Antioxidant system: By 3,9and15 WAE, significant variation in antioxidant enzyme such as SOD, POD, MDA, CAT, O^{2-}, APX were found for plants treated with EM treatments (Fig. 1). With the increased of SMC, SOD activity increased under control, light EM stress and medium EM stress, but dropped at heavy EM stress. There reduction in POD, MDA, and O^{2-} of leaf was consistent with the increase in SMC, when plants received light, medium, and heavy EM treatments. Among these, POD activity is higher at 3 WAE compared with 9 WAE and 15 WAE, while MDA and O^{2-} performed an opposite trend in different period compared with POD. CAT was significantly reduced by heavy EM treatment at 15 WAE but was not greatly decreased at 3 WAE and 9 WAE with all EM treatments. The change trend of APX and CAT along with SMC were basically identical, except that CAT declined at 15 WAE, while APX declined at 9 WAE.

These results showed that MDA content of sorghum was considerably reduced under EM stress which corroborated with The increased SOD activity induced the higher tolerance to oxidative stress which was similar with EM conditions, may be because sorghum was able to maintain POD activity in response to EM stress. This result is also consistent with the findings of other experiments, which have shown that SOD and CAT responded to EM stress by increasing their activity levels and led to the removal of the stress-generated (Diego *et al*., 2003).

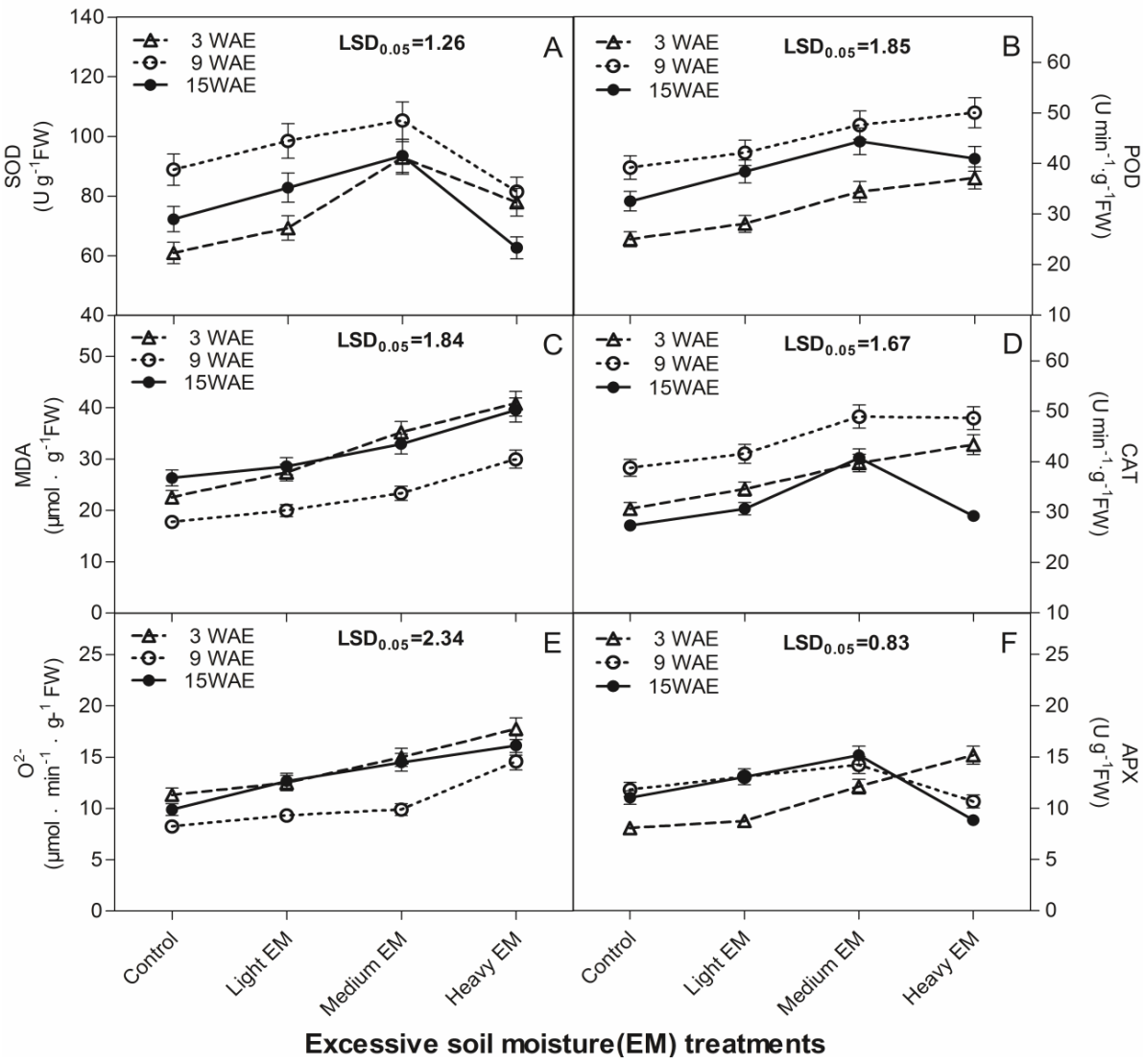

Fig. 1. Effect of excessive soil moisture (EM) stress on antioxidant enzyme of sweet sorghum during seeding, heading and grain filling stage in three combined experiments, 2011-2013, in Shenyang, China.†

Osmotic regulation system: By 3, 9 and 15 WAE, sweet sorghum soluble protein and free amino acid were linearly increased significantly by EM treatments as compared to stress-free control, while proline and soluble sugar performed a parabolic variation along with EMC (Fig. 2). Plants treated with heavy EM showed a decline in proline and soluble sugar, especially at 15 WAE, the average of which was significantly decreased by 21%, respectively, compared to control. In addition, the maximum value of proline, soluble sugar and soluble protein appeared in 9 WAE which was significantly increased as compared to control, while the maximum value of free amino acid appeared in 3 WAE and it is also significantly increased as compared to control.

In this study, osmotic regulation substances such as proline and free amine acid, descended along with EM stress. This may be because EM stress increased the leaf proline content, which might have contributed to osmotic adjustment and allowed the plant to maintain turgor pressure and adapt to EM availability (Anjum *et al*., 2011). This is conducive with previous reports on drought-induced proline accumulation (Fakhra *et al*., 2015). The decline of soluble sugar and soluble protein may be associated with plant metabolic disorders. These results are identical with Zaidi *et al*. (2003).

Relative water content: The interaction of EM treatment by WAE had significant reduction on leaf and root RWC, and thus, results are presented across evaluating dates to illustrate the differences in RWC. Sweet sorghum treated with medium or heavy EM treatments constantly displayed a low leaf and root RWC as compared to control (Table 2). Leaf RWC under light EM treatment was similar to that of the control by 3 WAE but was significantly less than that of control by 9 and 15 WAE. Root RWC at light EM treatment was similar to control by 3 WAE but was significantly declined by 9 and 15 WAE.

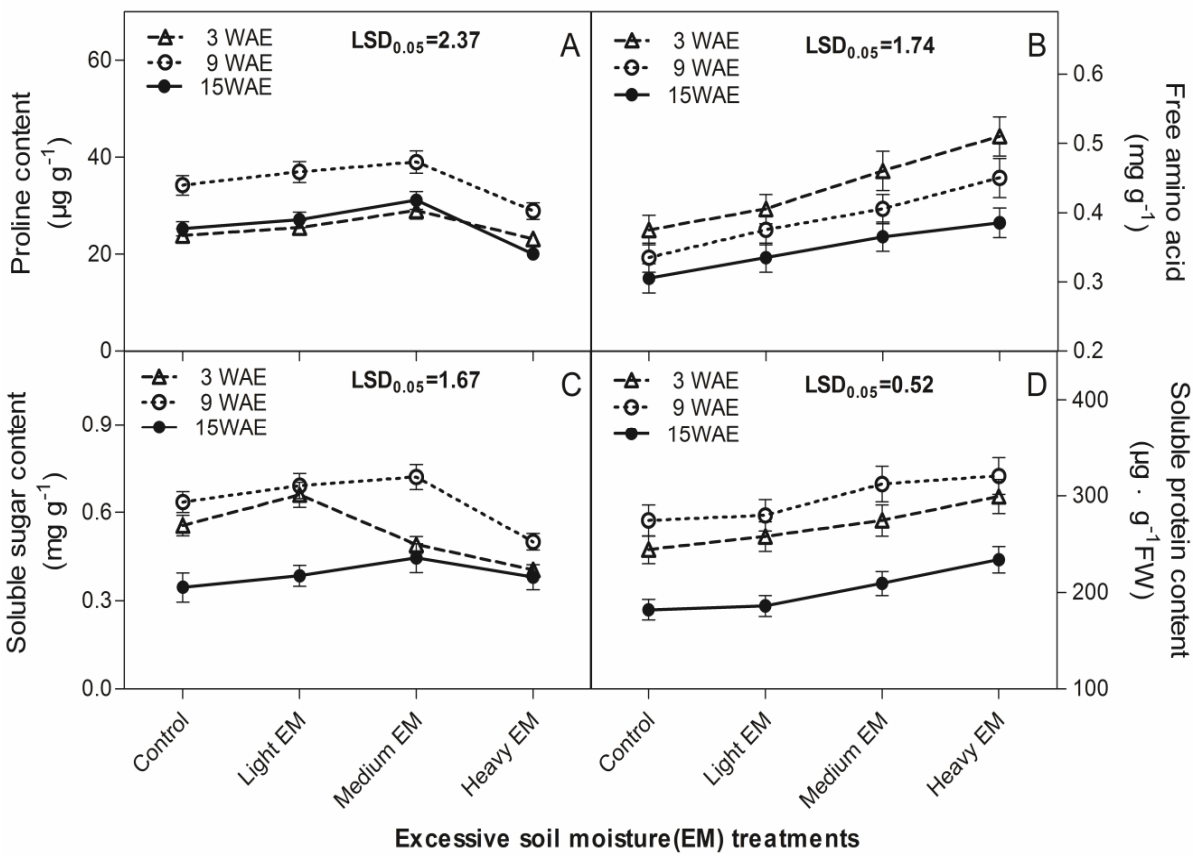

Fig. 2. Effect of excessive soil moisture (EM) stress on osmotic regulation substances of sweet sorghum during seeding, heading and grain filling stage in three combined experiments, 2011-2013, in Shenyang, China.†

Table 2. Effect of excessive soil moisture (EM)stress on relative water content (RWC) and root volume of sweet sorghum during seeding, heading and grain filling stage in three combined experiments, 2011-2013, in Shenyang, China.†

EM treatment‡	Leaf RWC			Root RWC		
	3 WAE	9 WAE	15 WAE	3 WAE	9 WAE	15 WAE
	%					
Control	89.0 ± 0.55a	78.3 ± 0.86a	76.4 ± 0.73a	82.3 ± 0.54a	75.8 ± 0.48a	72.4 ± 0.36a
Light	87.3 ± 0.47b	76.5 ± 0.63a	74.6 ± 0.82a	81.9 ± 0.98a	73.9 ± 0.53b	71.2 ± 0.86b
Medium	86.2 ± 0.76b	71.0 ± 0.79b	68.0 ± 0.45b	78.2 ± 0.69b	69.5 ± 0.44c	68.5 ± 0.82c
Heavy	79.8 ± 1.06c	66.9 ± 0.91c	62.8 ± 1.08c	73.7 ± 0.55c	65.1 ± 0.62d	64.7 ± 0.57d
ANOVA						
EM treatment	**	***	***	**	***	***

†Data are means across three years
‡Within a column, means followed by the same letters indicate that no significant difference at 0.05 probability level. Values in the table are mean ± SE (n = 9)
**Statistically significant at 0.01 probability level
***Statistically significant at 0.001 probability level

Table 3. Effect of excessive soil moisture (EM)stress on net photosynthesis (Pn), stomatal conductance (Gs), transpiration rate (Tr) of sweet sorghum during seeding, heading and grain filling stage in three combined experiments, 2011-2013, in Shenyang, China.†

EM treatment‡	Net photosynthetic (Pn)			Stomatal conductance (Gs)			Transpiration rate (Tr)		
	3 WAE	9 WAE	15 WAE	3 WAE	9 WAE	15 WAE	3 WAE	9 WAE	15 WAE
	ìmol CO_2 m^{-2} s^{-1}			mol H_2O m^{-2} s^{-1}			mmol m^{-2} s^{-1}		
Control	25.0±0.8a	28.5±0.7a	27.3±0.5a	0.35±0.04a	0.45±0.05a	0.43±0.03a	4.2±0.5a	6.2±0.3a	5.2±0. 2a
Light	24.3±1.1b	27.6±0.9a	26.4±0.8a	0.32±0.02b	0.42±0.04a	0.40±0.06a	3.9±0.3a	6.1±0.4a	5.1±0.4a
Medium	21.9±0.7c	23.1±1.2b	23.0±0.7b	0.29±0.03c	0.37±0.02b	0.35±0.02b	3.3±0.3b	5.7±0.5b	4.7±0.5b
Heavy	19.1±0.5d	20.3±0.6c	19.5±0.9c	0.25±0.03d	0.32±0.04c	0.31±0.01c	2.5±0.4c	5.2±0.2c	3.3±0.5c
ANOVA									
EM treatment	**	***	***	**	***	***	**	***	***

†Data are means across three years
‡Within a column, means followed by the same letters indicate that no significant difference at 0.05 probability level. Values in the table are mean ± SE (n = 9)
**Statistically significant at 0.01 probability level
***Statistically significant at 0.001 probability level

Results suggested that, when sweet sorghum was grown under EM stress, its leaf and root RWC were severely reduced as compared to control. Previous reports have noted that the decrease of the permeability of roots to water and leaf water potential under EM stress is responsible for plants wilting (Drew 1983; Hagan 1950; Jackson & Drew, 1984). The reduction of sweet sorghum RWC in our experiments is consistent with the findingsofother plant species response to EM stress (Davison &Tay, 1985; Kumutha *et al*., 2009). For example, it was reported that RWC of pigeon pea (*Cajanus cajan* L.) (Kumutha *et al*., 2009), Jarrah (*Eucalyptus marginata* Donn ex Sm.) (Davison &Tay, 1985), and welsh onion (*Allium fistulosum* L.) (Yiu *et al*., 2009) was reduced under EM stress.

Photosynthetic performance: By 3, 9 and 15 WAE, sweet sorghum net photosynthetic (Pn) and stomatal conductance (Gs) were significantly reduced by EM treatments as compared to stress-free control (Table 3). Plants treated with heavy EM showed the lowest net photosynthetic (Pn) and stomatal conductance (Gs), which was significantly decreased by 27% and 28% for the average of 3,9 and 15 WAE, respectively, over the control, and the maximum value of net photosynthetic (Pn), stomatal conductance (Gs), and transpiration rate (Tr) were all appeared in 9 WAE . Transpiration rate (Tr) was not significantly reduced by light EM treatment but was severely decreased by medium and heavy EM treatments, showing 12 and 29% lower compared to control for the average of 3, 9 and 15 WAE, respectively.

Stomatal closure is one of the leading responses to EM stress (Jackson & Drew, 1984). Reduced Gs was regarded as a defense strategy to avoid EM stress (Zainul *et al.,* 2015). In the present experiments, the significant reduction in Pn, Tr, and Ci under EM stress may be partially regulated by reduced Gs. Other reports (Ashraf, 2003; Huang *et al*., 1994) have also shown that stomatal closure is the principal factor affecting plant photosynthesis under EM conditions. In addition to stomatal closure, some reports (Zheng *et al.,* 2009) noted that non-stomatal limitations including the decreased photosynthesis II quantum yield and leaf chlorophyll content and the increased non photochemical quenching play a role to reduce plant Pn. In other plant species, EM stress resulted in reduced Gs Ci, Tr, and Pn in corn (Zaidi *et al*., 2007), lucerne (*Medicago sativa* L.) (Smethurst & Shabala, 2003), and mungbean (*Vigna radata* L. Wilczek) (Ahmed *et al*., 2002).

In previous reports, the reduced Trduring stress condition was considered as a defense mechanism to avoid the EM stress (Zaidi *et al*., 2003; Zaidi & Singh, 2001). It was documented that reduced Tr might have helped plants to reduce the translocation of toxic compounds synthesized in roots during EM stress to aboveground plant tissues (Zaidi & Singh, 2001).

Plant height, total biomass, stalk and grain yield: By 18 WAE, significant suppression in plant height was found for plants treated with EM (Table 4). The reduction in plant height was consistent with the increase in SMC, resulting in 21, 25, and 29% lower than that of control, when plants received light, medium, and heavy EM treatments, respectively. In addition, statistical analysis results showed that the three levels of EM treatments all presented a significant difference as compared to control. Fresh grain yield harvested in light EM treatment was found similar to control, but was significantly reduced in medium and heavy EM treatments, with 13 and 20% lower compared to control, respectively. Similarly, light EM treatment did not significantly reduce leaves and sheath yield compared to control but medium and heavy EM treatments significantly reduced leaves and sheath yield, showing 13 and 19% lower compared to control, respectively. Fresh stalk yield and fresh aboveground biomass were progressively decreased as SMC increased. Plants treated with light, medium and heavy EM treatments exhibited 5, 19, and 30% fresh stalk yield reduction and 5, 16, and 26% fresh aboveground biomass reduction from their respective controls.

Results of this research indicate that prolonged period of EM stress deleteriously affects sweet sorghum growth, resulting in the remarkable reduction of plant height, stalk yield, leaves and sheath yield. This finding is in agreement with the findings in maize plants: EM stress not only stunted growth but also delayed silking in maize plants, resulting in reduced grain yield (Zaidi & Singh, 2001; Zaidi *et al*., 2003, 2004, 2007).

Under EM stress, plants have suffer nutritional deficiencies due to poor nutrient uptake, which was caused by energy starvation under anaerobic conditions (Vartapetian, 1993). A waterlogging study conducted by Zaidi *et al*. (2007) showed that maize plants had comparatively less nitrogen uptake under EM stress compared to plants grown under optimum soil moisture conditions, even though the soils were well fertilized. Therefore, differences in sweet sorghum aboveground biomass accumulation between treatments in our experiments may also be partially attributed to the differential nutrient uptake.

Stalk juice trait and calculated ethanol yields: Light EM treatment did not significantly reduce stalk juice extraction rate but plants received medium and heavy EM treatments exhibited considerably lower stalk juice extraction rate as compared to control (Table 5). Stalk juice extraction rate was 45.0% for control plants, but was decreased to 43.0, 42.0, and 39.9% for those of plants received light, medium, and heavy EM treatments, respectively. The decrease in stalk juice extraction rate under EM stress might be attributed to reduced RWC in plant tissues.

EM stress had significant effect on stalk juice quality (Table 5). Significant decreases in stalk juice concentration were observed in plants grown under medium and heavy EM treatments. Stalk juice concentration of control plants was 17.8 °brix, while the value decreased to 17.1, 15.9, and 14.6 °brix under light, medium, and heavy EM treatments, respectively. The statistical analysis results showed that difference existed in medium and heavy EM treatments, while no difference existed at light EM treatments as compared to control.

Bagasse yields differed among EM treatments and ranged from 8.9 to 10.5 Mg ha^{-1}. In comparison, control plants yielded 10.8 Mg ha^{-1} bagasse (Table 5). Significant difference existed in medium and heavy EM treatments, while no difference existed at light EM treatments compared to control. SJY (stem juice yield) was 9.9, 12.2, and 14.9 Mg $ha^{-1}$for plants received light, medium, and heavy EM treatments, respectively. In comparison, control plants yielded 16.0 Mg ha^{-1} SJY. FSY (fermentable sugar yield) is calculated from both brix value and SJY and differed among EM treatments, ranging from 1.10 to 1.91 Mg ha^{-1}, while control plants yielded 2.11Mg ha^{-1}FSY.

Table 4. Effect of excessive soil moisture (EM) stress on various agronomic traits of sweet sorghum measured at physiological maturity in three combined experiments, 2011-2013, in Shenyang, China.†

EM treatment‡	Plant height cm	Bagasse yield	Fresh grain yield	Fresh stalk yield	Fresh aboveground biomass	Leaves and sheath yield
		----------Mg ha^{-1}----------				
Control	323.2 ± 0.72a	10.8 ± 0.26a	9.9 ± 0.16a	35.6 ± 0.62a	56.5 ± 1.18a	11.0 ± 0.17a
Light	256.1 ± 0.49b	10.5 ± 0.27a	9.5 ± 0.15a	33.9 ± 0.81b	54.5 ± 1.75b	10.9 ± 0.14a
Medium	242.0 ± 0.39c	9.4 ± 0.26b	8.7 ± 0.26b	29.0 ± 0.99c	47.4 ± 1.89c	9.6 ± 0.25b
Heavy	230.5 ± 0.52d	8.9 ± 0.31c	8.0 ± 0.28c	24.9 ± 1.05d	41.9 ± 2.10d	8.9 ± 0.52c
ANOVA						
EM treatment	***	***	**	***	**	***

†Data are means across the three years
‡Within a column, means followed by the same letters indicate that no significant difference at 0.05 probability level. Values in the table are mean ± SE (n = 9).
**Statistically significant at 0.01 probability level
***Statistically significant at 0.001 probability level

Table 5. Effect of excessive soil moisture (EM) stress on sweet sorghum stalk juice traits in three combined experiments, 2011-2013, in Shenyang, China.†

EM Treatment‡	Juice extraction rate	Stalk juice concentration	Stalk Juice yield	Fermentable sugar yield
	%	°Brix§	----------Mg ha^{-1}----------	
Control	45.0±0.64a	17.8±0.08a	16.0±0.82a	2.1±0.04a
Light	43.0±0.39ab	17.1±0.10a	14.9±0.91b	1.9±0.06b
Medium	42.0±0.53b	15.9±0.13b	12.2±0.56c	1.4±0.05c
Heavy	39.9±0.37c	14.6±0.15c	9.9±0.47d	1.1±0.04d
ANOVA				
EM Treatment	***	***	***	***

†Data are means across the three years
‡Within a column, means followed by the different letters indicate that significant difference at 0.05 probability level. Values in the table are mean ± SE (n = 9)
§1 degree of °brix is 1 g sucrose in 100 g solution
***Statistically significant at 0.001 probability level

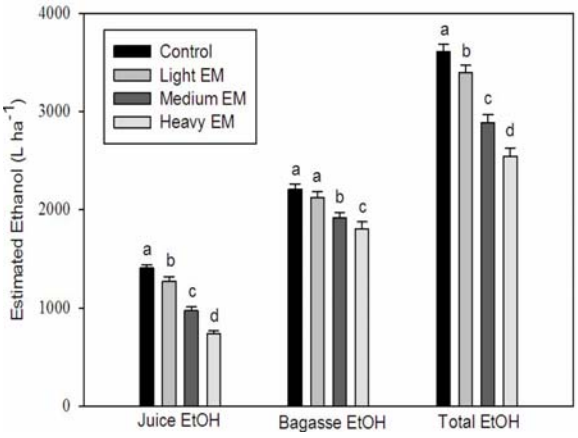

Fig. 3. Annual estimated ethanol (EtOH) yield of sweet sorghum received different levels of excessive soil moisture (EM) stress in Shenyang, China. Data are means across the three years. Bars with the same letters, within the same source of EtOH production, are not statistically different from one another at the 0.05 probability level. Vertical bars represent the mean of 9 measurements ± SE of the mean.

Estimated juice ethanol yield declined sharply as SMC increased (Fig. 3). Juice ethanol yield was proportional to FSY, and thus control plants yielded the highest FSY also produced the highest juice ethanol yield. Juice ethanol yield was 1407 L ha^{-1} for control plants but was decreased to 1272, 970, and 734 L ha^{-1}for plants received light, medium, and heavy EM treatments, respectively. Estimated bagasse ethanol yield was 2201L ha^{-1}for control plants, but was declined to 2121, 1911, and 1807 L ha^{-1} for plants received light, medium, and heavy EM treatments, respectively. As expected from juice and bagasse ethanol yields, differences in total ethanol yield were observed among EM treatments and control plants. Control plants were estimated to produce 3609 L ha^{-1} total ethanol yield, while plants received light, medium, and heavy EM treatments were estimated to produce 3394, 2822, and 2541 L ha^{-1} total ethanol yield, respectively.

Our findings showed that bagasse ethanol yield exceeded juice ethanol yield regardless SMC, indicating that cellulosic conversion to ethanol maight substantially increase total ethanol yield. The higher ethanol yield estimated from sweet sorghum bagasse than juice ethanol yield is consistent with the finding of Houx & Fritschi (2013). Nevertheless, it should be noted that, ethanol production from cellulosic sources has been generally lagged behind to its projections due to the conversion technologies have not been proven to be cost effective (Burks *et al*., 2012). In contrast, bioethanol production from sugar-based crops has been well established (Burks *et al*., 2012; Rao *et al*., 2013; Houx & Fritschi, 2013).

The results of this research suggest that prolonged period of EM stress is a significant problem for cultivating sweet sorghum in the lands where regularly subjected to this type of environmental stress at different growth stage. Prolonged EM stress, especially heavy EM, could severely reduce sweet sorghum agronomic productivity. SOD, CAT and APX was the key protective

enzyme of sorghum for with stand adversity, but unfortunately, they were declined at heavy EM stress. Sweet sorghum grown under prolonged EM stress has shown osmotic regulation substances increased (expected proline, soluble sugar at 15 WAE), arrested photosynthetic activity, reduced dry matter accumulation, SJY, stalk juice quality, and therefore, decreased bioethanol yield. Additionally, sweet sorghum planted in prolonged period of EM stress is likely to decrease plant RWC, which may further reduce stalk juice extraction rate. However, significant genetic variability may exist in sweet sorghum genotypes with regards to EM tolerance. Therefore, large-scale screening of sweet sorghum germplasm against the EM stress and generating the tolerant genotypes for adapting EM stress are needed.

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