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Heat and water stress tolerance of three Soybean (*Glycine Max* L.) varieties under two growing conditions

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ABSTRACT

In this study, the growth response of three soybean varieties to heat and water stress was assessed. The three soybean varieties were subjected to water stress under two growing temperature conditions (open field condition: 25-27°C and glass house: 30-35°C). The experiment was 2 x 2 x 3 factorial arranged in a complete randomized design with three replicates. Thus two (2) growing conditions (open field and glass house), two (2) watering regimes (50% and 100%) and three (3) soybean varieties (Afakyak', 'Songda' and 'Jenguma'). Data on plant height, stem diameter, number of leaves, leaf area, chlorophyll content (SPAD) value and fresh biomass of the plants were measured at 14, 28 and 42 days after sowing (DAS). The relative injury of the three soybean varieties were also determined using a cell membrane thermostability test. The results showed that the varieties exhibited similar growth rates under each growing condition for fresh biomass, leaf area, stem diameter, plant height, number of leaves and SPAD value. There was, however, no significant difference among the tested varieties in their response to heat stress, thus implying that the three soybean varieties assessed could adapt well in the savanna agroecology of Ghana.

Keywords: *Soybean varieties, cell thermostability test, relative injury, drought, heat tolerance*

INTRODUCTION

Crop plants cultivated in open fields are commonly exposed to multiple abiotic stresses including drought, high temperatures and nutrient deficiency (Niinemets, 2010; Khalid *et al.,* 2019). During the production of field crops, extreme temperatures and drought are usually encountered that can decrease yield by up to 70 % in field crops (Hossain *et al.*, 2016). These abiotic stresses, affects several plant species at critical growth and development stages and may cause complete crop failure (Guilioni *et al*., 2003; Fahad *et al*., 2017). The degree of crop tolerance to temperature extremes and drought is still the subject of ongoing studies (Basu *et al*., 2016).

The plant cell membrane is the initial site of stress injury, and its functions and structure can be severely damaged by abiotic stresses (McKersie and Lesheim, 2013). As a result, the evaluation of cellular membrane integrity in crop plants is important. It can be used as an index of their environmental stress tolerance (Bala and Sikder, 2017). So, measurement of cell membrane stress can be done using the cell membrane thermostability test (CMT) - a technique that employs the conductivity of electrolytes leaking from leaf tissues (Bala and Sikder, 2017). The technique has been successfully used as a method of screening for heat stress tolerance in some crop varieties in areas with drought (Tang *et al.*, 2007; Dias *et al.,* 2010). Thus, CMT can be a useful scientific tool for selecting the best crop varieties for cultivation in different agro ecologies. According to Bajji *et al.* (2002), factors such as the plant's age, organ sampled, degree of stress, growing time and the plant types are important consideration in assessing plant leaf response to stress using cell membrane thermostability test.

The potential risks of drought associated with global climate change and its variability are becoming more important in African agriculture (Li *et al.,* 2014; Muscolo *et al.,* 2015; Ning and Bradley, 2016). The importance of plant varieties that can tolerate drought and high temperatures at the same time cannot be overemphasized especially with the increasing impact of climate variability on crop development and overall agricultural productivity in sub-Saharan Africa (Ikeme, 2003). To achieve optimum crop yields, farmers must plant varieties that are adapted to growth in a given agro ecological zone. But the adaptation of crop plants to an agroecology is determined by complex functions of the crop species, genotype, and environmental factors (Evenson and Gollin, 2002).

Soybean (*Glycine max*) is one of the useful legume food crops of the world, and seems to be growing in importance (Anderson *et al.* 2019). It is a legume cultivated throughout sub-Saharan Africa, the crop yields more protein per unit of land (Loko *et al*. 2021), and is a versatile food crop that has the potential to supply most food nutrients (Alamu *et al.* 2017). Soybean products are widely used in animal nutrition, as a source of energy and protein in poultry feed formulations (Dourado *et al.* 2011). Throughout its growth cycle, soybean requires 450-700 mm of water per growing period and mean temperatures of 20-25 ^{O}C. Thus, soybean requires moderate soil moisture and can withstand some considerable amount of drought (FAO, 2002; Martins *et al*. 2013). These variables of soybean are an index of the crop's suitability to specific agroecology. The ability of soybean to withstand abiotic stress (water stress) and produce at least some yield when compared to other legumes is known (Talebi *et al*., 2013; Jumrani and Bhatia *et al*., 2018). Despite the recent advances in the release of crop varieties that are high yielding, their adaptation and yield production under climate change scenarios remain elusive. More research efforts are still required to understand the impact of heat and water stress tolerance in soybean varieties.

Although, a variety of conventional breeding approaches exists and have been used to develop drought-tolerant crop varieties for improved productivity (Datta, 2013; Hu and Xiong, 2014). Also, there is limited success in improving drought tolerance in crops due to its control by multiple genes. Therefore, the screening of existing soybean varieties for enhanced drought and heat tolerance remains a viable approach to increase crop yields under a changing climate. The aim of the present investigation was to evaluate the tolerance of three soybean varieties to heat (injury) and water stress using the Cell Membrane Thermo-stability (CMT) test method.

MATERIALS AND METHODS

Experimental site

Experiment one: The main objective of this experiment was to determine the effect of water stress on growth and development of soybean varieties under different growing temperature conditions. The experiment was conducted at the experimental field of the Department of Horticulture, University for Development Studies, Nyankpala Campus. Nyankpala is located in the northern Guinea savanna ecological zone on longitude 0º98W and latitude 9º41N and at an altitude of 183 m above sea level. The area experiences a mono modal annual rainfall of 1000 mm to 1200 mm from May to November (Savanna Agriculture Research Institute, 2004). The soil of the area is generally sandy loam with a medium to coarse texture.

Experimental design for experiment 1

The study was a 2 x 2 x 3 factorial involving two levels of water treatments (Water 1: Supplying 100 % water requirement of soybean once a day till the 42 days after planting. Water 2: Supplying 50 % water requirement of soybean once a day till the 42 days after plant planting); two levels of temperature conditions [open field at 25-27˚C and in glass house at 30-35˚C]; and three soybean varieties ("*Jenguma*" "*Afayak*" and "*Songda*."), making a total of 12 treatment combinations (Table 1). The treatments were arranged in a Complete Randomized Design and replicated three times.

Table: Treatment descriptions

Watering regime	Growing environment	Soybean variety	Treatment
100 %	Glass house	Afayak	**T1**
50 %	Glass house	Afayak	**T2**
100 %	Open field	Afayak	**T3**
50 %	Open field	Afayak	**T4**
100 %	Glass house	Jenguma	**T5**
50 %	Glass house	Jenguma	**T6**
100 %	Open field	Jenguma	**T7**
50 %	Open field	Jenguma	**T8**
100 %	Glass house	Songda	**T9**
50 %	Glass house	Songda	**T10**
100 %	Open field	Songda	**T11**
50 %	Open field	Songda	**T12**

Growing conditions, sowing and maintenance of plants in field and in glass house

Two experiments were conducted in field and in the glass house at the experimental site of the Department of Horticulture, University for Development Studies, Nyankpala Campus. The open field is an outdoor environment which we find out to be having a minimum temperature of 25 ºC and a maximum temperature of 27˚C during the study period. The glasshouse is entirely covered with glass. It minimum and maximum temperature during the study period were 30 ºC and 35 ºC. The mean values for the temperature were recorded with a simple digital thermometer which was taken daily.

The seeds were sown in plastic pots which were 0.22 m in height and 0.18 m in diameter and weighed 60.54 g. The pots were perforated at the bottom to allow excess water to drain off. Each pot was filled with 4 kg of top-soil taken from the field at a depth up to 0.20 m.

The three soybean varieties ('Afayak, 'Jenguma' and 'Songda') used in this study were obtained from the Savanna Agricultural and Research Institute (SARI), Nyankpala, Ghana. Two seeds were planted per pot and later thinned to one plant after two weeks of seedling emergence. The plants were watered with tap water (250 ml per pot) daily up to six weeks after sowing to enable them to maintain turgor.

Determination of the heat tolerance of three soybean varieties using Cell Membrane Thermostability (CMT) Test

Procedure for CMT

The CMT was used to assess heat (injury) tolerance in plants, and is similar to the procedure described by Martineau *et al.*, (1979), Nyarko *et al.,* (2008), Kebede *et al.,* (2012) but was slightly modified. Here, three young but fully developed leaves of the soybean varieties were picked at 42 days after planting, washed thoroughly under tap water, and then with distilled water to remove any soil particles. Paired adjacent sets (control and heat treatment) of ten leaf discs were cut using a cork borer avoiding the midrib from each leaf sample. Using the completely randomized design, the treatments consist of the control and the heat- treated leaves (see figure 1). The control and heat-treated leaf discs were placed into two separate test-tubes and washed thoroughly with distilled water. This removes exogenous electrolytes adhering to cut cells and leaf tissue surfaces at the periphery of the disc. After the final wash, the tubes were drained of excess water.

Sufficient amount of water remained on the disc tube interior to maintain a high humidity. Test tubes containing the heat-treated samples were then covered with saran plastic wrap and incubated in a thermostatically-controlled water bath for 15 minutes at 50˚C, while the control tubes were maintained at 25˚C for 15 min. After the elevated temperature treatment, tubes containing heat-treated samples were cooled at 25˚C, and both the control and the treatment tubes were filled with 15 ml distilled water and incubated overnight for 18 h at 10˚C to allow diffusion of electrolytes from the disc. The tubes were then transferred to a water bath at 25˚C, the content was thoroughly mixed for 5 seconds on a vortex mixer machine, and the initial conductance was measured using a conductivity meter (Jenway, Dunmow, UK, model 4071).

After this, both the control and heat treatment test tubes were covered with saran plastic wrap and autoclaved at 121˚C for 15 min to release all electrolytes. All tubes were cooled to 25˚C, the contents were thoroughly mixed, and final conductance was measured. The relative injury (RI) induced as a result of the initial 50˚C temperature treatment was then calculated as described by Sullivan (1972):
Relative Injury, RI (%) = $\{1 – ([1 – (S_1/S_2)] / [1 – (C_1/C_2)]\} \times 100$
Where, S and C refer to the conductance value for the heat treatment and control tubes, respectively and subscript 1 and 2 refer to the initial and final conductance reading, respectively.

Number of leaves per plant and plant height

The number of leaves produced by plants was counted at 14, 28 and 42 days after planting. The heights of plants were measured from the ground level to the apical point of the tagged plants with a meter rule at 14, 28, and 42 days after planting.

Leaf area (m^2)

This parameter was calculated using the linear measurement of leaves using a general relationship; Leaf Area = L x W x b where L=Leaf length; W= Leaf maximum width; b is a constant (0.75) as suggested by Montegomery (1911).

Fresh biomass per plant (g)

To determine total biomass, plants were uprooted and the weight of shoot and root were measured six weeks after sowing.

Stem girth (mm)
This was measured using an electronic caliper at 14, 28 and 42 days after planting.

Measurements were taken at 10 cm from the ground level.

SPAD value

This was measured using the SPAD meter (Model: Konica Minolta Chlorophyll Meter SPAD 502 PLUS) at 14, 28 and 42 days after sowing.

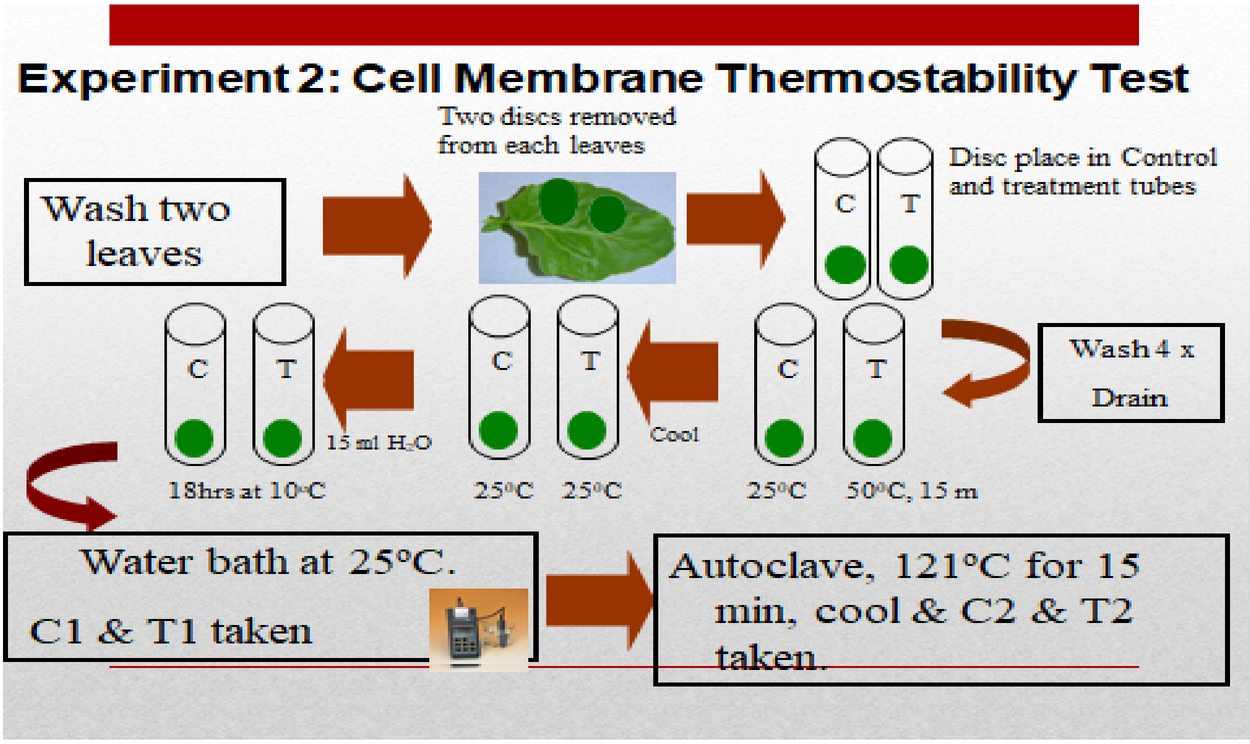

Figure 1: Flow chart of cell membrane thermostability test procedure
Data collection

Statistical analysis

The data gathered were subjected to Analysis of Variance (ANOVA) using the GENSTAT statistical package and differences between treatment means were determined using the standard error of difference (SED).

RESULTS AND DISCUSSION

Assessing soybean varieties growth indices under different watering regime and growing conditions

Climate change is expected to affect rainfall patterns leading to severe and frequent droughts. Globally, the change in climatic patterns has led to a decline in the developmental performance of field crops (Dai, 2013). Thus, this study assessed the growth and development response of three soybean varieties to different watering regime and temperature conditions. Crops respond to water and heat stress through various adjustments (Anjum *et al*., 2011). The stress response mainly depends on the force, rate and duration of contact and the stage of crop growth (Wajid *et al.,* 2004). In the current study, the three soybean varieties which were grown under glass house and open field conditions exhibited no significant difference among treatment for plant height, number of leaves, fresh biomass, leaf area, stem diameter and SPAD value as shown in Tables 2-7. From the study, the soybean varieties recorded plants height ranging from 13.9 to 35.36 cm. The highest plant height was recorded under the glass house growing condition in the present study. Similarly, an earlier study revealed that increasing temperature in growing environment influenced plant height and number of branches (Sionit *et al.,* 1987; Parsad *et al.,* 2006). Although, soybean is sensitive to heat or high temperature (Setiyono *et al*., 2007; Kumudini and Singh 2010).

When plants experience drought and heat stress, stem diameter shrinks resulting in decreased plant height in response to changes in internal water status (Simonneau *et al.,* 1993; Prasad *et al.,* 2006). In the current study, soybean varieties under the glass house growing condition generally experienced lower stem diameter due to high temperature conditions in the growing environment.

Fresh biomass is an essential component of crop yield (Araujo and Teixeira, 2008). *Songda* with 50% watering regime under both growing condition in the current study recorded the lowest plant biomass. This could be attributed to drying of the leaves, which leads to the reduction of the biomass as reported by Wych and Rasmusson (1983). So, soil moisture and growing conditions of a particular area are important for soybean production (Ghosh *et al,* 2000).

Leaf greenness (SPAD value) is a measure of chlorophyll content and determines the photosynthetic activities of a crop. In the present study, the soybean varieties grown in the glass house recorded the least leaf greenness. A study by Ghassemi-Golezani and Lotfi (2012) also reported leaf chlorophyll content index decreased with increasing water stress. Also, Isoda and Shahenshah (2010) in previous work opined that the higher the leaf temperature the lower the chlorophyll content.

Table 2: Effect of variety and water regime on plant height under different growing conditions

	Water regime (%)			
	Glass house (cm)		Open field (cm)	
Variety	50	100	50	100
Afayak	33.41	31.59	13.90	20.76
Jenguma	35.36	27.93	16.92	16.37
Songda	29.21	34.16	14.19	14.99
SED	3.443		3.276	
F pr.	0.051		0.248	

Table 3: Effect of variety and water regime on number of leaves

	Water regime (%)			
	Glass house		Open field	
Variety	50	100	50	100
Afayak	12.00	13.11	9.33	12.11
Jenguma	14.44	13.00	13.44	11.56
Sondga	12.89	12.33	11.33	9.67
SED	1.938		2.304	
F pr.	0.642		0.284	

Table 4: Effect of variety and water regime on fresh biomass

	Water regime (%)			
	Glass house (g)		Open field	
Variety	50	100	50	100
Afayak	4.69	5.44	9.24	6.88
Jenguma	6.39	5.00	9.32	9.35
Songda	3.93	5.22	7.44	7.22
SED	2.339		3.331	
p value	0.701		0.828	

Table 5: Effect of variety and water regime on leaf area

	Water regime (%)			
	Glass house (m^2)		Open field (m^2)	
Variety	50	100	50	100
Afayak	9.81	8.90	10.3	21.1
Jenguma	12.96	11.11	15.1	14.3
Songda	12.79	10.26	15.5	11.1
SED	1.706		4.48	
p value	0.800		0.055	

Table 6: Effect of variety and watering regime on stem diameter

	Water regime (%)			
	Glass house (mm)		Open field (mm)	
Variety	50	100	50	100
Afayak	2.08	2.04	2.50	2.90
Jenguma	2.46	2.38	2.93	2.64
Songda	2.79	1.93	2.86	2.36
s.e.d	0.405		0.372	
F pr.	0.281		0.212	

Variety x Watering regime interactions did not significantly ($P > 0.05$) affect stem diameter of the soybean varieties under the two growing conditions.

Table 7: Effect of variety and water regime on SPAD value

	Water regime (%)			
	Glass house		Open field	
Variety	50	100	50	100
Afayak	26.80	32.76	34.60	34.84
Jenguma	31.64	30.97	37.91	33.71
Songda	34.22	32.86	34.73	34.56
SED	2.696		2.053	
F pr	0.121		0.253	

Relative injury

For glass house growing condition, the relative injury ranged from 45.5 % to 66.0 %. However, the observed differences in the relative injuries of the varieties were not statistically different significant (Figure 2).

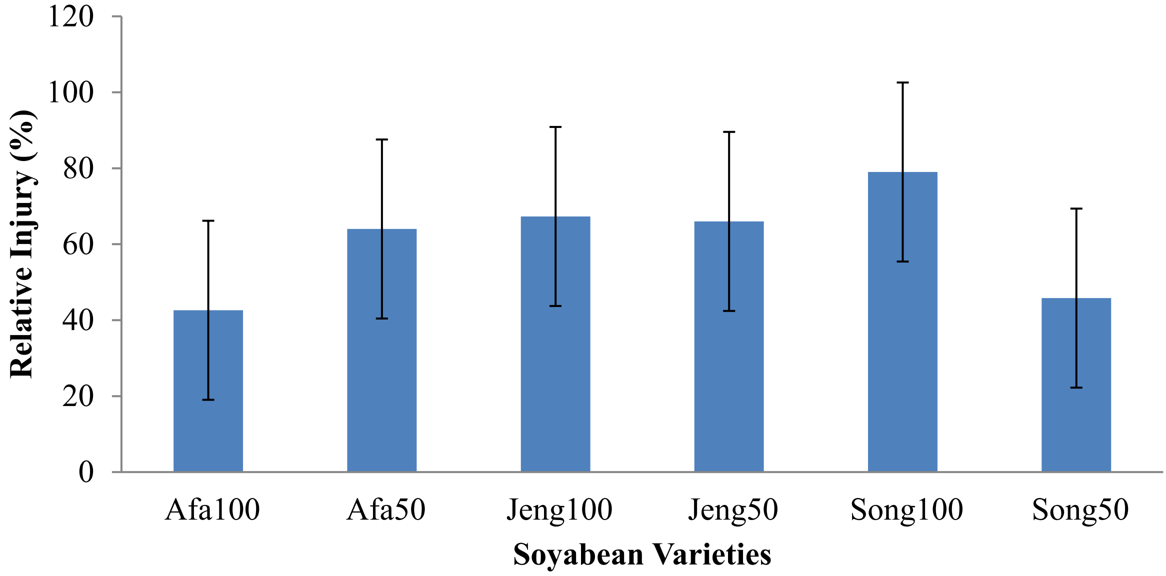

Figure 2: The effect of heat on three soybean varieties under glass house condition
Afa100 = Afayak with 100% watering regime; Afa50 = Afayak with 50% watering regime; Jeng50 = Jenguma with 100% watering regime; Jen50 = Jenguma with 50% watering regime; song50 = Songda with 5o% watering regime; sonf100% = Songda with 100% watering regime. Bars represent two standard errors of difference.

Soybean is sensitive to drought compared to other field crops as reported in previous studies (Ohashi *et al.,* 2006). Generally, a high relative injury is associated with high membrane leakage. Thylakoids and plasma membranes are considered the primary sites of attack during injury (Leshem, 1992) and this hinders the numerous biochemical and biophysical reactions. In this experiment, the three soybean varieties (*Afayak, Songda and Jenguma*) which were subjected to 50 % and 100 % of water requirement under glasshouse condition were not significantly different in terms of relative injury, implying that the soybean varieties have similar adaptation for growth under higher temperatures (30-35˚C) no variation in tolerance to heat stress.

CONCLUSION

The results of the study revealed that the growth of the three soybean varieties were the same in both growing environments (glasshouse and open field) with respect to the vegetative growth parameters. Glasshouse plants were generally taller and produced a greater number of leaves. Soybean varieties grown in pots kept under field conditions generally had higher biomass, leaf area, stem diameter and SPAD value. In determining the heat stress tolerance using the cell membrane thermostability technique, the results revealed no significant difference in heat tolerance among the three soybean varieties. Further studies should be conducted using different watering regimes and heat stress test methods

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