



Responses of Leaf Stomatal Parameters to Induced Water Stress and its Relationship with Stomatal Conductance in False Horn Plantain

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Authors' contributions

This work was carried out in collaboration between all authors. Authors BMD, JSA, SKD and RKB designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors JW and CS managed the literature searches and analyses of the study. Authors SKD, RKB and BMD managed the experimental process and data analyses. All authors read and approved of the final manuscript.

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ABSTRACT

Water deficit is one of the most important environmental factors affecting agricultural production especially in rainfed cropping systems. To understand how morphological and physiological factors interact, a study was conducted to assess the responses of leaf stomatal parameters to water status and its relationship with chlorophyll content in False Horn plantain. Each plant was grown in 120 kg soil in bowls and subjected to varying watering regimes (10 ml, 15 ml, 20 ml, 25 ml, 30 ml and control) without allowing natural rain into the bowls except the control. The result showed that

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False Horn plantain was sensitive to soil moisture stress; and this was reflected in changes in reduced growth through reduced plant height. The study also showed a systematic growth of the leaf with time. The patterns of leaf length growth remain similar under the different watering regimes. There was a significant difference in the leaf growth between the control and the varying amount of watering. The undulating pattern, however, showed that other factors could influence the leaf length. The large leaf area used for transpiration in Apantu is likely to be affected by soil water deficit. There was no correlation, however, between the leaf length to width ratio and the soil moisture content. The study further revealed that leaf length and width in False Horn plantain increases with growth, however, leaf sizes started to reduce when the plant approaches the flower primordial stage of growth. Watering regime influenced stomatal opening and closure in False Horn plantain and chlorophyll content. Leaf chlorophyll content was not highly influenced by the varying watering regimes; indicating that the presence of laticifer cells may be a factor for water stress tolerance in plantain.

Keywords: Plantain; physiology; stomata; water stress; chlorophyll content.

1. INTRODUCTION

Plantains are an important staple starch food and cash crop in humid sub-Saharan Africa. Plantains have been cultivated in the region over centuries but there is little information on their physiology and growth patterns. Knowledge on plantain crop physiology and growth patterns is important to establish the potential of the crop, explore the possibility of extending the crop to other areas and improve yields by resolving major yield constraints.

Water deficit is one of the most important environmental factors affecting agricultural production especially in rain-fed cropping systems. The consequences of water deficit include its adverse effects on plant phenology, development, assimilate partitioning, carbon assimilation, growth, and plant reproduction processes. Consequently, plant responses to drought have been extensively investigated from molecular, physiological, and whole plant to ecosystem levels [1]. Physiological responses to soil water deficit are the features that are most likely to determine the response of a crop to irrigation. Water stress could reduce leaf net photosynthetic assimilation (A_n) by both stomatal and metabolic limitations [2,1,3,4]. Also, many studies have reported that stomatal effects are significant under moderate stresses, but biochemical limitations are quantitatively important during leaf ageing or during severe drought [5,6]. An early response to water deficit in a plant is a reduction in leaf area and plant growth, which allows plants to reduce their transpiration, thus increasing water use efficiencies (WUE) [7-9] and promoting interspecific competition capacity under drought [10].

Plants subjected to abiotic stress use various defense mechanisms to cope with the stress. A common strategy is the synthesis and accumulation of osmoprotectants or compatible solutes like proline, glycine betaine, polyamines or trehalose. Tolerance to abiotic stresses can be acquired by pre-treatment with such a protective compound [11].

Several factors are known to influence stomatal opening and closure. Notable among them are light (intensity, duration and quality) internal water status of the leaf, carbon dioxide concentration of the surrounding air, and the leaf temperature. Soil water deficit coupled with other environmental factor create a complex mechanism that is very difficult to understand when not studied under control environment. Also it becomes complex to interrelate all these factors at the same time. Wind speed, soil moisture, solar radiation leaf temperature and relative humidity all contribute to influence the physiological responses.

Plant stomata are crucial gateways between plant and atmosphere and play a central role in plant/vegetation responses to environmental conditions. Several studies have been conducted and continue to be conducted from molecular and whole plant perspectives, as well as at ecosystem and global levels [12,4,13]. Studies carried out have shown stomatal density responses to various environmental factors, such as elevated CO_2 concentration [13], heat stress [14], salt stress [15], drought [16,17,18], precipitation change [19], and plant density [20]. Other studies have shown that water deficit leads to an increase in stomatal density [21,22], and a decrease in stomatal size [23,24,25] indicating could influence adaptation of plant to drought

[23,25,26]. While studies have shown that some selected abiotic factors influence physiological responses, others like wind speed, relative humidity, solar radiation, temperature and CO₂ also play a significant role in shaping the physiological responses.

Several authors have analyzed plant responses to water deficit in relations to various plant traits. While some authors related stress to stomatal conductance [27] others linked responses to photosynthesis [1]. Others also linked plant responses to environmental factors as a type of homeostasis [28]. A study showed that in some plants, stomata on the same leaf may respond homogeneously for heterogeneously to environmental conditions [29,27,30,31]. This behavior, named patchy stomatal closure, occurs when stomata at different locations on the same leaf show different responses to similar perturbations [32,33,30,31]. Some plants are known to rhythmically vary the width of the stomatal pores, to control the flux of CO₂ and H₂O between the leaf and the surround air in response to changes in environmental factors [32,33]. Although the stomatal response to the environment involves complex mechanisms influenced by many factors, stomatal movements appear to be governed by two principal control circuits, one involving gradients of CO₂ and the other involving gradients of H₂O vapour [34]. Patchwise stomatal closure is especially pronounced under stress situations, such as water deficit [35,33]. According to Cardon [36], during strong oscillatory behavior or even at a steady-state stomatal conductance, a surprising number of different behaviors in the dynamics of the photosynthetic activity may be found.

Leaf morphological traits, including stomatal density and distribution, and epidermal features are known to affect gas exchange quite remarkably and their relationships with key environmental factors such as light, water status, and CO₂ levels [13,12]. It is reported that increasing the blue-light response of stomata through red light is closely associated with guard cell chloroplast activity [37,38,39], indicating that leaf photosynthetic activity may involve stomatal movement and development. The balance between carbon gain through photosynthesis and water loss through transpiration was reported by Iyyakkutty [40] to also affect instantaneous water use efficiency (WUE [37,38]. Unfortunately, how stomatal density affects gas exchange has received little attention over the years.

Plantain as a giant monocot pose challenge to physiologists to measure indicators of water deficits, due to the presence of large air pockets within the leaves, and laticifers containing latex within the leaves, fruit, and corm that hinder the use of standard methods of measuring water relations [41]. Several methods have been used to measure physiological indicators of response [42,43,41]. However, none of these methods have been accepted as the only standard to be used, though [44] confirmed the reliability of method by [42] that is dependent on the refractive index of exuded latex. Although some authors [44,42] used leaf folding as an indicator of response to water deficit, in plantain under hot, arid conditions, leaf folding is not considered to be a reliable plant-based indicator of when to irrigate [45]. While physiological mechanisms of stomatal responses are complex and are not yet fully understood, it is even worse with False horn plantain as study describing how stomatal parameters respond to different water stresses, and their relationships with physiological processes are limited. The purpose of this study was to determine the stomatal response patterns to different water status, and to develop the relationship of stomatal parameters and photosynthetic processes.

2. MATERIALS AND METHODS

The study was conducted at the Crops Research Institute plantain orchard in Kumasi. The study area is located on latitude 06° 42'740"N; and longitude 001° 31' 827"W) with altitude of 300m above sea level. The first set of experiments was conducted between April 2013 and July 2013. The experiments were repeated between April 2014 and July 2014.

Planting materials of (False Horn plantain AAB subgroup) were generated using macro-propagation technique [46]. Healthy seedlings of uniform sizes with six leaves were selected and used for the study. The plantain plants were subjected to different water regimes for three months. Each plant was grown in 120 kg of sterile soil in large plastic bowls. The bases of the bowls were perforated to avoid water logging. The surfaces of the bowls were covered with black polyethylene sheets to prevented external rain water from entering. The soils were initially soaked to field capacity at planting. The bowls were then subjected to various water regimes- 10 ml, 15 ml, 20 ml, 25 ml, 30 ml per week. The surfaces of the bowls with control plants were uncovered to receive natural rain. Irrigation was

done with a large syringe through a pore at the base of the pseudostem. Fertilizer (NPK) was applied weekly at 15 g per plant. Fertilizer application was at the time of watering.

Each treatment had 15 plants replicated four times in a randomized complete block design and repeated three times. The Data were taken weekly from the first week to the 12th week on leaf emergence, leaf length, leaf width petiole length of fully opened new leaf. Plants were released from the stress condition after the 12th week. The plants were subjected to one month water stress again from the 37th to the 42nd (flowering) leaf stage of growth. Data was collected on the leaf length and leaf width. Data was analysed using Analysis of variance (ANOVA) (P= 0.05).

2.1 Leaf Stomatal Density and Guard Cell Size

The impression approach was used to determine leaf stomatal density, which was expressed as the number of stomata per unit leaf area [47]. The leaves selected were those for which chlorophyll content was also measured. Micro-morphological observations were carried out 12 weeks after planting using a digital microscope (Amscope, USA). The stomatal density (SD) stomatal length (SL) and stomatal width (SW) were determined from the underside (abaxial surface) of each leaf using prints made with nail varnish. SL was measured between the junctions of the guard cells at each end of the stoma as defined by [48,49]. The SW was measured perpendicularly to maximum width, which represents the maximum potential opening of the stomatal pore, but not the aperture of opening that actually occurs. SD (number of stomata per mm²) was determined as described by [47]. Stomatal surface (SS) stomatal shape coefficient (SSC), potential conductance index (PCI) and relative stomatal surface (RSS) was obtained using equations 1 to 4 (equations from Wang et al. [50] with some modifications).

$$SS = (SL * SW * \pi) / 4 \quad (1)$$

$$SSC = 100 * SW / SL \quad (2)$$

$$PCI = (SL)^2 * SD * 10^{-4} \quad (3)$$

$$RSS = SPS * SD * 100 \quad (4)$$

The SL and SW values are measured as micrometers (μm), SS in (μm)² and RSS in percentages.,

Stomatal conductance = (Stomatal aperture length)² * Total stomatal density * 10⁻⁴ [51].

The number of guard cells was estimated by doubling the number of counted stomata in the same leaf area [47]. Stomatal size was defined as the length in micrometres between the junctions of the guard cells at each end of the stoma, and may indicate the maximum potential opening of the stomatal pore, but not the aperture of opening that actually occurs [48,49].

2.2 SPAD Value

Chlorophyll content was determined from intact leaves using a chlorophyll meter (SPAD 502 Manitol) Reading of SPAD values was done on weekly basis. Three measurements were made per plant, three leaves were chosen from each plant (lower, middle and upper leaves of a plant), and three different regions of each leaf (middle and two ends of leaf) were used for tests. The chlorophyll meter was used to estimate the nitrogen status of the crops. The instrument measures transmission of red light at 650 nm, at which chlorophyll absorbs light, and transmission of infrared light at 940 nm, at which no absorption occurs. On the basis of these two transmission values the instrument calculates a SPAD value that is quite well correlated with chlorophyll content [52,53].

3. RESULTS AND DISCUSSION

The study area experiences bimodal rainy season with the major rainy season starting from March to July and the minor rainy season from September to November. Subjecting False Horn plants to water stress affected physiological morphological and biochemical processes. The average temperature of the study area was 26°C and the relative humidity was 82% (Table 1).

The minimum and maximum annual temperatures recorded during the period were 24°C and 28°C respectively (Table 1). The lowest temperatures occurred in the months between the major and the minor rainy seasons (Table 1); whereas the maximum was observed during the peak (February) of the dry season. This deviates widely from the notion that April is the hottest month in the middle belt of Ghana. In April, the relative humidity is often high coupled with the high temperature, the weather becomes humid and hot; unlike in February when the weather is dry hazy with the north-west westerly

winds. The lowest relative humidity was recorded in the dry season (January). This is not surprising as the weather is often dry and hazy in January with the north-west westerly winds bringing in a lot of dust from the Sahara desert. It is interesting to observe the strongest winds experienced in September and October. However, the heaviest lodging in plantain is experienced between March and April when the rains are starting after a long drought. The beginning of the rains comes along with strong winds; and because the plants are dehydrated as a result of the long drought there is severe lodging.

Strong winds are known to cause stem and root lodging in plantain but not at tender ages. However, strong wind coupled with low relative humidity could influence water loss from the leaves; as these could influence stomatal opening and closure to conserve water in the tissues.

Water stress adversely affected plantain growth and development. Leaf emergence was highly influenced by water stress [41]. The control maintained regular leaf emergence compared to

the other treatments (Table 2). Under 25 ml and 30 ml of water per week regimes, False Horn produced one leaf in every seven days; however, as the watering reduced, leaf emergence was delayed to a leaf in 12 days. Though there was no sign of dehydration of the leaves, it was observed that leaf necrosis was very high. The numbers reduced with time especially after week three. This agrees with Kallarackal, et al. [43] and Turner and Thomas [41] that plantain response to water deficit by stomatal closure and that as the soil water decreases, the plants remain highly hydrated. However, weekly leaf emergence is affected by water stress. This prolongs the crop cycle. False Horn plantain, as a determinate plant is expected to produce a fixed number of leaves (about 42) leaves before flowering. Under good irrigation conditions coupled with soil nutrient flowering starts after six months and harvesting at the ninth month. However, with new leaf emergence at fortnightly intervals, flowering would start after 12 months and harvesting at the fifteenth month. This situation would contribute to high cost of production.

Table 1. Mean of weather conditions at the experimental location (2013-2014)

Month	Temperature (°C)	Rainfall (mm)	Relative humidity (%)	Wind speed (m/s)	Solar readiation (W/m ²)
January	26.5	8.0	56.6	0.7	155.8
February	28.1	17.8	65.8	0.9	165.3
March	27.2	82.4	81.5	1.0	172.5
April	27.0	152.6	80.9	0.9	185.9
May	26.4	169.4	87.2	0.7	162.1
June	25.7	199.2	89.1	0.9	147.5
July	24.6	43.6	89.1	1.0	117.8
August	23.9	7.4	90.1	1.1	101.5
September	23.7	255.4	90.1	128.9	121.4
October	24.7	215.2	90.1	139.4	154.9
November	26.2	41.4	85.2	0.6	156.0
December	25.9	40.8	80.1	0.6	149.1

Table 2. Analysis of variance of leaf emergence of false horn plantain under water stress

Treatment (ml)	Total number of leaves with time (weeks)										
	1	2	3	4	5	6	7	8	9	10	11
Control	7.0	7.0	7.0	7.0	7.0	7.0	6.0	6.0	5.0	5.0	5.0
10	6.0	7.0	6.0	5.0	5.0	5.0	5.0	5.0	5.0	4.0	4.0
15	7.0	7.0	7.0	5.0	6.0	6.0	6.0	6.0	4.0	4.0	4.0
20	7.0	7.0	8.0	5.0	6.0	6.0	6.0	5.0	5.0	4.0	4.0
25	7.0	7.0	7.0	5.0	6.0	5.0	5.0	5.0	5.0	5.0	5.0
30	7.0	9.0	9.0	8.0	5.0	5.0	4.0	4.0	4.0	4.0	4.0
SE	0.4	0.4	0.4	0.5	0.4	0.5	0.3	0.4	0.5	0.3	0.4
CV%	5.7	5.1	6.7	9.5	6.8	7.4	5.9	7.0	10.4	6.0	10.1
LSD (p<0.05)	1.2	1.2	0.8	0.8	0.8	0.8	0.7	1.1	1.0	1.2	1.4

The sensitivity of False Horn plantain to soil moisture stress is reflected in changes in reduced growth through reduced plant height (Table 3). There was a significant difference ($p < 0.5$) in plant height between the treatments (Table 3). Soil water stress affected plant growth. While plant height was increasing at a weekly average rate of 5.7 cm in the control, it was an average of 3.1 cm in the 10 ml, 2.9 cm in 15 ml, 3.1 cm in 20 ml, 3.3 cm in 25 ml and 3.0 cm in 30 ml of water per week. The results showed that False Horn plantains need more than 30 ml of water per week to grow and achieve its optimum potential. Though there was no sign of dehydration in the pseudostem the results showed that plantain has an internal mechanism for water stress endurance. Growth is determined by multiplication of cells and expansion of cells, hence any environmental factor that can hinder water loss can influence growth. In their study, [43,41] reported that, plantains are sensitive to soil water deficits; expanding tissues such as emerging leaves and growing fruit are among the first to be affected. They also observed that as the soil begins to dry, stomata close; however, leaves remain highly hydrated. Turner, [54] observed that stomatal closure under soil water deficits conditions may likely be linked to a signal from the roots rather than water deficit in the leaves. In another experiment [55] reported that drying part of root system of banana had no effect on leaf water status but did close the stomata. However, severing the roots on the dry side caused the stomata to reopen. These observations therefore support the view that the roots produce a signal that is transported to the leaves. This mechanism conserves the plant's water, but reduces carbon assimilation and productivity. The concept that plantains use large amounts of water could not have a strong physiological basis as the plants

remain hydrated under severe soil moisture deficit.

The new leaves produced continue to increase in length with severe soil water deficit. There was a significant difference between the control and the treatments (Table 4). There was no significant difference between in the leaf length between treatments 10 ml, 15 ml, 20 ml and 25ml at week 4 (Table 4). This showed that other factors would also be relevant to the growth of the plant. It is observed that under thick and prolonged cloud (about 2-6 weeks) plantains and banana cease to growth. This has been a major challenge for commercial banana growers in Ghana. It is observed that during a period when day and night temperature and almost the same, there is lack of rains, but with high humidity and thick cloud cover, fruits cease to grow irrespective of the amount of water and nutrient applied. This often occurs between March and April every year in Ghana.

There was a significant difference in leaf width between the control and the other treatments from week one to week eleven (Table 6). Leaf width experienced reduced rate of expansion between week 3 to week 6 for all treatments. The leaf surface area is the main photosynthetic organ and reduction affects the photosynthetic process of the plant. Plantain with broad leaf surface and the large number of stomatal pores would contribute to the physiological processes. However, water stress is known to reduce stomatal development, size and density; and hence affect photosynthetic activities [39,56]. This could be attributed to other environmental factors. The weather could be contributing to the reduced growth. The high humidity, thick cloud cover without rains and the narrow difference between day and night temperatures could have contributed to the reduced growth as photosynthesis ceases.

Table 3. Analysis of variance of plant height of false horn plantain under water stress

Treatment (ml)	Plant height (cm) with time (weeks)										
	1	2	3	4	5	6	7	8	9	10	11
Control	22.0	23.0	32.0	36.0	39.0	47.0	60.0	63.0	65.0	66.0	79
10	17.0	20.0	24.3	27.8	28.8	33.3	42.3	43.0	43.7	43.7	48.5
15	18.1	19.9	24.8	27.5	29.0	29.8	37.2	43.8	44.2	44.5	45.5
20	15.6	19.7	25.8	29.2	30.8	33.3	42.8	42.8	46.2	46.5	50.3
25	18.0	21.2	28.7	31.3	33.7	34.7	41.7	42.5	45.0	47.8	51.8
30	18.9	21.6	26.8	26.9	28.9	31.7	37.5	38.9	42.1	43.9	44.8
SE	2.28	2.32	3.08	2.74	2.89	1.25	1.66	1.39	1.55	1.43	2.71
CV%	6.0	4.9	3.5	2.6	2.6	3.6	3.8	3.0	3.3	3.0	5.1
LSD ($p < 0.05$)	3.49	4.71	4.71	4.20	4.42	5.11	7.46	8.35	8.70	8.92	12.48

Table 4. Analysis of variance of leaf length of false horn plantain under water stress

Treatment (ml)	Leaf length (cm) with time (weeks)										
	1	2	3	4	5	6	7	8	9	10	11
Control	25.0	28.0	44.0	46.0	47.0	51.0	64.0	68.0	65.0	71.0	72.0
10	20.1	21.8	31.3	34.0	35.2	34.8	41.8	44.5	47.8	47.0	51.5
15	20.9	20.9	29.2	34.0	35.5	36.0	43.0	43.8	40.9	46.0	49.0
20	19.9	20.0	32.2	35.5	37.0	39.3	48.3	51.3	51.3	53.8	57.2
25	20.2	22.9	32.3	34.2	38.0	37.8	46.3	47.8	47.7	50.5	53.7
30	21.0	23.6	32.5	37.8	37.3	38.7	43.9	52.8	42.6	51.9	52.2
SE	1.37	3.09	0.54	0.93	0.82	0.34	1.22	1.58	1.82	1.2	1.4
CV%	6.5	3.7	1.6	2.5	2.2	0.9	2.6	3.1	3.7	2.3	2.6
LSD (p<0.05)	2.7	4.7	7.5	6.2	5.8	5.2	6.8	7.6	8.8	7.0	8.2

Table 5. Leaf length-width ratio of false horn plantain under varying water regimes

Watering regimes (ml)	Mean leaf length (cm) and SD	Mean leaf width (cm) and SD	Leaf length: Width ratio
10	51.9±4	30.2±6	1.7
15	42.2±5	21.9±7	1.9
20	55.8±7	32.1±4	1.7
25	63.4±5	35.1±6	1.8
30	53.5±4	31.4±3	1.7
Control	71.7±10	38.2±8	1.9
LSD (p <0.05)	5.5	2.4	

Table 6. Analysis of variance of leaf width of false horn plantain under varying water regimes

Treatment (ml)	Leaf width (cm) with time (week)										
	1	2	3	4	5	6	7	8	9	10	11
Control	13.0	14.0	23.0	25.0	22.0	28.0	34.0	38.0	68.0	40.0	39.0
10	8.2	9.2	14.0	15.3	15.4	16.9	22.2	23.7	27.7	25.8	28.0
15	8.9	9.6	12.8	15.3	15.0	15.6	22.8	24.3	24.8	23.3	27.4
20	9.0	10.0	14.5	16.4	17.6	18.6	24.7	27.8	28.8	29.9	31.0
25	9.6	11.5	14.3	14.8	17.3	18.7	28.3	24.8	44.0	26.3	29.2
30	10.6	12.0	14.6	17.6	20.0	20.7	25.1	27.8	53.9	29.6	29.9
SE	0.7	0.6	0.7	0.8	0.5	0.7	2.6	1.6	2.3	1.3	1.1
CV%	7.0	5.2	4.7	4.7	2.8	3.7	9.9	5.7	5.6	4.4	3.5
LSD (p<0.05)	1.8	3.1	3.8	4.4	3.8	3.6	6.7	5.1	8.1	5.8	5.4

Petiole length was not significantly influenced by soil water stress (Table 7). However, there was a reduction growth between weeks 3 and 6 as a result of other weather conditions. The control however, had a frog leap in length after week 10. The length of the petiole has an influence the leaf lamina as it the base that holds the lamina and exposes to the sun. The length would contribute to the orientation of the lamina for effective photosynthesis.

The study showed a systematic growth of the leaf with time (Table 5). However, the pattern of the leaf length shows moderately undulating patterns. The patterns of leaf length growth remain similar under the different watering regimes (Table 5). There was a significant

difference between the control and the varying amount of water application. The undulating pattern, however, showed that other factors could influence the leaf length growth. The large leaf area used for transpiration in False Horn is likely to be affected by soil water deficit. The large leaf surface area coupled with the long crop cycle under rain-fed conditions, False Horn plantain must therefore develop an appropriate mechanism for withstand soil water deficit.

There was no significant difference between the leaf length to width ratio and the soil moisture content. There was no correlation between watering regimes and the leaf length: leaf width ratio. This could be linked to the study by Turner and Thomas [41] in which they stated that plants

remain hydrated under severe soil moisture deficit. Thus water-use efficiency in plantain could come from a closer match between plant water use and the amount of water applied.

False Horn produces on the average 42 leaves before flowering. It was observed that leaf length and width in False Horn plantain increases with growth, however, these features start to reduce when the plant approaches the flower primordial stage of growth (Table 8). Leaf length and width reduction starts from 39th leaf to the 42nd (flag leaf). The leaf length: width ratio did not change much with growth (Table 8) when the plants were subjected to water stress for one month before flowering.

Under the control experiment, the leaf length and width also reduces as the plant approached the flower primordial stage of growth (Table 9).

However, there was no difference between the leaf length: width ratio during growth and development. The reduction in leaf length and width proportionately could be an innate characteristic of the crop. It showed that ripeness to flower primordial initiation results in energy storage hence reduction in vegetative growth.

The study revealed a homogeneous closure of stomata in response soil water deficit in False Horn. However, under severe soil moisture deficit, stomata closure is pronounced (Plate 1a).

The water regime influenced stomatal opening and closure. Under the 10ml of water per week regime, the stoma closes as compared to 20ml and the control (Plates 1, 2 and 4). Soil water deficit is known to reduce stomatal conductance and leaf size [43] increased leaf senescence [54].

Table 7. Analysis of variance of petiole length of false horn plantain under water stress

Treatment (ml)	Petiole length (cm) with time (week)										
	1	2	3	4	5	6	7	8	9	10	11
Control	3.8	5.0	5.0	5.2	5.7	8.0	8.7	10.3	9.0	10.2	14.2
10	3.0	4.5	4.1	4.1	3.8	5.5	6.5	6.8	7.2	7.6	7.5
15	3.4	3.5	4.1	4.3	4.4	4.7	6.5	6.5	7.0	7.1	7.1
20	3.3	3.9	4.3	4.6	4.3	6.2	6.8	7.0	7.8	8.1	8.0
25	3.4	4.8	4.2	4.1	5.5	5.5	7.5	7.0	7.5	6.8	9.4
30	3.9	4.2	4.1	4.1	4.6	4.7	6.3	7.0	7.2	7.0	7.4
SE	0.2	0.2	0.3	0.2	0.3	0.5	0.5	0.3	0.4	0.5	0.5
CV (%)	6.2	3.9	6.5	5.2	7.1	9.3	6.6	4.1	4.7	6.9	5.9
LSD (p<0.05)	1.0	0.8	0.8	0.5	0.7	0.9	1.3	1.1	1.0	1.2	2.1

Table 8. Flower primordial growth stage leaf length-width ratio of false horn plantain under water stress

Leaf number	Mean leaf length (cm) and SD	Mean leaf width (cm) and SD	Leaf length: Width ratio
37 th	189±14	82±12	2.3
38 th	171±11	70±13	2.4
39 th	153±14	61±11	2.5
40 th	142±11	57±16	2.4
41 st	118±12	49±10	2.4
LSD (p< 0.05)	6.0	7.0	

Table 9. Flower primordial growth stage leaf length: Width ratio of false horn plantain under well watering regime

Leaf number	Mean leaf length (cm) with SD	Mean leaf width (cm) with SD	Leaf length: width ratio
37 th	194±12	86±19	2.3
38 th	187±14	77±13	2.4
39 th	163±18	68±16	2.4
40 th	142±16	60±14	2.4
41 st	120±19	51±11	2.4
LSD (p< 0.05)	5.6	4.7	

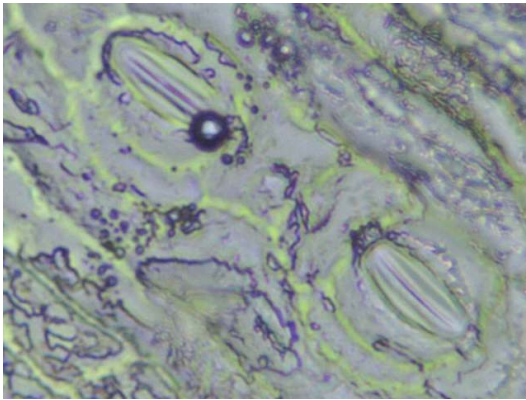


Plate 1. (a) Stomatal shape under 10 ml water regime



Plate 2. (b) Stomatal shape under 20 ml water regime

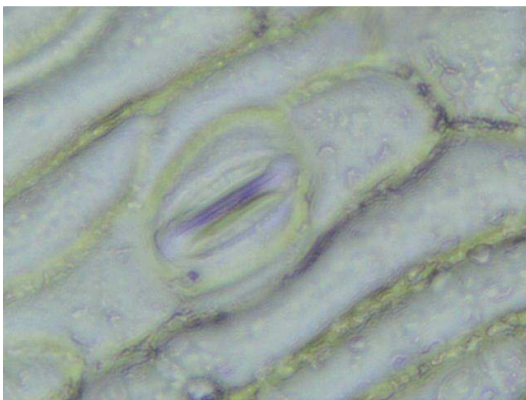


Plate 3. Stomatal shape under 30 ml water regime

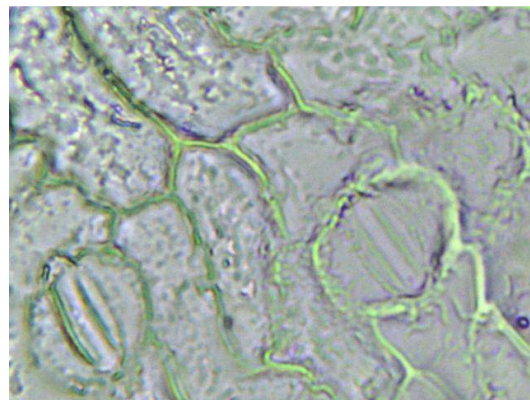


Plate 4. Stomatal shape under the control

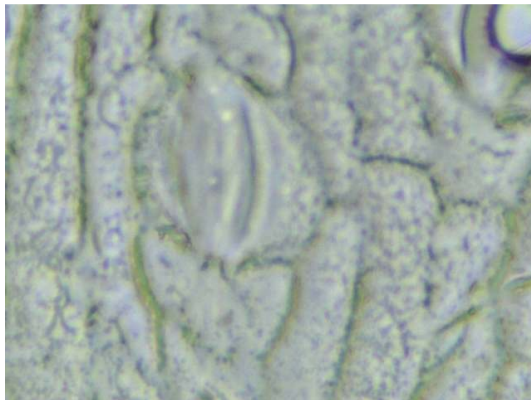


Plate 5. Stomatal shape under 15 ml water regime

Plantains (*Musa* spp.) rarely attain their full genetic potential for yield due to limitations imposed by abiotic factors which limit the plant's photosynthesis. Plantains are reported to be

particularly sensitive to changes in the environment [57]. This thus affect the productivity of the crop because there is often early closure of stomata to conserve water and hence affect photosynthetic activities.

Leaf folding is on characteristic feature of plants to response to water deficit. Leaf folding results in reduction in surface area. In their study soil water deficit and banana responses, [41] reported that there was a link between leaf folding and water deficit. In another study Lu et al. [58] did not find any link between leaf folding and soil water deficit as they compared sap flow system with gravimetric system to measure drought resistance in banana. In this leaf folding was not observed as a characteristic in response to water deficit. It was observed that leaves of False Horn plantain remained hydrated throughout. Leaf drying and delayed leaf emergence were rather observed during the experimental period.

Table 10. Changes in guard cell length, stomatal density and potential conductance index of false horn plantain with varying water regimes

Watering regimes	Guard cell length (µm) with SD	Stomatal density (count/mm ²) with SD	Potential Conductance Index (PCI) with SD
10 ml	151.4±0.6	464±0.8	106.4±0.5
15 ml	225.0±0.4	352±0.5	178.2±0.3
20 ml	167.3±0.6	304±0.3	85.1±0.4
25 ml	178.2±0.3	320±0.6	85.1± 0.6
30 ml	209.7±0.4	304±0.2	63.5±0.5
Control	155.2±0.3	304±0.8	85.1±0.4
LSD (p< 0.05)	5.3	2.7	5.4

Table 11. Analysis of variance of SPAD chlorophyll conductance of false horn plantain before routine application of water

Treatment (ml)	Chlorophyll conductance with time (Week)										
	1	2	3	4	5	6	7	8	9	10	11
Control	49.7	49.3	32.5	45.2	44.7	49.5	50.1	42.7	43.6	46.8	45.5
10	42.8	51.1	45.0	44.9	45.7	53.6	53.6	51.6	48.5	49.1	49.9
15	41.3	49.3	44.7	55.6	46.4	52.7	53.5	59.3	55.8	49.6	46.4
20	44.9	48.7	43.8	49.1	45.5	51.6	50.3	45.4	47.0	44.9	42.3
25	48.0	50.8	41.8	46.6	45.1	49.6	48.6	46.6	48.5	47.6	44.7
30	50.3	47.9	36.5	42.2	49.7	50.6	60.4	41.6	38.6	42.1	45.3
SE	1.7	1.7	3.4	1.9	2.3	3.3	3.4	5.7	3.7	3.1	1.4
CV (%)	3.7	3.4	8.4	3.9	5.6	6.3	6.5	12.0	7.8	6.5	3.0
LSD (p,0.05)	6.0	5.6	6.9	9.0	4.9	6.1	6.7	15.3	8.5	8.4	6.2

All the plants recovered after three months of water stress. The result therefore showed that water stress at early stages of growth has little influence on the final growth and yield of crops with long gestation.

Table 12. Stomatal conductance index (PCI) of false horn plantain under varying watering

Watering regime (ml)	Stomatal density	Stomatal length (µm)	PCI
10	464±8	151.4±6	106.4
15	352±7	225.0±8	178.4
20	304±8	167.3±5	85.1
25	304±6	178.3±7	85.1
30	320±5	209.7±6	63.5
Control	304±7	141.0±9	85.1

P<0.05

The chlorophyll content did not show any significant trend in response to watering regime. The chlorophyll content showed undulating characteristics indicating that there was no relationship between soil moisture stress and chlorophyll content. Studies showed that the presence of large air pockets within the leaves, and laticifers containing latex within the leaves, fruit, and corm that hinder the use of standard

methods of measuring water relations [41]. This peculiar characteristic of plantains and bananas make them adjust quickly to water stress without showing significant physiological changes. In a study with two tobacco species, there was a significant relationship between the heavy metal concentration and stomatal parameters [29].

Stomatal conductance is linked to stomatal density and guard cell length. The study has not shown any straightforward link between water deficit and physiological responses with growth in False Horn (Table 12). There was no correlation between stomatal density and length stomatal conductance index (PCI) in False Horn. It is known that stomatal densities and stomatal lengths have a correlation with stomatal conductance index [51]. These parameters are also influenced by water stress; however, in some plants like plantain the presence of large air pockets within the leaves, and laticifers containing latex within the leaves, fruit, and corm influence physiological responses. Stomatal conductance is known to be a function of stomatal density, size and the degree of opening of the stomatal pores [59]. However, in their study, Lawson and Blatt, [59] reported that stomatal density and size can be negatively correlated as the stomatal opening is induced by abiotic factors.

Variation in the abiotic factors (water, temperature, light, wind speed and CO₂) affect stomatal aperture within minutes and can also induce long term changes in stomatal density, hence determine the limits for maximum stomatal conductance [60].

The world-wide water shortage and uneven distribution of rainfall as a result of climate variability makes drought resistance important factor to consider in physiological studies. The physiological changes within leaves such as changes in stomatal aperture or leaf folding or leaf elongation in response to soil water deficit did not correspond with changes in leaf water status measured. False Horn plantain productivity is greatly affected by environmental stresses such as drought, however, the crop responds and adaption to these stresses to survive could be at the molecular and cellular levels as well as at the physiological and biochemical levels.

4. CONCLUSION

The False Horn plant was sensitive to soil moisture stress. This was reflected in reduced growth through reduced plant height. Response to water stress reflects equally on the leaf length and width hence the leaf length: Leaf width ratio. The study has shown that False Horn plantain for that matter plantain genotypes have different inbuilt mechanisms for resistance to drought stress. Physiological responses of plantain to stress require that each parameter be studied under control environment without other having influence.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chaves MM, Maroco JP, Pereira JS. Understanding plant responses to drought-from genes to the whole plant. *Functional Plant Biology*. 2003;30:239–264.
2. Farquhar GD, Sharkey TK. Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology*. 1982;33:317–345.
3. Ghannoum O, Conroy JP, Driscoll SP, Paul MJ, Foyer CH, Lawlor DW. Nonstomatal limitations are responsible for drought-induced photosynthetic inhibition in four C4 grasses. *New Phytologist*. 2003;159:599–608.
4. Ripley BS, Gilbert ME, Ibrahim DG, Osborne CP. Drought constraints on C4 photosynthesis: Stomatal and metabolic limitations in C3 and C4 subspecies of *Alloteropsis semialata*. *Journal of Experimental Botany*. 2007;58:1351–1363.
5. Grassi G, Magnani F. Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. *Plant, Cell and Environment*. 2005;28:834–849.
6. Gallé A, Haldimann P, Feller U. Photosynthetic performance and water relations in young pubescent oak (*Quercus pubescens*) trees during drought stress and recovery. *New Phytologist*. 2007;174:799–810.
7. Xu ZZ, Zhou GS. Effects of water stress and nocturnal temperature on carbon allocation in the perennial grass, *Leymus chinensis*. *Physiologia Plantarum*. 2005;123:272–280.
8. Monclus R, Dreyer E, Villar M, Delmotte FM, Delay D, Petit J-M, Barbaroux C, Thiec D, Bréchet C, Brignolas F. Impact of drought on productivity and water use efficiency in 29 genotypes of *Populus deltoides* × *Populus nigra*. *New Phytologist*. 2006;169:765–777.
9. Aguirrezabal L, Bouchier-Combaud S, Radziejwoski A, Dauzat M, Cookson SJ, Granier C. Plasticity to soil water deficit in *Arabidopsis thaliana*: Dissection of leaf development into underlying growth dynamic and cellular variables reveals invisible phenotypes. *Plant, Cell and Environment*. 2006;29:2216–2227.
10. Xu H, Li Y, Xu G, Zou T. Ecophysiological response and morphological adjustment of two Central Asian desert shrubs towards variation in summer precipitation. *Plant, Cell and Environment*. 2007;30:399-409.
11. Stolker R. Combating abiotic stress using trehalose. Thesis Report, Wageningen University & Research Centre. 2010;42.
12. Nilson SE, Assmann SM. The control of transpiration. Insights from arabidopsis. *Plant Physiology*. 2007;143:19-27.
13. Woodward FI. Stomatal numbers are sensitive to increases in CO₂ from pre-industrial levels. *Nature*. 1987;327:617–618.

14. Beerling DJ, Chaloner WG. The impact of atmospheric CO₂ and temperature change on stomatal density: Observations from *Quercus robur* Lammad leaves. *Annals of Botany*. 1993;71:231–235.
15. Zhao S, Chen W, Ma D, Zhao F. Influence of different salt level on stomatal character in rice leaves. *Reclaiming and Rice Cultivation*. 2006;6:26–29.
16. Lecoeur J, Wery J, Turc O, Tardieu F. Expansion of pea leaves subjected to short water-deficit: Cell number and cell-size are sensitive to stress at different periods of leaf development. *Journal of Experimental Botany*. 1995;46:1093–1101.
17. Zhao RX, Zhang QB, Wu XY, Wang Y. The effects of drought on epidermal cells and stomatal density of wheat leaves. *Inner Mongolia Agricultural Science and Technology*. 2001;6:6–7.
18. Galmés J, Flexas J, Savé R, Medrano H. Water relations and stomatal characteristics of mediterranean plants with different growth forms and leaf habits: Responses to water stress and recovery. *Plant and Soil*. 2007;290:139–155.
19. Yang L, Han M, Zhou G, Li J. The changes of water-use efficiency and stoma density of *Leymus chinensis* along Northeast China Transect. *Acta Ecologica Sinica*. 2007;27:16–24.
20. Zhang XY, Wang HM, Hou ZD, Wang GX. Stomatal density and distributions of spring wheat leaves under different planting densities and soil moisture levels. *Acta Phytocologica Sinica*. 2003;27:133–136.
21. Yang HM, Wang GX. Leaf stomatal densities and distribution in *Triticum aestivum* under drought and CO₂ enrichment. *Acta Phytocologica Sinica*. 2001;25:312–316.
22. Zhang YP, Wang ZM, Wu YC, Zhang X. Stomatal characteristics of different green organs in wheat under different irrigation regimes. *Acta Agronomica Sinica*. 2006;32:70–75.
23. Cutler JM, Rains DW, Loomis RS. The importance of cell size in the water relations of plants. *Physiologia Plantarum*. 1977;40:225–260.
24. Quarrie SA, Jones HG. Effects of abscisic acid and water stress on development and morphology of wheat. *Journal of Experimental Botany*. 1977;28:192–203.
25. Spence RD, Wu H, Sharpe PJH, Clark KG. Water stress effects on guard cell anatomy and the mechanical advantage of the epidermal cells. *Plant, Cell and Environment*. 1986;9:197–202.
26. Martinez JP, Silva H, Ledent JF, Pinto M. Effect of drought stress on the osmotic adjustment, cell wall elasticity and cell volume of six cultivars of common beans (*Phaseolus vulgaris* L.) *European Journal of Agronomy*. 2007;26:30–38.
27. Souza GM, Ferraz de Oliveira R, Cardoso VJM. Temporal dynamics of stomatal conductance of plants under water deficit: Can homeostasis be improved by more complex dynamics? *Brazilian Archives of Biology and Technology*. 2004;47(3):423–431.
28. Kauffman AS. *The origins of order*. New York: Oxford University Press; 1993.
29. Ocren N, Nazarian G, Gharibkhani M. The responses of stomatal parameters and SPAD values in Asian tobacco exposed to chromium. *Pol. J. Environ. Stud*. 2013; 22(5):1441–1447.
30. Mott KA, Denne F, Powell J. Interactions among stomata in response to perturbations in humidity. *Plant Cell Envir*. 1997;20:1098–1107.
31. Haefner JW, Bunckley TN, Mott KA. A spatially explicit model of patchy stomatal responses to humidity. *Plant Cell Envir*. 1997;20:1087–1097.
32. Terashima I. Anatomy of non-uniform photosynthesis. *Photosynthesis Research*. 1992;31:195–212
33. Larcher W. *Physiological plant ecology*. 3th ed. Berlin: Springer-Verlag; 1995.
34. Shabala S, Delburgo R, Newman I. Observations of bifurcation and chaos in plant physiological responses to light. *Australian Journal of Plant Physiology*. 1997;24:91–97.
35. Terashima I, Wong SC, Osmond CB, Farquhar GD. Characterization of non-uniform photosynthesis induced by abscisic acid in leaves having different mesophyll anatomies. *Plant Cell Physiol*. 1988;29:385–394.
36. Cardon ZG, Mott KA, Berry JA. Dynamics of patchy stomatal movements, and their contribution to steady-state and oscillating stomatal conductance calculated using gas-exchange techniques. *Plant. Cell. Envir*. 1994;17:995–1007.

37. Roelfsema MRG, Konrad K, Marten H, Psaras G, Hartung W, Hedrich R. Guard cells in albino leaf patches do not respond to photosynthetically active radiation, but are sensitive to blue light, CO₂ and abscisic acid. *Plant, Cell and Environment*. 2006;29:1595–1605.
38. Shimazaki K-I, Doy M, Assmann SM, Kinoshita T. Light regulation of stomatal movement. *Annual Review of Plant Biology*. 2007;58:219–247.
39. Elio Conte, Maria Pieralice. Estimation of fractal dimension on inner structure of leaf samples by using the box counting method. *IJRR*. 2013;16(4):48-59.
40. Iyyakkutty Ravi, Subbarava Uma, Mathu Mayil Vaganam, Mohamed M Mustafa. Phenotyping bananas for drought resistance. *Front Physiol*. 2013;4:9.
41. Turner DW, Thomas DS. Measurement of plant and soil water status and their association with leaf gas exchange in banana (*Musa* spp): A laticiferous plant. *Scientia Hort*. 1998;77:177–193.
42. Milburn JA, Kallarackal J, Baker DA. Water relations of the banana. I. Predicting the water relations of the field grown banana using the exuding latex. *Aust. J. Plant Physiol*. 1990;17:57–68.
43. Kallarackal J, Milburn JA, Baker DA. Water relations of the banana. III. Effects of controlled water stress on water potential, transpiration, photosynthesis and leaf growth. *Aust. J. Plant Physiol*. 1990;17: 79–90.
44. Thomas DS, Turner DW. Banana (*Musa* sp.) leaf gas exchange and chlorophyll fluorescence in response to soil drought, shading and lamina folding. *Scientia Hort*. 2001;90:93-108.
45. Thomas DS, Turner DW. Leaf gas exchange of droughted and irrigated banana cv. Williams (*Musa spp.*) growing in hot, arid conditions. *J. Hort. Sci. Biotechnol*. 1998;73:419–429.
46. Dzomeku BM, Darkey SK, Wunsche J, Bam RK. Response of selected local plantain cultivars to pibs (plants issus de bourgeons secondaires) technique. *J. Plant Develop*. 2014;21:117–123.
47. Radoglou KM, Jarvis PG. Effects of CO₂ enrichment on four poplar clones. II. Leaf surface properties. *Annals of Botany*. 1990;65:627–632.
48. Malone SR, Mayeux HS, Johnson HB, Polley HW. Stomatal density and aperture length in four plant species grown across a subambient CO₂ gradient. *American Journal of Botany*. 1993;80:1413–1418.
49. Maherali H, Reid CD, Polley HW, Johnson HB, Jackson RB. Stomatal acclimation over a subambient to elevated CO₂ gradient in a C₃/C₄ grassland. *Plant, Cell and Environment*. 2002;25:557–566.
50. Wang H, Shi H, Yang R, Liu J, Yu Y. Stomatal characteristics of greening plant species in response to different urban atmospheric environments in Xi'an, China. *J. Food Agric. Environ*. 2012;10(3&4): 1524-1529.
51. Holland N, Richardson AD. Stomatal length correlates with elevation of growth in four temperate species. *Journal of Sustain Forestry*. 2009;28:63-73.
52. Markwell J, Osterman JC, Mitchell JL. Calibration of the minolta SPAD-502 leaf chlorophyll meter. *Photosynth Res*. 1995; 46:467-472.
53. Wood CW, Reeves DW, Himelrick DG. Relationships between chlorophyll meter readings and leaf chlorophyll concentration, N status and crop yield: A review. *Proceedings. Agronomy Society of New Zealand*. 1993;23:1-9.
54. Turner DW. Factors affecting the physiology of the banana root system. Methodologies for root system assessment in bananas and plantains (*Musa* spp.), in: *Banana root system: Towards a better understanding for its productive management, proceedings of an international symposium*, eds Turner DW, Rosales FE, editors. (Montpellier, France: INIBAP). 2005;107-113.
55. Thomas DS. The influence of the atmospheric environment and soil drought on the leaf gas exchange of banana (*Musa* spp). PhD thesis, the University of Western Australia; 1995.
56. Anastasia Matrosova. New insights into the regulation of stomatal movement by red light, carbon dioxide and circadian rhythms. PhD Thesis. Faculty of Forest Sciences, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, Umea. 2015;67.
57. Bhattacharya RK, Madhava Rao VN. Leaf production of robusta banana as influenced by soil covers and soil moisture regimes. *Banana Newsletter*. 1988;11:4-5.

58. Lu P, Woo KC, Liu ZT. Estimation of whole plant transpiration of bananas using sap flow measurements. *Journal of Experimental Botany*. 2002;53:1771-1779.
59. Lawson T, Blatt MR. Stomatal size, speed and responsiveness impact on photosynthesis and water use efficiency. *Plant Physiology*. 2014;164:1556-1570.
60. Haworth M, Elliot-Kingston C, McElwain JC. Stomatal control as a driver of plant evolution. *Journal of Experimental Botany*. 2011;62:2419-2423.

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