JOURNAL OF AGROBIOTECHNOLOGY 2019, VOL 10(2):73-85
e-ISSN: 2180-1983
https://journal.unisza.edu.my/agrobiotechnology/index.php/agrobiotechnology/index

Influence of Water Stress in Association with Aplication of Brassinolide and Minerals on Growth, Physiological and Biochemical Changes of Banana (*Musa acuminata* cv. Berangan)

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Received: 13/04/2018, Accepted: 03/11/2018, Available Online: 17/11/2019

ABSTRACT

Water stress or synonymy referring to the drought season is the major abiotic stress which affect growth, physiology and biochemical activity in plant and cause major losses to agriculture production sector. This study was aimed to determine the effects of exogenous application of brassinolide (BR) and combination of minerals on growth performance, physiological and biochemical changes of banana plantlets (Musa acuminata cv. Berangan) under water stress condition. The leaves of the whole plantlets were foliar sprayed for every two weeks interval with three treatments; (i) BR as control, (ii) magnesium carbonate (MgCO3) + calcium carbonate (CaCO3) and (iii) combination of BR + MgCO3 + CaCO3. The plants were also subjected to water stress treatments: 50%, 75% and 100% of the field capacity. The treatments were assigned as split-plot design in randomized complete block design (RCBD) arrangement. Water stress had significantly reduced major growth parameters (plant height, pseudo-stem diameter and total leaf area) but enhanced accumulation of proline and malondialdehyde content in leaves tissue. These findings also provided profound new insights and water stress by regulating the changes on stomata conductance and vapour pressure deficit under severe water stress condition.

Keywords: Water stress, banana, brassinolide, physiological indices, minerals

INTRODUCTION

Drought is an outcome of the climatic changes that may affect productivity of the plant, thus reducing yield in many region around the world (Riccardi et al., 2016). In Malaysia, adaptation to climate change is a great challenges to sustain agricultural productivity and attain food security (Alam et al., 2011). Tropical environments with unfavourable water stress condition may lead to low survival rate of banana plants since they are very sensitive to dry soil as well as various environmental changes. Banana plant requires uniformly warm and moist conditions for optimum growth and yield production (Razi et al., 2004). However, plant responds to water stress differently according to water stress level and growth stage of plant development. Plants may respond to abiotic and biotic stress. However, abiotic stress is critically induced alteration in morphological, physiological and biochemical changes of the plants.

The usage of anti-stress typed such as application of plant growth regulator (PGR) is one of the most effective strategies to minimize effect of water stress on banana. Brassinolide (BR) has been reported may help farmers to increase plant stress tolerance and their crop yield due to unfavourable environmental conditions (Sasse, 2003). Application of minerals such as magnesium carbonate ($MgCO_3$) and calcium carbonate ($CaCO_3$) could be another way to improve tolerance of plant to water stress. $MgCO_3$ has been reported suitable to be used as anti-transpirant at concentration of 5% applied on the leaves and it may reduce transpiration of plant and thus diminish the waste of water input throughout the growing season of banana plants (El-Kader et al., 2006). While, the application of $CaCO_3$ on top and bottom of the leaves has been found are able to increase total chlorophyll content under stress conditions compared to control of wheat plants (Maswada and El-Rahman, 2014). Hence, the main objective of this experiment is to evaluate the effects of exogenous application of BR and combination of minerals on growth performance, physiology changes and biochemical analysis of banana plantlets (*Musa acuminata* cv. Berangan) under water stress condition.

MATERIAL AND METHODS

Experimental site, plant materials and design

The experiment was conducted under a rain shelter house located in Field 15 at Faculty of Agriculture, Universiti Putra Malaysia (UPM), Serdang, Selangor. The planting material used in this experiment was *Musa acuminata* cv. Berangan plantlets obtained from NNS Permata Holdings Sdn. Bhd. located at Kuala Pilah, Negeri Sembilan. Soil sampling was collected before cultivation to analyse on physicochemical characteristics and the result was presented in Table 1. A uniform and healthy of one week old banana tissue culture seedling was grown in each polybag with the size of 15 cm × 15 cm. The experiment was laid out as split-plot in randomized complete block design (RCBD) with three replicates and three subsamples per replication. Water stress and PGR were assigned as main-and sub-plot treatments, respectively.

Treatments and plant maintenance

The leaves of the whole plantlets were foliar sprayed with three treatments: (i) BR as control, (ii) $MgCO_3$ + $CaCO_3$ (1:1, v/v) and (iii) BR + $MgCO_3$ + $CaCO_3$ (1:1:1, v/v). The solutions for BR, $MgCO_3$ and $CaCO_3$ were prepared by dissolving 6.88 g, 0.23 g and 3.96 g into 1 Litre (L) of distilled water, respectively. Every treatment (50 ml) was applied at two weeks interval on apex and base of leaf surface area of established Berangan banana. Banana plant was established for two weeks prior three water stress levels based on field capacity (FC)(50%, 75% and 100% FC) were subjected to the plant throughout the experiment period. Plant irrigated with 100% FC was served as a control. The banana plants were fertilized with NPK fertilizer (15:15:15) about 5 g per polybag at 5 weeks after transplanting.

Table 1. Selected physicochemical characteristics of studied soil (Bungor series).

Soil properties	Values
Soil texture	Fine sandy loam
Sand (%)	44.00
Silt (%)	9.91
Clay (%)	46.08
Bulk density(gcm^{-3})	1.10
Porosity (%)	58.65
Field capacity (FC) %	20.00
Soil pH	5.35
Electrical conductivity ($mScm^{-1}$)	0.17
Total N (%)	0.17
Total C (%)	2.13
Cation exchange capacity($cmol_c\ kg^{-1}$)	9.20

Determination of plant growth traits

Plant height of the plantlets were taken from the soil surface level until the first internode located at the top of plant shoots and the data measured at weekly basis using a measuring tape (expressed in centimetre). Meanwhile, pseudo-stem diameter of banana was also measured at weekly using vernier calliper throughout the experimental period. Total leaf number and total leaf area were counted or measured by using a ruler manually for each plant at the end of the experiment. The leaf area was calculated using the formula:

$$LA = [\text{leaf length} \times \text{maximum width} \times 0.755]$$

Where, 0.755 was a fix correction factor by following Robinson and Nel (1985)

After two months, the plants were destructively sampled for further analysis. Harvested plant were washed with water to remove any dirt and left for the air dried. Plants were separated into section (shoot and root) to measure it separately. All parts of plant placed in the oven-dried at 60±1°C for 72 hours until stable weight was achieved and then weighed by electronic weighing machine (Sartorious A and D FX200Iwp, Germany). Dry weight of each section was determined after oven-dried and recorded in the book. The root shoot ratio was calculated on dry weight basis. Root morphological including root length, root diameter, root surface area and root volume were measured using root scanner (Model: EPSON Flatbed Scanner 1680).

Measurement of leaf gas exchange

The rate of net photosynthesis, stomatal conductance, transpiration rate and vapour pressure deficit were determined using Li-COR (Model: L1-6400, Li-COR, USA). The measurement was made on the third fully expanded leaf at standard time (0800 – 1100 a.m) throughout the data collection by clipping the leaf on the chamber. The reading of photosynthesis, stomata conductance, transpiration rate and VPD were expressed as μmol CO_2 $m^{-2}s^{-1}$, mmol $m^{-2}s^{-1}$, mmol H_2O $m^{-2}s^{-1}$ and mol H_2O $m^{-2}s^{-1}$, respectively.

Determination of chlorophyll content

Total chlorophyll content (Chl_{a+b}) was determined following the method described by Coombs (1985). Four gnaw of banana leaf were done on each leaf by using cork borer. Samples were put into vial (readily covered with aluminium foil) which contained 20 mL of 80% (v/v) acetone. The samples were kept for 7 days in a dark place until all the chlorophyll was extracted from leaves. The chlorophyll a, chlorophyll b and total chlorophyll content were determined by using spectrophotometer (Model UV3101 PC, Shimadzu, Japan) at wavelength of 664 nm and 647 nm and all were expressed as mg cm^{-2} fresh weight (FW) of leaf. Chlorophyll a, chlorophyll b and total chlorophyll contents were calculated as follows:

$$\text{Chlorophyll a content} = 13.19\ (A_{664}) - 2.57\ (A_{647})$$
$$\text{Chlorophyll b content} = 22.1\ (A_{647}) - 5.26\ (A_{664})$$
$$\text{Total chlorophyll content} = 3.5\ (\text{chl a} + \text{chl b})/4$$

Where, A_{647} and A_{664} represent absorbance of the solution at 647 and 664 nm, respectively, while 13.19, 2.57, 22.1 and 5.26 are the absorption coefficients, 3.5 was total volume used in the analysis taken from the original solution (ml) and 4 was the total discs area (cm^2).

Determination of relative water content

Relative water content (RWC) of leaves was estimated according to the method of Fallon et al. (2015) and expressed in percentage (%). Sample of the fresh leaf (0.5 g) were saturated in 100 mL distilled water for 24 hours at 4℃ in the dark and their turgid weights (TW) were recorded. Then, oven-dried at 65℃ for 48 hours and their dry weights (DW) were recorded. RWC was calculated as follows:

$$RWC\ (\%) = [(FW - DW)/ (TW - DW)] \times 100$$

Where, FW, DW and TW were defined as fresh weight, dry weight, and turgid weight, respectively.

Determination of electrolyte leakage

The electrolyte leakage (EL) of membrane was assessed as their membrane permeability according to Lutts et al. (1996). Leaf discs (1 cm in diameter), were taken from the well-developed leaves and washed with deionized water then placed in individuals vials containing 10 mL of deionized water. These samples were incubated at room temperature (25℃) on a shaker with the running speed of 100 rpm for 24 hours. Electrical conductivity (EC) of bathing solution (EC1) was read after incubation. The same samples were placed in an autoclave at 120℃ for 20 min, and the second reading (EC2) was determined after cooling to room temperature. The EL was expressed following the formula:

$$EL = (EC1/EC2) \times 100$$

Determination of proline and malondialdehyde content

Determination of proline content was carried out as described by Brake et al. (2017). The plant material (0.5 g) was homogenized with 10 ml 3% sulfosalicylic acid. The homogenate was filtered. Acetic acid (2 mL) and acidic ninhydrin reagent (2 mL) were added to a 2 mL aliquot. The mixture was thoroughly stirred and incubated in a boiling water bath for 1 hour. Then it was transferred to ice bath and warmed to room temperature. Toluene (4 mL) was added to the mixture and the extinction of upper toluene level was measured at 518 nm by using a spectrophotometer (Shimadzu UV-160A Visible Recording Spectrophotometer, Japan).

Determination of malonyldialdehyde (MDA) content, a product of lipid peroxidation was conducted by following the method described by Habiba et al. (2015). One gram of fresh banana leaf sample was macerated in 3 ml of 0.1% thrichloroacetic acid (TCA) and then, homogenate was centrifuged at 10,000×g for 20 minutes. A 0.5 mL, aliquot of the supernatant was mixed with 1.5 mL solution of 20% TCA containing 0.5% thiobarbituric acid (TBA). The mixture was heated at 95°C for 30 minutes and cooled quickly on ice bath and made it warm at room temperature. The extinction was measured at 532 nm and 600 nm by using a spectrophotometer (Shimadzu UV-160A Visible Recording Spectrophotometer, Japan). Lastly, the MDA content was calculated and expressed as μmol MDA per gram FW.

Statistical analysis

All data collected were analysed using analysis of variance (ANOVA) by Statistical Analysis System (SAS 9.4) to determine the significance difference between the treatment means. Differences between means separated was made using least significant difference (LSD) at $P<0.05$ level. Correlation analysis was performed to establish relationship between VPD vs. stomata conductance and RWC vs. proline content.

RESULTS AND DISCUSSION

Plant growth traits

Water stress is one of the limiting factor at the initial phase of plant growth and establishment where it will be greatly suppressed the cell expansion and cell growth for many plants. There was no significance interaction ($P<0.05$) between water stress and PGR treatments on the various growth traits of banana plantlets (Table 2). However, plant treated under water stress significantly reduced plant height, pseudo-stem diameter, total leaf number and total lead area. Banana plant exposed with 50% FC was significantly reduced 31.6%, 28.76%, 16.18% and 60.44% of plant height, pseudo-stem diameter, total leaf number and total leaf area as compared to control treatment (100% FC), respectively. Exogenous application of combination treatment BR + $CaCO_3$ + $MgCO_3$ under water stress were significantly increased in plant height (23.2%), pseudo-stem diameter (21.7%) and total leaf number (19%) as compared to control (BR alone) but total leaf area did not differ in water-stressed and control plants.

Such has been reported earlier, BR are effective in reducing abiotic stresses including salinity, moisture, drought, low and high temperature (Tester and Davenport, 2003; Ozdemir et al., 2004; Hasan et al., 2008). Application of BR with minerals was found to alleviate the harmful effect of water stress as reflected from the result of the study by increasing of plant height, pseudo-stem diameter and total leaf area. Growth reduction such as in plant height

under water stress condition would be attributed to the reduce turgor pressure and cell enlargement in the plant system, whereas osmotic regulation is able to help the maintenance of cell turgor for survival under severe drought conditions such has been reported in pearl millet (Shao et al., 2008).

However, plants which received sufficient amount of water shows better effect on plant growth including plant height, pseudo-stem diameter and leaves width as a result of good cell growth formation, thus smooth the morphology development process (Khan et al., 2008). As the water stress increased up to 50% FC, the total leaf area and plant height per plant were also decreased. The loss of water under water stress conditions also has been reported to reduce leaf growth of Populus plant, however the suction efficiency increases in leaves sufficient to maintain the rate of water uptake to the rate of water loss (Wullschleger et al., 2005).

Table 2. Effect of water stress and plant growth regulator (PGR) treatments on plant height, pseudo-stem diameter, total leaf number and total leaf area.

Treatment Factors	Plant Height (cm)	Pseudo-stem Diameter (cm)	Total Leaf Number	Total Leaf Area (cm^2)
Main Plot Means (FC)				
50%	10.52[b]	1.61[b]	6.69[c]	457.20[b]
75%	13.91[a]	2.04[a]	7.56[b]	917.80[a]
100%	15.38[a]	2.26[a]	8.22[a]	1155.80[a]
LSD (P<0.05)	1.49**	0.27 **	0.43**	323.92**
Sub Plot Means (PGR)				
BR	11.52[c]	1.75[c]	7.00[b]	742.09[a]
$CaCO_3$ + $MgCO_3$	13.30[b]	1.94[b]	7.33[b]	821.32[a]
BR + $CaCO_3$ + $MgCO_3$	15.00[a]	2.22[a]	8.33[a]	967.29[a]
LSD (P<0.05)	0.72***	0.12***	0.54***	NS
Significance Interaction	NS	NS	NS	NS

Means followed by the same letter within a column are not significantly difference at (P>0.05) by least significant difference (LSD) with n = 27. ** and *** significantly difference at P<0.01 and 0.001, respectively. NS = not significant and FC = field capacity.

Variance analysis results of studied traits in Table 3 showed that no significance interaction (P<0.05) between different water stress levels and PGR treatments. As water stress increased, severe stress treatment (50% FC) significantly reduced 56.96%, 59.12% and 67.74% of root length, root surface area and root volume as compared to control plants (100% FC), respectively. No significant different was found between root diameter of water-stressed and control plants. Soha et al. (2011) reported that soil drying due to increase in water stress retarded growth of stem and root elongation of *Jatropha curcas* L. including root length, root diameter, root surface area as well as root volume. Reduction in plant growth under water stress probably due to inhibition of cell enlargement and cell division besides decrease in the activity of meristematic tissues which responsible for elongation (Siddique et al.,1999).

However, application of PGR treatments on top and bottom of the leaves under water stress conditions did not show any significant effect on root length, root diameter, root surface area and root volume of banana plantlets. In fact, excess water levels will cause root to rot and oxygen cannot penetrate the media. Meanwhile, insufficient water supply cause nutrients needed by the plants cannot be taken up by the root due to unhealthy roots condition which may cause the plant cannot grow well. Therefore, adequate level of water supply according to plants needed is very important to ensure the plants can growth healthily.

Table 3. Effect of water stress and growth regulator treatments on root length, root diameter, root surface area and root volume.

Treatment Factors	Root Length (cm)	Root Diameter (mm)	Root Surface Area (cm^2)	Root Volume (cm^3)
Main Plot Means (FC)				
50%	199.13[b]	2.16[a]	140.35[b]	6.88[b]
75%	343.11[ab]	2.19[a]	238.58[ab]	13.40[ab]
100%	462.68[a]	2.42[a]	343.35[a]	21.33[a]
LSD (P<0.05)	174.48*	NS	128.53*	9.15*
Sub Plot Means (PGR)				
BR	293.19[a]	2.20[a]	199.50[a]	11.03[b]
$CaCO_3$ + $MgCO_3$	341.88[a]	2.19[a]	245.79[a]	13.19[b]
BR + $CaCO_3$ + $MgCO_3$	369.85[a]	2.38[a]	277.00[a]	17.39[a]
LSD (P<0.05)	NS	NS	NS	3.95*
Significance Interaction	NS	NS	NS	NS

Means followed by the same letter within a column are not significantly difference at (P>0.05) by least significant difference (LSD) with n = 27.* significantly difference at P<0.05. NS = not significant and FC = field capacity.

According to Table 4, results showed that there were no significance interactions between different levels of water stress and PGR on shoot fresh weight, shoot dry weight, root fresh weight, root dry weight and root to shoot ratio between these factors. Water stress significantly reduced shoot dry weight and root dry weight of banana plantlets. The maximum and minimum shoot fresh weight and shoot dry weight were recorded when the plant subjected to 100% FC and 50% FC, respectively. At 75% FC, shoot fresh weight and shoot dry weight reduced 47.26% and 42.09% as compared to control (BR). A similar result was reported by Mohammadian et al. (2005) who found that sugar beet plant grown under mild water stress condition affect the shoot dry weight, even though the loss on shoot dry weight was greater than root dry weight under severe-stressed. The root fresh weight and root dry weight of banana plant subjected to 50% FC decreased 79.30% and 69.49%, respectively, as compared to 100% FC (control). Moreover, the results showed that there were no significant in root to shoot ratio between water stress treatments. Petropoulos et al. (2008) reported that reduced root dry matter biomass was also seen in water stressed *Petroselinum crispum*. The exogenous application of combination treatment BR + $CaCO_3$ + $MgCO_3$ had significantly higher 18.56% and 32.58% of shoot fresh weight and shoot dry weight, respectively, than control (BR). Reduction of total chlorophyll content under drought stress may influence plant photosynthetic efficiency of *Ocimum basilicum* plant and consequently fresh weight and dry weight decreased (Khalil et al., 2010). Similar results were reported by Ismail et al. (2002) that total leaf area and leaves numbers decreased and may influence total dry matter weight of pepper plant as influenced by water stress. However, exogenous application of $CaCO_3$ + $MgCO_3$ significantly increased 0.61% and 4.65% of root fresh weight and root dry weight, respectively, compared to control (BR), whereas root shoot ratio did not differ between PGR treatments.

Table 4. Effect of water stress and plant growth regulator (PGR) treatments on shoot fresh weight, shoot dry weight, root fresh weight, root dry weight and root to shoot ratio.

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Treatment Factors	Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Root Fresh Weight (g)	Root Dry Weight (g)	Root to Shoot ratio
Main Plot Means (FC)					
50%	33.11^{c}	2.89^{c}	11.41^{c}	0.72^{b}	0.27^{a}
75%	67.33^{b}	4.80^{b}	21.78^{b}	1.37^{ab}	0.28^{a}
100%	127.67^{a}	8.29^{a}	55.11^{a}	2.36^{a}	0.29^{a}
LSD (P<0.05)	21.16***	1.86**	9.52***	1.01*	NS
Sub Plot Means (PGR)					
BR	27.26^{b}	1.32^{b}	74.22^{b}	4.95^{b}	0.26^{a}
$CaCO_3$ + $MgCO_3$	28.72^{b}	1.39^{b}	74.67^{b}	5.18^{ab}	0.27^{a}
BR + $CaCO_3$ + $MgCO_3$	32.32^{a}	1.75^{a}	79.22^{a}	5.87^{a}	0.30^{a}
LSD (P<0.05)	2.15*	0.26*	4.07***	0.77**	NS
Significance Interaction	NS	NS	NS	NS	NS

Means followed by the same letter within a column are not significantly difference at (P>0.05) by least significant difference (LSD) with n=27. *, ** and *** significantly difference at P<0.05, 0.01 and 0.001, respectively. NS= not significant and FC=field capacity.

Leaf gas exchange

There was no significance interaction (P>0.05) between different water stress levels and PGR treatments on leaf gas exchange (Table 5). However, photosynthesis can slow down or even stop due to water stress conditions. Dorji et al. (2005) noted that sufficient supply amount of water may promote better plant growth as well as physiological process especially photosynthesis whereby the exchanging of gas in the leaves to allow the transport of water and sugar to create its own food. The results indicated that 100% FC (control) have the highest net photosynthesis with mean value of 25.62 μmol CO_2 $m^{-2}s^{-1}$ as compared to 50% FC with mean value of 23.99 μmol CO_2 $m^{-2}s^{-1}$. Reduction in leaf area especially plant grown under drought condition causes reduction in crop photosynthesis in grapevine plants (Escalona et al., 2003). That is why development of optimal leaf area is very important to photosynthesis rate and dry matter yield production. Furthermore, the plants treated under well-watered (100% FC) also significantly decreased transpiration rate by 13.01% compared to water-stressed plant (50% FC), whereas stomata conductance and VPD did not differ in water-stressed and control plants. Differences among PGR also found that exogenous application of combination treatment BR + $CaCO_3$ + $MgCO_3$ significantly increased the photosynthesis rate (6.96 %) and VPD (18.18%), while the results also revealed the role of PGR significantly decreased rate of stomata conductance and transpiration rate by 28.13% and 9.45%, respectively, as compared to control (BR). Application of minerals such as $CaCO_3$ and $MgCO_3$ as anti-transpirant could be one of the factors caused the reduction of transpiration rate activity besides these minerals also promoted the closure of stomata, so may result to decrease in the rate of stomata conductance. Banana is very sensitive to dry soil whereby root pressure caused the stomata started to close and leaves remain highly hydrated (Turner and Rosales, 2005). Similar result was reported by Oliver et al. (2016) who had mentioned that when water potential of the soil is more negative than in the leaves of tropical rainforest trees in response to experimental drought, the leaf cells then lost turgor pressure and stomata closure occurs in purposely.

Table 5. Effect of water stress and plant growth regulator (PGR) treatments on photosynthesis, stomata conductance, transpiration rate and vapour pressure deficit (VPD).

Treatment Factors	Photosynthesis ($\mu mol\ CO_2\ m^{-2}s^{-1}$)	Stomata Conductance ($mmol\ m^{-2}s^{-1}$)	Transpiration rate ($mmol\ H_2O\ m^{-2}s^{-1}$)	VPD ($mol\ H_2O\ m^{-2}s^{-1}$)
Main Plot Means (FC)				
50%	23.99[b]	0.48[a]	4.17[a]	0.91[a]
75%	25.54[a]	0.61[a]	3.72[b]	0.77[a]
100%	25.62[a]	0.58[a]	3.69[b]	0.80[a]
LSD (P<0.05)	0.97*	NS	0.20**	NS
Sub Plot Means (PGR)				
BR	24.13[b]	0.64[a]	4.02[a]	0.77[b]
$CaCO_3 + MgCO_3$	25.21[a]	0.57[ab]	3.89[a]	0.81[ab]
$BR + CaCO_3 + MgCO_3$	25.81[a]	0.46[b]	3.67[b]	0.91[a]
LSD (P<0.05)	0.73**	0.11*	0.16**	0.12*
Significance Interaction	NS	NS	NS	NS

Means followed by the same letter within a column are not significantly difference at (P>0.05) by least significant difference (LSD) with n = 27. *, ** significantly difference at P<0.05, 0.01 respectively. NS = not significant and FC = field capacity.

Physiological attribute and biochemical content

No significance interaction was recorded between different levels of water stress and PGR on physiological attribute and biochemical content (Table 6). As water stress increased, banana plant subjected to 50% FC had significantly 23.11%, 24.84%, 23.49% and 44.49% lower chlorophyll a, chlorophyll b, Chl_{a+b} and RWC, respectively, but 28.86% higher EL than control plants (100% FC). Meanwhile, plant treated with combination BR + $CaCO_3$ + $MgCO_3$ treatment showed significantly 7.78% higher RWC as compared to combination $CaCO_3$ + $MgCO_3$ treatment, whereas Chl a, Chl b and Chl_{a+b} did not differ among the sub-plot treatments. The result was corresponding with what has been found by Asharaf et al. (1990) who stated that Chl_{a+b} of the leaf for *Brassica* sp. declined under water stress conditions. Manivannan et al. (2007) stated that drought condition may reduce the Chl a, Chl b, and Chl_{a+b} in all sunflower plants due to water deficit. Similar result was reported by Alachew et al. (2016) that water stress adversely affected growth of *Pisum sativum* L. cultivars and also may reduce the pigment concentration such as Chl a, Chl b and Chl_{a+b} in the leaves as stress level was increased. So, the results revealed that Chl_{a+b} and RWC decreased, but increased in EL with increased in water stress conditions. Accumulation of large amount of proline and MDA content significantly increased when the plant under water stress condition, however plant subjected to 50% FC gained the highest mean value of 36.86 $\mu mol\ g^{-1}$ and 0.41 $\mu mol\ g^{-1}$, respectively as compared to others treatment. Among the sub-plot treatments, exogenous application of combination $CaCO_3$ + $MgCO_3$ treatment significantly reduced proline content by 1.12% and MDA content by 30.77% compared to control (BR). In response to water stress, the plant will break down carbohydrate into proline which is needed for their stress defend mechanism.

Table 6. Effect of water stress and plant growth regulator (PGR) treatments on chlorophyll a (Chl a), total chlorophyll b (Chl b), total chlorophyll content (Chl $_{a+b}$), relative water content (RWC), electrolyte leakage (EL), proline and malonyldialdehyde (MDA) content.

Treatment Factors	Chl a (mg cm^{-2})	Chl b (mg cm^{-2})	Chl $_{a+b}$ (mg cm^{-2})	RWC (%)	EL (%)	Proline (µmol g^{-1} FW)	MDA (µmol g^{-1} FW)
Main Plot Means (FC)							
50%	4.16^{b}	1.21^{c}	4.69^{b}	48.71^{c}	67.62^{a}	36.86^{a}	0.41^{a}
75%	4.85^{ab}	1.43^{b}	5.49^{a}	69.27^{b}	54.27^{b}	35.90^{b}	0.32^{ab}
100%	5.41^{a}	1.61^{a}	6.13^{a}	87.75^{a}	38.76^{c}	33.27^{c}	0.28^{b}
LSD (P<0.05)	0.73*	0.18**	0.78*	6.60***	7.72**	0.86***	0.11*
Sub Plot Means (PGR)							
BR	4.76^{a}	1.41^{a}	5.39^{a}	65.20^{b}	53.93^{a}	35.27^{a}	0.39^{a}
$CaCO_3$ + $MgCO_3$	4.91^{a}	1.45^{a}	5.56^{a}	67.63^{b}	53.58^{a}	35.69^{a}	0.27^{c}
BR + $CaCO_3$ + $MgCO_3$	4.75^{a}	1.39^{a}	5.36^{a}	72.89^{a}	53.13^{a}	35.06^{a}	0.34^{b}
LSD (P<0.05)	NS	NS	NS	3.36**	NS	NS	0.04***
Significance Interaction	NS	NS	NS	NS	NS	NS	NS

Means followed by the same letter within a column are not significantly difference at (P>0.05) by least significant difference (LSD) with n = 27. *, ** and *** significantly difference at P<0.05, 0.01 and 0.001, respectively. NS = not significant and FC=field capacity.

Relationship between vapour pressure deficit and stomata conductance

There was a significant negative relationship between VPD and stomata conductance under water stress condition (Figure 1). These results suggested that an increase in VPD could simultaneously decrease stomata conductance which indicating that the VPD driven transfer of water vapour from the leaf was becoming limited by stomata closure. Similar results were obtained by Grassi and Magnani (2005) who concluded that increasing VPD and decreasing stomata conductance on the same leaf of oak tree was happened as influenced by drought condition. The $MgCO_3$ te is potentially to be used as anti-transpiration that able to help to close the stomata and thus, affect metabolic process in the leaf tissues as well as water use efficiency in the plant (Nermeen and Emad, 2011).

Relationship between relative water content and proline content

The internal water status of banana plant was associated with the level of proline accumulation in banana leaf tissue. A significant negative relationship was recorded between RWC and proline content under different water conditions (Figure 2). The level of proline started to increase from 32.57 µmolg^{-1} FW to a value of 37.86 µmolg^{-1} FW when the RWC of banana leaf tissue started to decrease from 95% to 38% under different water stress treatment. Similar results had been reported by Kameli and Losel (1996) that decrease in RWC in wheat plant after onset of water stress treatment. Amount of proline content in wheat plants was also found increases by Vendruscolo et al. (2007) after water stress application.

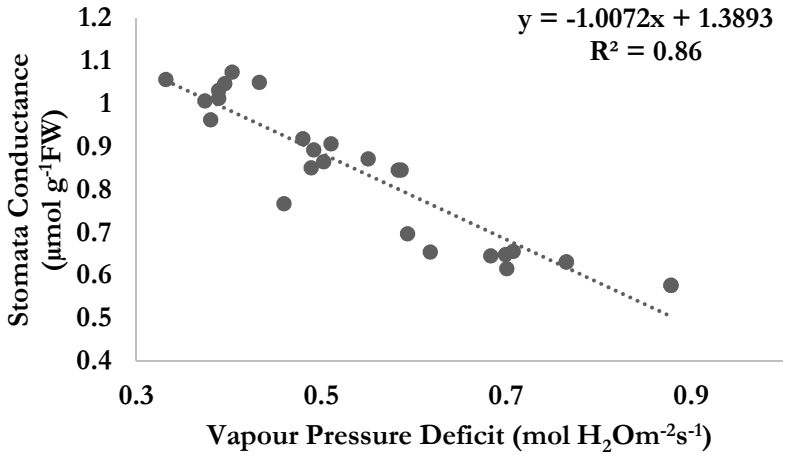

Figure 1. The correlation analysis between vapour pressure deficit (VPD) and stomata conductance as influenced by water stress and plant growth regulator (PGR) treatments.

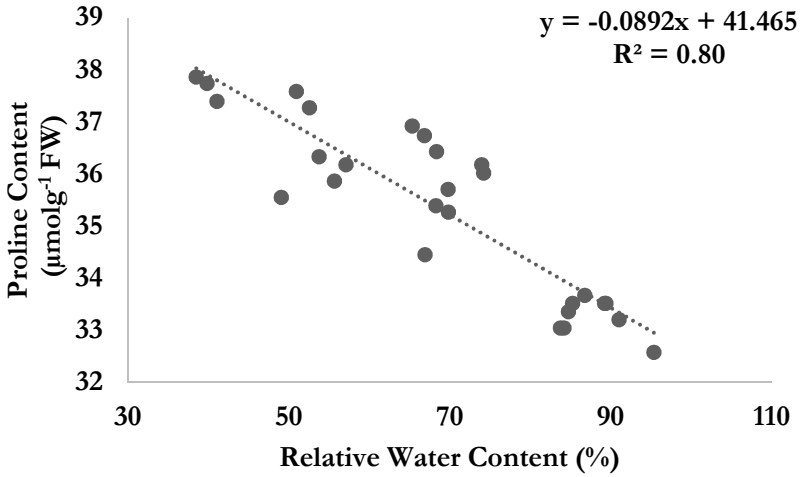

Figure 2. The correlation analysis between relative water content (RWC) and proline content as influenced by water stress and plant growth regulator (PGR) treatments.

CONCLUSION

Water stress negatively affected the growth and physiological parameters of *Musa acuminata* cv. Berangan. It was noted that, plant height, pseudo-stem diameter, total leaf area, root length, root surface area, root volume, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, Chl_{a+b}, photosynthesis rate and stomata conductance were reduced as the water stress increased up to 50% FC with association of BR and minerals combination. Meanwhile, the increment of water stress level enhanced the concentration of proline, and the activities of lipid peroxidation as well as EL in the leaves tissue of banana. However, root diameter and root to shoot ratio, transpiration rate and VPD were not affected by these treatments. The application of exogenous application of combination $CaCO_3$ + $MgCO_3$ and BR + $CaCO_3$ + $MgCO_3$ treatment on banana leaves increased VPD but reduced stomata conductance. Water stress and PGR treatments significantly influenced the plant growth development at vegetative stage of banana plantlets. Application of combination BR + $CaCO_3$ + $MgCO_3$ treatment under increased water stress conditions of banana plantlets was found to reduce deleterious effect of water stress and the application of BR together with minerals mixture able to tolerate water stress by increased in major growth characteristics and physiological traits. However, more study need to be undertaken in the future to reveals the mechanism behind this process in order to strengthen the fact of beneficial effect of supplementing BR with minerals combination.

ACKNOWLEDGEMENTS

The authors wish to thank the Agrow Synergy (M) Sdn. Bhd., Greenearth Intl Holdings Sdn. Bhd. and the staff of Crop Science Department for tremendous technical support. This project was funded by the Faculty of Agriculture, Universiti Putra Malaysia. We declare that we have no conflict of interest.

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How to cite this paper:
Zakaria, M.A.T., Sakimin, S.Z., Ramlan, M.F., Jaafar, H.Z., Baghdadi, A., & Mohd Din, S.N. (2019). Influence of water stress in association with aplication of brassinolide and minerals on growth, physiological and biochemical changes of banana (*Musa acuminata* cv. Berangan). Journal of Agrobiotechnology, *10*(2), 73-85