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EFFECTS OF BRASSINOLIDE AND ANTI-TRANSPIRANTS UNDER WATER STRESS CONDITIONS ON PLANT GROWTH AND PHYSICO-CHEMICAL CHANGES OF Musa acuminata COLLA cv. BERANGAN

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By

MD AIMAN TAKRIM BIN ZAKARIA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Chair : Assoc. Prof. Siti Zaharah Sakimin, PhD
Faculty : Agriculture

Banana *(Musa acuminata)* cv. Berangan relatively sensitive to dry soil condition which may influence good performance of growth, physiology and biochemical changes of banana. Global water supply crisis and uneven distribution of rainfall are some of limiting factors in agriculture sector for production of banana under Malaysia climate condition. The study was aimed to investigate the plant growth, physiology and biochemical changes on yield of banana as influenced by brassinolide (BR) and minerals solely or in combination under water stress conditions. BR represents one of the plant growth regulators essential in multiple developmental processes in plants including cell division, cell elongation and also reproductive development. One month old banana seedlings were transplanted into the 15 cm × 15 cm size of polybag and placed under rain shelter. Banana seedlings were foliar sprayed with different concentrations of BR (0, 3, 6 and 12 gL^{-1}) for every two weeks intervals. The results showed that, BR concentration gave significant effects on the growth and physiology of banana plant (*Musa* acuminata cv. Berangan). As the BR concentrations increased from 0 to 6 gL^{-1}, plant height, pseudo-stem diameter, total leaf numbers, total leaf area, fresh and dry weight of shoot were markedly increased from week 3 to week 8 after transplanting. The BR-induced increase in chlorophyll content which contributed the increase in photosynthesis rate. The root size and distributions were, however, not significantly affected by BR. However, exogenous application of BR at 6.88 gL^{-1} was the best concentration for banana plant at nursery stage as it was able to increase the plant height, pseudo-stem diameter, total leaf numbers and total leaf area of the species. Additionally, water stress or synonym 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. The impacts of water stress in combination with exogenous application of plant growth regulator and minerals were also studied under rain shelter and open field plot. Under rain shelter experiment, one week old banana seedlings were transplanted in the polybag and the plants were grown under optimized concentration of

BR (6.88 gL^{-1}) from previous experiment. The leaves of the whole banana seedlings were foliar sprayed with three treatments: (i) BR as control, (ii) $CaCO_3$ + $MgCO_3$ (1:1, v/v) and (iii) BR + $CaCO_3$ + $MgCO_3$ (1:1:1, v/v). The solutions for BR, $CaCO_3$ and $MgCO_3$ were prepared by dissolving 6.88 g, 3.96 g and 0.23 g into 1 Litre (L) of distilled water, respectively. The plants were also subjected to water stress treatments: 50%, 75% and 100% of the FC. Water stress at 50% FC 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. Concurrently, two months old banana seedlings were transplanted at open field plot in Field 15, Faculty of Agriculture, UPM. The similar treatments [(i) 6.88 gL^{-1} BR as control, (ii) $CaCO_3$ + $MgCO_3$ and (iii) BR + $CaCO_3$ + $MgCO_3$] were applied at every 2 weeks interval from 2 months after transplanting until flowering stage. Banana plants have been subjected to two different water regimes: (i) rain-fed and (ii) irrigated by micro-sprinkler system as control treatment for a continuous periods about 12 months. According to the results showed that, banana plants grown under rain-fed condition significantly reduced morphological characters such as plant height, pseudo-stem, canopy diameter, but enhanced accumulation of proline and malondialdehyde content in leaves tissue. Overall, the results proved that both conditions of water stress (50% FC) and rain-fed were significantly reduced major growth parameters (plant height, pseudo-stem diameter, total leaf area and canopy diameter), but enhanced accumulation of proline and malondialdehyde content in leaves tissue, respectively. These results suggest that application of BR + $CaCO_3$ + $MgCO_3$ on banana leaves increased vapour pressure deficit but reduced stomata conductance. Foliar application of BR with combination of $CaCO_3$ and $MgCO_3$ is recommended to regulate mechanism of drought adaptation to Berangan banana plant under field condition that facing global water supply criss and uneven distribution of rainfall, without negative effect on the harvested yield.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN BRASSINOLIDE DAN ANTI-TRANSPIRAN DALAM KEADAAN KETEGASAN AIR TERHADAP PERTUMBUHAN TANAMAN DAN PERUBAHAN FIZIKO-KIMIA *Musa acuminata* COLLA cv. BERANGAN

Oleh

MD AIMAN TAKRIM BIN ZAKARIA

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Pisang *(Musa acuminata*) kultivar Berangan agak sensitif terhadap keadaan tanah kering yang mungkin mempengaruhi prestasi pertumbuhan, fisiologi dan perubahan biokimia pisang. Krisis bekalan air global dan taburan hujan tidak sekata adalah beberapa faktor yang menyekat dalam sektor pertanian untuk pengeluaran pisang di bawah keadaan iklim di Malaysia. Kajian ini bertujuan untuk mengkaji pertumbuhan tanaman, fisiologi dan perubahan biokimia pada hasil pisang seperti yang dipengaruhi oleh brassinolide (BR) dan mineral semata-mata atau gabungan dalam keadaan ketegasan air. BR mewakili salah satu pengawal atur tumbuhan yang penting dalam pelbagai proses pembangunan dalam tumbuhan termasuk pembahagian sel, pemanjangan sel dan juga perkembangan pembiakan. Anak pokok berusia satu bulan ditanam ke dalam 15 cm x 15 cm saiz polibeg dan diletakkan di bawah rumah kalis hujan. Anak-anak pokok disembur dengan kepekatan BR yang berbeza (0, 3, 6 dan 12 gL^{-1}) untuk setiap selang dua minggu. Keputusan menunjukkan bahawa kepekatan BR memberi kesan yang signifikan terhadap pertumbuhan dan fisiologi pokok pisang (*Musa* sp.) kultivar Berangan. Peningkatan BR dari 0 hingga 6 gL^{-1} telah meningkatkan secara ketara terhadap ketinggian pokok, diameter pseudo-batang, jumlah jumlah daun, jumlah kawasan daun, berat pucuk segar dan berat pucuk kering dari minggu 3 hingga minggu 8 selepas pemindahan. Aplikasi BR meningkatkan kandungan klorofil yang menyumbang peningkatan kadar fotosintesis. Bagaimanapun, saiz dan pembahagian akar tidak memberi perbezaan yang ketara dengan rawatan BR. Walau bagaimanapun, aplikasi BR secara luaran dengan kadar 6.88 gL^{-1} adalah merupakan kepekatan yang terbaik untuk pokok pisang di peringkat nurseri kerana ia dapat meningkatkan ketinggian pokok, diameter pseudo-batang, jumlah daun dan jumlah keluasan daun. Selain itu, ketegasan air atau sinonim yang merujuk kepada musim kemarau adalah tekanan utama abiotik yang mempengaruhi pertumbuhan, fisiologi dan aktiviti biokimia dalam tanaman dan menyebabkan kerugian besar kepada sektor pengeluaran pertanian. Kesan ketegasan air dalam gabungan dengan pengawal atur tumbuhan dan mineral juga dikaji di bawah perlindungan hujan dan plot lapangan terbuka. Eksperimen di bawah rumah kalis hujan, anak pokok berusia satu

minggu dipindahkan ke dalam polibeg dan disembur dengan kepekatan BR yang optimum (6.88 gL^{-1}) daripada eksperimen sebelumnya. Keseluruhan daun anak pokok disembur dengan tiga rawatan berbeza untuk setiap selang dua minggu; (i) BR sebagai kawalan, (ii) $CaCO_3$ + $MgCO_3$ (1: 1, v/v) dan (iii) BR + $CaCO_3$ + $MgCO_3$ (1: 1: 1, v/v). Campuran untuk BR, $CaCO_3$ dan $MgCO_3$ disediakan dengan melarutkan 6.88 g, 3.96 g dan 0.23 g ke dalam 1 liter (L) air suling. Anak-anak pokok juga diberi rawatan ketegasan air: 50%, 75% dan 100% daripada kapasiti lapangan. Ketegasan air pada 50% kapasiti lapangan telah mengurangkan parameter pertumbuhan utama dengan ketara (ketinggian pokok, diameter pseudo-batang dan jumlah keluasan daun) tetapi pengumpulan kandungan proline dan malondialdehid meningkat dalam tisu daun. Pada masa yang sama, anak pokok pisang berusia dua bulan dipindahkan di plot lapangan terbuka di Ladang 15, Fakulti Pertanian, UPM. Rawatan yang sama [(i) 6.88 gL^{-1} BR sebagai kawalan, (ii) $CaCO_3$ + $MgCO_3$ dan (iii) BR + $CaCO_3$ + $MgCO_3$] digunakan pada setiap selang dua minggu bermula dua bulan selepas pemindahan sehingga tahap berbunga. Anak-anak pisang telah didedahkan kepada dua rejim air yang berbeza: (i) hanya bergantung kepada hujan dan (ii) pengairan oleh sistem pemercik mikro sebagai rawatan kawalan untuk tempoh berterusan kira-kira 12 bulan. Menurut hasil kajian menunjukkan, tanaman pisang yang ditanam di bawah keadaan bergantung hujan secara ketara mengurangkan ciri morfologi seperti ketinggian tumbuhan, pseudo-batang dan diameter kanopi, tetapi meningkatkan pengumpulan kandungan proline dan malondialdehid dalam tisu daun. Secara keseluruhannya, hasil kajian membuktikan bahawa kedua-dua keadaan ketegasan air (50% kapasiti lapangan) dan bergantung kepada hujan mengurangkan pertumbuhan utama secara ketara (ketinggian tumbuhan, diameter pseudo-batang, jumlah keluasan daun dan diameter kanopi), tetapi peningkatan pengumpulan proline dan kandungan malondialdehid dalam tisu daun, masing-masing. Hasil kajian menunjukkan bahawa penggunaan BR + $CaCO_3$ + $MgCO_3$ pada daun pisang meningkat defisit tekanan wap tetapi mengurangkan konduktiviti stomata. Aplikasi foliar BR dengan gabungan $CaCO_3$ dan $MgCO_3$ disarankan untuk mengatur mekanisme adaptasi kemarau kepada tanaman pisang Berangan di bawah keadaan lapangan yang meghadapi krisis bekalan air global dan taburan hujan yang tidak rata, tanpa memberi kesan negatif terhadap hasil tuaian.

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I certify that a Thesis Examination Committee has met on 13 August 2018 to conduct the final examination of Md Aiman Takrim bin Zakaria on his thesis entitled "Effects of Brassinolide and Anti-Transpirants under Water Stress Conditions on Plant Growth and Physico-Chemical Changes of *Musa acuminata* Colla cv. Berangan" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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%	Percentage
<	Less than
>	Greater than
*	Significantly difference at P<0.05
**	Significantly difference at P<0.01
***	Significantly difference at P<0.001
ANOVA	Analysis of Varience
BR	Brassinolide
C	Carbon
CEC	Cation Exchange Capacity
CO_2	Carbon Dioxide
cm	Centimetre
cv.	Cultivar
℃	Degree Celcius
Chl a	Chlorophyll a
Chl b	Chlorophyll b
Chl_{a+b}	Total Chlorophyll
$CaCO_3$	Calcium Carbonate
DW	Dry Weight
et al.	And Friends
EL	Electrolyte Leakage
FAOSTAT	Food and Agriculture Organization Statistical Database
FC	Field Capacity
FW	Fresh Weight
g	Gram
gL^{-1}	Gram Per Litre
gcm^{-1}	Gram Per Centimetre
ha	Hectare
H_2O	Water
IAA	Indole-3-Acetic Acid
kg	Kilogram
kPa	Kilo Pascal
L	Litre
LA	Leaf Area
LSD	Least Significant Difference
LAI	Leaf Area Index
m	Metre
MDA	Malonyldialdehyde
µg	Microgram
µmol	Micromole
mL	Millitre
mm	Millimetre
mmol	Millimole
$MgCO_3$	Magnesium carbonate
NPK	Nitrogen Phosphorus Potassium
n	Number of Respondence
ns	Not Significant
pH	Measurement of Acidity / Alkalinity
RWC	Relative Water Content

RCBD	Randomized Complete Block Design
s	second
SAS	Statistical Analysis System
TW	Turgid Weight
UPM	Universiti Putra Malaysia
USA	United States of America
v/v	Volume per Volume
VPD	Vapour Pressure Deficit
vs.	Versus
WAT	Week After Transplanting

CHAPTER 1

INTRODUCTION

Banana (*Musa acuminata*) belong to the family Musaceae has a lot of potential as a sustainable crop with multi-uses product. Global water supply crisis and uneven distribution of rainfall are some of limiting factors in agriculture sector for production of banana under Malaysia climate condition. The most important source of natural water for crop growth is through rainfall. When rainfall is insufficient, thus irrigation system need be installed to guarantee a good harvest. The changes and uneven distribution of rainfall in Malaysia fluctuated heavily from -30% to +30% coupled with the rise of temperature by 0.3℃ to 4.5℃ and rise in sea level is expected to be about 95 cm over a hundred years (Alam et al., 2011). However, this crop is very sensitive to the climate change and may reduce banana growth performance (Ranjitkar et. al., 2016). Malaysia is one of the centers of origin for many banana varieties in the world (Siti Hawa, 1992) and cv. Berangan is the most popular one. Banana has been planted either in large or small scale for commercial in Malaysia. There were 27, 296 ha planted areas of banana in Malaysia with total production of 343, 061 metric tonnes (DOA, 2015). The largest world producers are Latin America and Carribean. Latin America and Carribean exports banana about 13.4 million tonnes in year 2013. Whereas in Asia, India is the largest producer and the Philipines is the largest exporter. In 2013, Asia region exports banana about 2.9 million tonnes (FOASTAT, 2015).

According to Lascano et al. (2007), it is expected that human population in the world will be increased 8 to 9 billion by the year 2025, meanwhile the demand or consumption of the food will be higher than present. Global issues concerning now on the climate change and food security due to human population growth and stagnating productivity of food. Other than our concent on the food productivity, there is evidence that more than 40% of the people in our planet are affected by water scarcity. By the year 2025, about 1.8 billion people will be living in the countries with absolutely lack of water resources (Lascano et al., 2007). There is also an evidence that two-third of the world's population suffered from water stress (Forouzani et al., 2012). Impact of the water stress is closely related to the fenomena of drought which is an outcome of the climatic changes that directly affected 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). Forouzani et al. (2012) reported that management of water resources in a sustainable way is very important for growing population as well as the need more of food production drives the agricultural sector to manage availabl usage properly.

Banana plant requires uniformly warm and moist conditions for optimum growth and yield production (Razi et al., 2004). 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. Water is one the most important element for plant to grow well especially for transporting important nutrients through the plant. Zingaretti et al. (2011) reported that during vegetative stage growth, water is essentially required by plant to obtain maximum yield, but inadequate water uptake in this stage may reduce crop productivity.

Applications of plant hormone as well as foliar spray of mineral as anti-transpirant are possible strategies for improving adaptability of plant with climate change. Plant hormone widely used in modern agriculture at low dosages and typically applied via foliar sprays with water as a carrier that affect developmental or metabolic processes in higher plants such as banana (Rademacher, 2015). Brassinolide (BR) is one of the phytohormones essential for plant growth and development as well as important for cell division and expansion which may increase crop yield and able to alleviate various biotic and abiotic stress (Sasse, 2003; Jager et al., 2008). According to Ingram and Bartels (1996), stated that either by endogenous molecular systems or exogenous applied compounds able to protect the plant cell from severely damage and to mitigate the plant from environmental stress. Exogenous application of BR also has been reported influences growth and development processes as well as mitigates the abiotic stresses of tomato plants (Montoya et al., 2005).

Through understanding of physiological responses of plant to water stress and mechanism water use efficiency, foliar application with minerals such as magnesium carbonate ($MgCO_3$) and calcium carbonate ($CaCO_3$) as anti-transpirant on upper and lower part of the leaves able to enhance photosynthetic efficiency. Carmen et al. (2014a), noted that the application of lithovit as foliar fertilizer consist of calcium carbonate on the leaf surface of tomato plant resulted in highest photosynthesis intensity. In the modern agriculture, improving the plant growth performance and crop productivity by using anti-transpirant materials potentially reduce transpiration rate and increasing turgidity of leaves as well as stomata guard cell for increasing water stress use efficiency and water stress resistance (Davenport et al., 1972).

Therefore, the study was aimed to investigate the plant growth performance, physiology, biochemical changes and yield of Berangan banana as influenced by BR and minerals under water stress conditions. The general objectives of the study were:

(1) To identify banana seedlings response toward usage of plant hormone and mineral of anti-transpirants with different concentrations on morphological and physiological changes.
(2) To observe morphological and physiological response biochemical changes of banana seedlings as influenced by plant hormone and anti-transpirants under water stress conditions.
(3) To analyse biochemical changes as affected by plant hormone, anti-transpirants and water stress conditions on yield production of banana.

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