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ALLEVIATION OF WATER STRESS IN ROSELLE (Hibiscus sabdariffa L.) USING ZEOLITE

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

To all my well wishe

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

ALLIVIATION OF WATER STRESS IN ROSELLE (*Hibiscus sabdariffa* L.) USING ZEOLITE

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MEHAR UN NISA

August 2018

Chairman : Puteri Edaroyati Megat Wahab, PhD
Faculty : Agriculture

Water stress is one of the major limiting factors for plant growth and yield. Water stress affects plants by imposing numerous morphological, physiological and molecular changes. There are various approaches for mitigation of plant under water stress. One of the approaches is through the zeolite application by improving growth, physiological and biochemical attributes. The response of roselle plant variety UMKL-1 subjected to different soil moisture content (100%, 67% and 33% FC) was investigated under rain shelter. The objective of this study was to determine the effects of different soil moisture content on growth performance and physiological changes, calyx yield and quality in *H. sabdariffa*. Severe water stress (33% FC) significantly decreased dry matter production, harvest index and photosynthetic rate due to limitation in the efficiency of assimilate translocation. LWP, relative growth rate and nutrient content (N, P, and K) in *H. sabdariffa* was also significantly decreased at 33 % FC. Root to shoot ratio, accumulation of proline were increased when plants at 33 % FC. The changes in the number of branches and chlorophyll content were not significant. Moreover, *H. sabdariffa* calyx yield was not significantly different at 100 % and 67 % FC. On the other hand, higher content of total soluble solids, ascorbic acid and anthocyanin were observed at 33% FC. In the 2nd experiment, zeolite used to minimize the adverse effects of water stress on growth, and calyx yield under rain shelter condition. Zeolite application improved growth and yield due to improved dry matter production, nutrient content (N, P, and K) stomatal conductance and photosynthetic rate. Quantitative gene expression studies indicate that the expression pattern of *P5CS* gene was up-regulated and *PDH* gene was down-regulated at 33% FC as compared to 67% FC. When zeolite was applied under water stress conditions, the expression pattern of *P5CS* gene was down-regulated and *PDH* gene was up-regulated. These results suggested that severe water stress (33% FC) had negative effects on *H. sabdariffa* yield by reducing growth, photosynthetic rate and nutrient uptake. Zeolite application can be useful to

save water that leads more yield under water stress conditions. 67 % FC with 2.50 g zeolite kg^{-1} soil was found to be more effective in improving growth and calyx yield. This study also established and validated *Actin*-7 and *alpha tubulin*-4 as the reference genes in *H. sabdariffa* under water stress conditions and provides a powerful tool for the quantitative expression analysis of *P5CS* and *PDH* in *H. sabdariffa*. Furthermore, *P5CS* could participate in the drought resistance process by regulating proline content in *H. sabdariffa* leaves.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGURANGAN TEKANAN KEMARAU PADA ROSELLE (*Hibiscus sabdariffa* L.) MENGGUNAKAN ZEOLITE

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Tekanan kemarau adalah salah satu tekanan abiotik tumbuhan utama yang menghadkan pertumbuhan dan hasil tanaman. Tekanan kemarau memberi kesan kepada tumbuhan dengan memberi banyak perubahan morfologi, fisiologi dan molekul. Terdapat beberapa pendekatan untuk mengurangkan tekanan kemarau pada tumbuhan. Salah satu pendekatan adalah melalui aplikasi zeolite dengan mempertingkatkan pertumbuhan, fisiologi dan ciri biokimia. Tindak balas pokok roselle variasi UMKL-1 terhadap kandungan kelembapan tanah yang berbeza (100, 67 dan 33% FC) telah diselidik di bawah perlindungan hujan. Objektif kajian ini adalah untuk menentukan kesan kandungan kelembapan tanah yang berbeza terhadap corak pertumbuhan dan perubahan fisiologi, hasil kelopak buah dan kualiti *H. sabdariffa*. Tekanan kemarau yang teruk (33% FC) merendahkan penghasilan berat kering, indeks penuaian dan kadar fotosintesis secara signifikan disebabkan oleh kecekapan pergerakan asimilasi yang terhad. LWP, kadar pertumbuhan relatif dan kandungan nutrien (N, P dan K) pada *H. sabdariffa* juga menurun secara signifikan pada 33% FC. Nisbah akar kepada pucuk, pengumpulan prolin telah meningkat apabila tumbuhan diairi pada 33% FC. Perubahan bilangan cabang dan kandungan klorofil tidak signifikan. Selain itu, hasil kelopak buah *H. sabdariffa* tidak berbeza secara signifikan pada 100% dan 67% FC. Perubahan pada bilangan cabang setiap pokok dan kandungan klorofil tidak signifikan. Selain itu, hasil kelopak *H. sabdariffa* tidak berbeza secara signifikan pada 100% dan 67% FC. Sebaliknya, kandungan tertinggi jumlah pepejal larut, asid askorbik dan antosianin dilihat pada 33% FC. Di dalam eksperimen ke-2, zeolite digunakan untuk meminimumkan kesan buruk tekanan kemarau pada pertumbuhan, dan hasil kelopak di bawah keadaan lindungan hujan. Aplikasi zeolite meningkatkan pertumbuhan dan hasil disebabkan oleh penghasilan berat kering yang meningkat, kandungan nutrien (N, P dan K), konduksi stomata dan kadar fotosintesis. Kajian kuantitatif ekspresi gen menunjukkan corak ekspresi gen *P5CS* meningkat dan *PDH* gen telah menurun

pada 33% FC berbanding dengan 67% FC. Apabila zeolite digunakan di bawah tekanan kemarau, corak ekspresi gen *P5CS* telah menurun dan gen *PDH* telah meningkat. Keputusan ini menunjukkan bahawa tekanan kemarau yang teruk (33%) memberi kesan negatif terhadap hasil *H. sabdariffa* dengan mengurangkan pertumbuhan, kadar fotosintesis dan pengambilan nutrien. Penggunaan zeolite boleh digunakan untuk menjimatkan air yang membawa kepada hasil yang lebih banyak di bawah keadaan tekanan kemarau. Didapati 67% FC dengan 2.50 g zeolite kg^{-1} tanah lebih efektif dalam meningkatkan pertumbuhan dan hasil kelopak. Kajian ini juga ditubuhkan dan Actin-7 dan alpha tubulin-4 disahkan sebagai gen rujukan untuk *H. sabdariffa* di bawah tekanan kemarau dan dijadikan bahan yang berguna untuk analisis ekspresi kuantitatif *P5CS* dan *PDH* pada *H. sabdariffa*. Tambahan pula, *P5CS* boleh digunakan di dalam proses rintangan kemarau dengan mengawal kandungan prolin dalam daun *H. sabdariffa.*

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

A	Photosynthetic rate
ACT	Actin
CB9	Clean buffer 9
cDNA	Complementary DNA
CEC	Cation exchange capacity
Ci	Intracellular CO_2 concentration
Ct	Threshold cycle
DAT	Days after transplanting
DNA	Deoxyribonucleic acid
dNTP	Deoxy nucleotide triphosphate
E	Transpiration rate
Gs	Stomatal conductance
LWP	Leaf water potential
MPa	Mega Pascal
Min	Minute
PCR	Polymerase chain reaction
PDH	Proline dehydrogenase
P5CS	Δ1-Pyrroline-5-carboxylate synthetase
qRT-PCR	Quantitative reverse transcription Polymerase Chain Reaction
RFU	Relative fluorescence unit
RNA	Ribonucleic acid
RSR	Root shoot ratio
SLA	Specific leaf area
TA	Titratable acidity
TAE	Tris-acetate-EDTA
TSS	Total soluble solids
TUBA	Alpha tubulin
µl	Microliter
WAT	Weeks after treatments
WB9	Wash buffer 9

CHAPTER 1

INTRODUCTION

Roselle (*Hibiscus sabdariffa* L) is an important medicinal and fiber plant. The commercially important part of the roselle is the calyx. The calyx used in the production of jelly, jam, syrup, juice, tea, wine, gelatine, pudding, cake and flavouring. The calyx's juice is a healthy drink due to its high content of vitamin C, anthocyanins and other antioxidants (Mohamad *et al*., 2002; Louis *et al*., 2013; Mohamad *et al*., 2015). Drought stress diminishes yield of medicinal plants by three main mechanisms: First, whole canopy absorption of incident photosynthetically active radiation may be decreased. Second, drought stress reduced the efficiency with which absorbed photosynthetically active radiation is used to produce new dry matter. Third, drought stress may reduce the yield by limiting the harvest index (Earl and Davis, 2003). It has been estimated that increasing drought stress will cause up to 30% loss in global crop production by 2025 (Zhang, 2011). The predictions were also that irrigation demand considerably increases in the future to alleviate climate change consequences with more frequent and severe drought (Luquet *et al*., 2005).

As arid and semi-arid lands are naturally under the threat of drought, crop yields are prone to drastic reduction in such areas. This is also the case with lands where the soil has a limited supply of water due to high evapo-transpiration. Presently, in most places on earth, the natural water supply used for irrigation is declining. The prospects for water shortage are explicable in terms of climate changes with temperature increases and a shortage of rainfall. Given such circumstances, it is crucial to investigate crop responses to water shortages in specific environments so that to mitigate the impact of water stress on crop production.

Several measures are available to alleviate the effects of water stress, which include use of growth enhancers, crop management practices and use of drought tolerant varieties. Zeolite application could be one possible approach to reduce the effects of water stress on plant productivity. Zeolites are crystalline aluminosilicates of alkali and micro porous with alkaline materials which possess higher internal surface area (Silberbush *et al*., 1993). The unique cation exchange, adsorption, and catalytic properties of zeolite have prompted the slow release of fertilizers (Artiola, 1991). Therefore, zeolite can decrease soil moisture loss, especially in arid as well as semi-arid zones by improving soil physical properties. Zeolite can preserve soil moisture for a longer time (Zamanian, 2008). In many reports it is concluded that zeolite application had beneficial impacts in alleviating the negative impacts of drought stress on yield (Gholizadeh *et al*. 2006; Khashei *et al*., 2008).

At present, the role of zeolite on *H. sabdariffa* in relation to the alleviation of water stress is not examined. Therefore, this study is undertaken with the main objective to alleviate the effects of water stress on growth and yield of *H. sabdariffa* L. by zeolite application.

The specific objectives of this study were to:

1. Determine the effects of different soil moisture content on growth performance and physiological changes, calyx yield and quality in *H. sabdariffa*.
2. Alleviate the effects of water stress on growth, physiological attributes, yield and quality of calyces in *H. sabdariffa* L. by zeolite.
3. To investigate the interaction effect of zeolite levels and water stress on some physiological, phytochemical characteristics, and growth performance.
4. Optimize qRT-PCR primers condition for genes related to proline synthesis and degradation (*P5CS* and *PDH*) and reference genes (*ACT* and *TUBA*) in *H. sabdriffa.*
5. Analyze the expression pattern of *P5CS* (Δ1-pyrroline-5-carboxylate synthetase) and *PDH* (proline dehydrogenase) genes in *H. sabdriffa* under water stress condition with zeolite application.

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