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CHARACTERISTICS OF NaCl-SALINIZED CUCURBITS AND IMPACT OF SILICON IN ALLEVIATING SALT STRESS

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By

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Members of *Cucurbitaceae* are salt-sensitive plants and continuous fertilizer application without sufficient leaching may create saline environment that consequently reducing their growth and yield. Among other methods, silicon fertilization was reported to have some ability to reduce negative salinity effects on many plant species. This study was carried out to evaluate the physiological responses of four selected *Cucurbitaceae* to salinity stress, and to investigate the possible role of silicon in alleviating salinity effects on the crops. Four types of *Cucurbitaceae* viz. cucumber (*Cucumis sativa*), pumpkin (*Cucurbita moschata*), bitter gourd (*Momordica charantia*) and bottle gourd (*Lagenaria siceraria*) were subjected to four levels of NaCl (0, 25, 50, 75 mM) and data at vegetative stages; growth, leaf relative water content (RWC), mineral content in leaf, stem, root, relative chlorophyll content and proline concentration were collected. In the second experiment, a study involving two species of *Cucurbitaceae* that have shown highest (bitter gourd) and lowest (cucumber) salinity tolerant were performed. The species were subjected to two levels of NaCl (0, 50 mM) and three silicon concentrations (0, 50, 100 mg/L sodium silicate) and data on growth, leaf RWC, leaf mineral content, net photosynthesis and stomatal conductance at vegetative stage were collected.

Among all species, bitter gourd was least salt-sensitive while cucumber was most salt-sensitive. In terms of growth, bitter gourd had the lowest reduction of leaf area, plant height and stem dry weight while cucumber had the highest reduction of leaf area. Total reduction of plant dry matter as salinity increased to 75 mM NaCl was the least in bitter gourd, 44.40% and the highest in cucumber, 67.84%. Significant reduction of chlorophyll content was recorded at 75 mM in cucumber and pumpkin while in other species shown no significant

reduction. At 75 mM NaCl, cucumber leaf water status was significantly impaired by salinity which marked by highest accumulation of proline (3.55 times compared to control) and strong negative correlation between proline and RWC (r= - 0.83, p ≤ 0.01), whereas in other species no significant association was recorded.

When treated with 100 mg/L silicon, growth of salt-stressed and non-salt stressed plants for both species was significantly improved, with more beneficial effects recorded on the salt-stressed plants and salt-sensitive species. In terms of Na^+ ion, plants treated with 50 mg/L silicon had 11.11% significantly lower Na^+ ion in leaf compared to 0 mg/L silicon, regardless of species and salinity condition. Moreover, treatment of 100 mg/L silicon significantly increased net photosynthesis and stomatal conductance by 12.13 and 30.14% respectively, compared to 0 mg/L silicon treatment. Besides that, plants supplied with silicon at 100 mg/L also had significantly higher RWC compared to non-silicon supplied plants.

In conclusion, based on reduction in plant dry matter, accumulation of Na^+ and Cl^- ions in leaf, degradation of chlorophyll content and impaired leaf water status, bitter gourd exhibited a better adaptation to salinity stress than other *Cucurbitaceae* tested and application of silicon can alleviate salinity stress in both on salt-sensitive species (cucumber) and on a less salt-sensitive species (bitter gourd) by reducing sodium toxicity, increasing photosynthetic activity and improving leaf water status.

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

CIRI-CIRI CUCURBIT YANG MENGALAMI KEMASINAN NaCl DAN IMPAK SILIKON DALAM MENGURANGKAN TEGASAN KEMASINAN

Oleh

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Tumbuhan dari kalangan *Cucurbitaceae* adalah peka kemasinan dan pembajaan berterusan tanpa proses larut lesap yang mencukupi akan menyebabkan persekitaran bergaram yang mengurangkan pertumbuhan dan hasil. Antara kaedah-kaedah lain, pembajaan silikon dilaporkan berupaya mengurangkan kesan negatif kemasinan terhadap pelbagai spesies tumbuhan. Kajian ini telah dijalankan untuk menilai gerak balas fisiologi empat *Cucurbitaceae* terpilih terhadap tegasan kemasinan, dan menyelidik peranan silikon dalam mengurangkan kesan kemasinan terhadap tumbuhan. Empat jenis *Cucurbitaceae* iaitu timun (*Cucumis sativa*), labu manis (*Cucurbita moschata*), peria katak (*Momordica charantia*) dan labu botol (*Lagenaria siceraria*) telah diberikan empat tahap kemasinan NaCl (0, 25, 50, 75 mM) dan data peringkat vegetatif; pertumbuhan, kandungan air daun relatif, kandungan mineral daun, batang, akar, kandungan klorofil relatif dan kepekatan prolin telah diambil. Pada eksperimen kedua, kajian yang melibatkan dua spesies *Cucurbitaceae* yang menunjukkan ketahanan kemasinan tertinggi (peria katak) dan terendah (timun) telah dijalankan. Spesies-spesies tersebut telah diberikan dua tahap kemasinan NaCl (0, 50 mM) dan tiga kepekatan silikon (0, 50, 100 mg/L sodium silika) dan data pertumbuhan, kandungan air daun relatif, kandungan mineral daun, fotosintesis dan aliran stomata telah diambil pada peringkat vegetatif.

Antara semua spesies, peria katak adalah paling kurang peka kemasinan manakala timun adalah paling peka kemasinan. Dari segi pertumbuhan, pengurangan keluasan daun, ketinggian pokok dan berat kering batang, peria katak adalah yang terendah manakala timun mempunyai pengurangan keluasan daun yang tertinggi. Jumlah pengurangan bahan kering pokok

apabila kadar kemasinan meningkat ke 75 mM NaCl adalah terendah pada peria katak, 44.40% dan tertinggi pada timun, 67.84%. Pengurangan kandungan klorofil yang signifikan direkodkan pada 75 mM untuk timun dan labu manis manakala untuk spesis lain, tiada pengurangan signifikan direkodkan. Pada 75 mM NaCl, status air daun pada timun terjejas secara signifikan oleh kemasinan yang dijelaskan oleh pengumpulan prolin yang tertinggi (3.55 kali lebih tinggi berbanding kawalan) dan korelasi negatif antara prolin dan kandungan air daun relatif (r= - 0.83, $p \leq 0.01$), manakala tiada korelasi signifikan direkodkan pada spesies lain.

Apabila dirawat dengan 100 mg/L silikon, pertumbuhan pokok yang tegas kemasinan dan tidak tegas kemasinan untuk kedua-dua spesies bertambah baik secara signifikan, dengan kesan bermanfaat direkodkan pada pokok yang tegas kemasinan dari spesies yang peka kemasinan. Dari segi ion Na^+, pokok yang dirawat dengan 50 mg/L silikon mempunyai 11.11% lebih rendah ion Na^+ secara siginifikan berbanding 0 mg/L sillikon, tidak kira spesies atau keadaan kemasinan. Tambahan lagi, rawatan 100 mg/L silikon juga meningkatkan fotosintesis dan aliran stomata masing-masing sebanyak 12.13 dan 30.14% berbanding 0 mg/L rawatan silikon. Selain itu, pokok yang diberikan silikon pada kadar 100 mg/L juga mempunyai kandungan air daun relatif yang secara signifikannya lebih tinggi berbanding pokok tanpa penambahan silikon.

Sebagai rumusan, berdasarkan pengurangan bahan kering pokok, pengumpulan ion-ion Na^+ dan Cl^- pada daun, pengurangan kandungan klorofil dan status air daun, peria katak menunjukkan penyesuaian terhadap tegasan kemasinan yang lebih baik berbanding *Cucurbitaceae* lain di dalam kajian ini dan aplikasi silikon boleh mengurangkan tegasan kemasinan pada spesies yang peka kemasinan (timun) dan kurang peka kemasinan (peria katak) dengan mengurangkan ketoksikan sodium, meningkatkan aktiviti fotosintesis dan menambahbaik status air daun.

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

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LIST OF ABBREVIATIONS

mM	Milimolar
mmol/L	milimolar per litre
RWC	Relative water content
Pn	Net photosynthesis
g_s	Stomatal conductance
µmol	micromole
DW	Dry weight
FW	Fresh weight
TW	Turgid weight
EC	Electrical conductivity
MDA	Malondialdehyde
ROS	Reactive oxygen species
dS/m	Decisiemens per metre

CHAPTER 1

INTRODUCTION

1.1 Background information

In 2015, the world population had reached 7.3 billion and with approximately 83 million addition increased annually, the current population is projected to reach 8.5 billion by 2030 (UN, 2015). Agricultural productivity in contrast, is not increasing at a required rate to keep up with the food demand. Many factors contributed to this problem such as availability of agricultural land, fresh water resources, ever-increasing biotic and abiotic stresses, and low economic activity in agricultural sector (Athar and Ashraf, 2009). It is believed that abiotic stresses are considered to be the key source of yield reduction (Munns and Tester, 2008). Salinity is one of the major abiotic stresses that affect production in agriculture. According to FAO statistics, over 800 million hectares of land throughout the world is presently salt-affected, equivalent to more than 6% of the world's total land area. Continuing salinization of arable land is expected to result in a 30% loss of agricultural land over the next 25 years and up to 50% loss by 2050 (Vahdati and Leslie, 2013).

Salinity causes detrimental effects on plant's life. Growth reduction is a consequence of numerous physiological responses including modifications of ion balance, water status, mineral nutrition, stomatal behavior, photosynthetic efficiency, carbon allocation and utilization (Kusvuran, 2011). The degree to which growth is reduced by salinity differs greatly among species and to a lesser extent among varieties within a species (Ghoulam *et al*., 2002).

In combating salinity problem, numerous approaches and studies have been done in recent years. Traditional breeding programmes have proven to be time-consuming and very challenging due to the complexity of the traits since salt tolerance is the result of complex genetics and physiological interactions (Flowers, 2004). Alternatively, grafting onto rootstocks that capable in ameliorating salt-induced damage to the shoot is feasible but limitations may occur in compatibility of the scions and rootstocks (Cohen *et al*., 2007) while chemical amendments or leaching salts application is not environmentally friendly (Cuartero *et al*., 2006).

Silicon (Si) has essential function in healing plants in response to environmental stresses (Sahebi *et al*., 2015). Application of silicon is a promising method that has been proven in alleviating salt stress in various important crops namely rice (Gong *et al*. 2006), barley (Liang *et al*., 2003), wheat (Tuna *et al*., 2008), sugarcane (Ashraf *et al*., 2010), soybean (Lee *et al*., 2010), tomato (Romero-Aranda *et al*., 2006), and zucchini (Savvas *et al*., 2009). Salinity tolerance in plants treated with silicon is associated with the ability of the silicon to reduce ion toxicity and decrease oxidative damage under salt stress.

Cucurbitaceae family primarily comprises species consumed as food worldwide which consists of about 130 genera and 800 species (Jeffrey, 2005). Genetic diversity within the family is tremendous and the ability of *Cucurbitaceae* to tolerate salinity stress differs considerably among species. Majority of the cucurbits are reported moderately sensitive to salt stress such as cucumber (*Cucumis sativus* L.) and musk melon (*Cucumis melo* L.) while bitter gourd (*Momordica charantia* L.) is reported to have salinity tolerance (Pessarakli, 2016).

While studies of *Cucurbitaceae* species in response to salinity stress has been done extensively in other regions, information on salinity responses in local Malaysia *Cucurbitaceae* species is still lacking. Due to the tremendous genetic diversity within *Cucurbitaceae* family, the effects of salinity on Malaysian *Cucurbitaceae* species might be different from studies reported in other regions. Information gained from studies on the effects of salinity on physiological and biochemical aspects of *Cucurbitaceae* species could lead to identification of salt tolerant species among *Cucurbitaceae* in Malaysia. Furthermore, the alleviating effects of silicon against salinity stress could be beneficial in improving production in salt-stressed *Cucurbitaceae* as well as giving new perspectives in *Cucurbitaceae* research in the future.

1.2 Problem statements

Traditional breeding, grafting onto salt-tolerant rootstock and application of chemical amendments have its own limitation in overcoming salinity problems. Alternatively, agronomic practice approach which can alleviate salt stress such as application of silicon is more efficient and promising. While studies on effects of salinity stress on Cucurbit has been done extensively in other regions, information on salinity responses in local Malaysia Cucurbit species is still lacking and no comparative studies has been done previously in comparing their salinity tolerance level. Besides that, the alleviate effects of silicon in reducing salt stress in both susceptible and less susceptible Cucurbits are still equivocal and need to be elucidated further.

1.3 Aim and objectives of the study

The aim of the study is to differentiate salinity tolerance level among selected *Cucurbitaceae* species and to enhance salinity tolerance using silicon at vegetative stage. In achieving that, the objectives of this study include:

i. To characterize the growth and physiological responses of *Cucurbitaceae* species to different levels of NaCl-induced salinity.
ii. To determine the susceptible and salt-tolerant species among the *Cucurbitaceae* species studied.
iii. To investigate the effects of varying concentrations of NaCl salinity and silicon application on physiological and biochemical properties in both susceptible and tolerant species.

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LIST OF PUBLICATIONS

Najib, O.G., Yahya, A. and Firdaus, I. (2016). Assessing four *Cucurbitaceae* species for salt-tolerant rootstock using plant growth parameters as indicators. *National Seed Symposium, 23-24 August 2016, Sepang, Poster Presentation.*

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