UNIVERSITI PUTRA MALAYSIA

RESPONSE TO SALT STRESS AND STRATEGIES TO IMPROVE SALT TOLERANCE IN CHINESE KALE (Brassica oleracea var. alboglabra)

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By
AMIN TAYEBI-MEIGOONI

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

June 2012

DEDICATION

I lovingly dedicate this thesis to

My motherland IRAN,

My beloved Parents,

My lovely Wife,

My dear daughterViana,

My beloved Brother & lovely Grandma,

Whose supported me each step of the way.

Abstract of thesis presented to senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

RESPONSE TO SALT STRESS AND STRATEGIES TO IMPROVE SALT TOLERANCE IN CHINESE KALE (*Brassica oleracea* var. *alboglabra*)

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Chairman: **Assoc. Prof. Yahya Awang, PhD**

Faculty: **Agriculture**

Despite the large body of literature on salinity stress, our knowledge about the effects of salt stress on Chinese kale (*Brassica oleracea* var. *alboglabra*) is still little. Therefore, there is a pressing need to know in more detail how an important vegetable plant as Chinese kale responds and adapts to such conditions. With regard to the importance of Chinese kale in term of their benefits and high consumption in human diets, understanding the possible effects of salt stress on Chinese kale have never been exposed to salt stress can be significant. Therefore attempts to provide some practical, safe and environmentally sound techniques to retain productivity under salt stress is critical. The purposes of this study were to unveil the mechanism contributing to salinity tolerance during vegetative stage and to test for possible contribution of exogenous ascorbic acid and pre-treatment with low

concentration of hydrogen peroxide in association with the tolerance mechanisms during vegetative development of the crop.

Preliminary study involving four cultivars of Chinese kale ['Standard kailan', 'Hong Kong kailan', 'Kale Curly Leaf' and 'Hong Kong Stem Flower'] and four salinity levels (0, 25, 50 and 75) showed that final growth parameters, relative water content, concentration of photosynthetic pigments and maximum quantum yield of PSII (*Fv*/*Fm*) were significantly reduced by salinity. The treatment elevated plant's proline, hydrogen peroxide and lipid peroxidation. Among cultivars tested, cv. 'Standard kailan' showed minimum reduction of biomass. Cv. 'Standard kailan' also showed relatively higher adaptability to salinity due to its ability to regulate hydrogen peroxide (H_2O_2) generation and moderate oxidative damage to cell membranes as shown by malondialdehyde (MDA) content in leaf tissues at all salinity. Based on the results, cv. 'Standard kailan' was more salt-tolerant than other cultivated varieties and has been used for further experimentation.

Biochemical analysis of the leaves revealed that salinity also produced significant negative impacts on protein synthesis and leaf pigments (chlorophylls and carotenoid). The results may imply the increase in the energy dissipation *via* chlorophyll fluorescence along with reduction of chlorophylls maybe is a manifestation to preserve a balance between

harvesting and utilizing energy in order to reduce threat of oxidative stress. Consistent with results in the first experiment, detrimental effects of salinity were further illustrated in the generation and accumulation of hydrogen peroxide and MDA. However, salt stressed plants contained relatively higher concentrations of proline and activity of antioxidant enzymes especially ascorbate peroxidase (APX) and peroxidase (POX). Chinese kale was shown to be not affected by low salinity up to 25 mM and can be tolerant up to 75 mM NaCl.

Subsequent study further confirmed the results of the first experiment where the overall reduction in plant growth was coupled with reduced leaf expansion and net assimilation rate. Negative effects of salinity are clearly shown as reduction in net assimilation rate (NAR) and relative growth rate (RGR) and this was coupled with alteration of ionic balance in the plant tissue. Salinity reduced N, K^+, Ca^{2+} and Mg^{2+} in leaves and roots. P was also decreased by the treatment in leaves, while it was increased in root. The concentrations of Na^+ and Cl^- in leaves were markedly enhanced in plants tissues as the NaCl in the root zone increases. Reduction of N and Mg^{2+} availability for various processes such as chlorophyll biosynthesis, lack of P of bioenergetics pathway, besides imbalance of K^+ and Ca^{2+} and negative effects of Na^+ and Cl^- to different bioactive processes were also considered to be responsible for growth reduction in this plant.

Interactions of foliar application of ascorbic acid (AsA) and salt stress on the plants were also evaluated in this study. Application of AsA reduced portion of antioxidant enzyme activity catalase (CAT) and peroxidase (POX) may be due to its own talent for scavenging of reactive oxygen species. APX (acts by utilizing AsA) was stimulated by exogenous AsA, which assist the plants for higher resistance under salinity stress. Application exogenous H_2O_2 as a stress priming factor has resulted in a noticeable reduction of endogenous H_2O_2 and lipid peroxidation. Stimulation of POX and APX activities by exogenous H_2O_2 were greater than CAT activity, suggesting that POX and APX have had a higher affinity to scavenge ROS in these circumstances, and this could increase the plant's tolerance level to salinity.

Overall, results of the study showed that Chinese kale was sensitive to NaCl salinity stress but the plant can tolerate up to 75 mM NaCl. However, the deleterious effects of salinity can be partially moderated *via* application of exogenous ascorbic acid and H_2O_2 priming.

Abstrak tesis untuk dikemuakan kepada senat, Universiti Putra Malaysia
bagi memenuhi keperluan ijazah Doktor Falsafah

TINDAK BALAS KEPADA STRES KEMASINAN DAN STRATEGI UNTUK MEMPERBAIKI TAHAP KEMASINAN DALAM KAILAN (*Brassica oleracea* var. *alboglabra*)

Oleh

AMIN TAYEBI-MEIGOONI

Jun 2012

Pengerusi: **Profesor Madya Yahya Awang, PhD**

Fakulti: **Pertanian**

Kajian dan literasi berkaitan tegasan kemasinan adalah sangat meluas, namun pengetahuan mengenai kesan tegasan kemasinan terhadap kailan (*Brassica oleracea* var. *alboglabra*) sukar diperolehi. Oleh itu, pengetahuan tentang keupayaan dan cara bagaimana cara tanaman sayuran seperti kailan beradaptasi terhadap tekanan kemasinan sangat diperlukan. Berdasarkan kepada kepentingan kalian khususnya dari segi diet pemakanan dan permintaan, adalah sangat penting dan signifikan bagi menjalankan sebuah kajian untuk memahami kesan tegasan kemasinan terhadap tanaman ini. Selain daripada mengekalkan produktiviti di bawah tegasan kemasinan, salah satu perkara yang perlu dititikberatkan ialah untuk menyediakan suatu teknik pengeluaran yang praktikal, selamat dan mesra alam. Tujuan kajian ini dijalankan ialah untuk mendedahkan mekanisma yang terlibat

dalam toleransi terhadap tegasan kemasinan serta mengkaji peranan asid askorbik eksogenous dan pra-rawatan hidrogen peroksida pada kepekatan yang rendah terhadap mekanisma toleransi kailan pada peringkat perkembangan vegetatif.

Kajian permulaan yang melibatkan empat kultivar kailan menunjukkan bahawa parameter tumbesaran, kandungan relatif air, kepekatan pigmen fotosintesis dan hasil kuantum maksimum PSII (*Fv/Fm*) menurun secara signifikan oleh kemasinan. Rawatan yang diberikan telah meningkatkan kandungan proline, hidrogen peroksida dan lipid peroksida (sebagai malondialdehyd, MDA) dalam kailan. Kultivar 'Standard kailan' menunjukkan pengurangan biomass yang minimum berbanding kultivar-kultivar yang lain. Secara perbandingannya, 'Standard kailan' juga menunjukkan adaptasi kemasinan yang tertinggi pada semua tahap kemasinan berdasarkan kemampuannya mengawal pengeluaran hidrogen peroksida dan kesan pengoksidaan yang sederhana pada membran sel seperti yang ditunjukkan oleh kandungan MDA di dalam tisu daun.

Analisis biokimia daun menunjukkan kemasinan memberikan kesan negatif yang signifikan ke atas sintesis protein dan pigmen daun (klorofil dan karotenoid). Keputusan ini mungkin menunjukkan peningkatan dalam pelesapan tenaga menerusi klorofil floresens dengan pengurangan klorofil sebagai suatu menifestasi untuk mengekalkan keseimbangan di antara

penuaian dan penggunaan tenaga demi mengurangkan tegasan oksidatif. Konsisten dengan keputusan eksperimen yang pertama, kesan kemasinan dihasilkan berdasarkan kepada penghasilan dan pengumpulan hidrogen peroksida dan MDA. Walaubagaimanapun, kailan yang mengalami tegasan kemasinan mengandungi prolin dan aktiviti enzim antioksida khususnya aksorbat peroksida dan peroksida pada kepekatan yang tinggi. Kailan menunjukkan ketahanan terhadap kemasinan sehingga kepada 75 mM.

Kajian yang berikutnya mengesahkan keputusan eksperimen yang pertama iaitu penurunan pertumbuhan tumbuhan ditambah pula dengan penurunan pengembangan daun dan kadar fotosintesis bersih. Kesan negatif kemasinan ditunjukkan secara jelas oleh penurunan kadar asimilasi bersih (NAR) dan kadar pertumbuhan relatif (RGR) dan ini ditambah lagi dengan gangguan pada keseimbangan ion pada tisu tumbuhan. Kemasinan menurunkan N, K^+, $Ca2^+$ dan Mg^{2+} di dalam daun dan akar. Kepekatan P di dalam daun menurun dengan rawatan NaCl. Peningkatan kepekatan Na^+ dan Cl^- di dalam tisu daun jelas dengan peningkatan NaCl di kawasan akar. Penurunan N dan Mg^{2+} yang berfungsi dalam pelbagai proses seperti biosintesis klorofil, kekurangan P untuk tindakan bioenergetik, disamping ketidakseimbangan K^+ dan Ca^{2+}, dan kesan negatif Na^+ dan Cl^- terhadap pelbagai proses bioaktif adalah merupakan antara faktor-faktor yang bertanggungjawab terhadap penurunan pada pertumbuhan kailan.

Kesan interaksi di antara rawatan asid askorbik (AsA) dan kemasinan terhadap tumbuhan juga diperhatikan di dalam kajian ini. Aplikasi AsA telah menyebabkan penurunan aktiviti enzim antioksida (CAT dan POX) dan ini mungkin disebabkan oleh sifat semulajadinya yang mengikat oksigen yang reaktif. APX (menggunakan AsA untuk berfungsi) telah dirangsang oleh AsA untuk membantu tumbuhan untuk lebih tahan kepada tegasan kemasinan. Aplikasi H_2O_2 yang merupakan faktor utama penyebab tegasan telah menyebabkan penurunan H_2O_2 dalam tisu pokok dan lipid peroksida. Rangsangan terhadap aktiviti POX dan APX oleh H_2O_2 adalah sangat besar berbanding aktivit CAT, di dalam situasi begini POX dan APX mempunyai afiniti yang tinggi untuk mengikat ROS dan seterusnya meningkatkan ketahanan tumbuhan terhadap kemasinan.

Secara keseluruhannya, keputusan kajian menunjukkan bahawa kailan sangat sensitif kepada tekanan kemasinan NaCl tetapi kailan mempunyai ketahanan sehingga 75 mM NaCl. Walaubagaimanapun, kesan kerosakan oleh tegasan kemasinan boleh dikurangkan melalui aplikasi AsA dan H_2O_2.

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APPROVAL

I certify that a Thesis Examination Committee has met on June 11, 2012 to conduct the final examination of Amin Tayebi-Meigooni on his thesis entitled "**Response to salt stress and strategies to improve salt tolerance in Chinese kale (*Brassica oleracea* var. *alboglabra*)**" 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 Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the Degree of **Doctor of philosophy**. The members of the Supervisory Committee were as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

AMIN TAYEBI-MEIGOONI

Date: 11 June 2012

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