UNIVERSITI PUTRA MALAYSIA

GENETIC DIVERSITY AND EFFECT OF SALINITY ON MORPHO-PHYSIOCHEMICAL PROPERTIES OF PURSLANE (Portulaca oleracea L.) GERMPLASM COLLECTIONS

MD. AMIRUL ALAM

GENETIC DIVERSITY AND EFFECT OF SALINITY ON MORPHO-PHYSIOCHEMICAL PROPERTIES OF PURSLANE (*Portulaca oleracea* L.) GERMPLASM COLLECTIONS

By

MD. AMIRUL ALAM

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

December 2014

DEDICATION

To those people of the world who are still combating only for daily meals.

To my beloved father and departed soul (13/01/2015) of my mother; for their sacrifice and continuous blessings

To my wife Lubna, to digest loneliness, patience and happily forgiving my routinely delay arriving at home and passing the least time with her entire period of my PhD study.

Also to my only princes Bushratul Jannah, who always wait for my arrival at house.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Doctor of Philosophy

GENETIC DIVERSITY AND EFFECT OF SALINITY ON MORPHO-PHYSIOCHEMICAL PROPERTIES OF PURSLANE (*Portulaca oleracea* L.) GERMPLASM COLLECTIONS

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Chairman: Professor Abdul Shukor Juraimi, PhD
Faculty: Agriculture

Global warming is already having significant and costly effects on our climate, our health, our environment and overall agricultural system. Global warming is also accelerating the rate of sea level rise, increasing flooding risks to low-lying communities, high-risk coastal properties, causing severe salinity and making agricultural land unsuitable for crop production. Considering such situation environmental friendly technologies with stress tolerant crop species is the only solution for sustainable agricultural development. Purslane (*Portulaca oleracea* L.) is a pioneer vegetable crops having moderate to high salt tolerant capability containing vitamins and minerals and rich in antioxidant properties than any other vegetable crops ever reported. The present study was therefore designed to select high salt tolerant purslane accessions and their morpho-physiological variation due to salinity stress as well as impact on antioxidant properties. A survey was conducted to collect available purslane accessions from seven states (Selangor, Melaka, Negeri Sembilan, Kedah, Perak, Penang and Perlis) of Western Peninsular Malaysia. A total of 45 purslane samples were collected and divided into seven different groups based on distinct morphological variation. Morpho-physiological and mineral variations were also evaluated among collected purslane accessions. Highly significant variations were observed among accessions as well as measured parameters. Variations were also recorded among the same groups may be due to collection from different location. Twenty eight ISSR and ten EST-SSR markers based genetic diversity analysis was carried out among collected purslane germplasms. Analysis of molecular variance (AMOVA) showed 89% and 71.87% variation within the populations while variation among the populations accounted for 11% and 28.13% using ISSR and EST-SSR markers respectively. A dendrogram was constructed by Unweighted Pair Group Method with Arithmetic Average (UPGMA) based on Nei's genetic distance grouped the whole germplasms into 7 and 8 distinct clusters respectively from ISSR and EST-SSR markers. Based on the information from this dendrogram, accessions that are far from each other by virtue of genetic origin and diversity index are strongly recommended to be used as parent for improved variety development. Another glass house experiment was conducted with initially selected 25 purslane accessions and subjected to five salinity (0.0, 10.0, 20.0, 30.0 and 40.0 dS m^{-1} NaCl) treatments to screen out high salt tolerant purslane accessions. Overall salinity effect on

morphology and reduction of dry matter content of two accessions were graded as tolerant (Ac7 and Ac9), six accessions were moderately tolerant (Ac3, Ac5, Ac6, Ac10, Ac11 and Ac12) and five accessions were moderately susceptible (Ac1, Ac2, Ac4, Ac8 and Ac13) for biomass production and the rest 12 accessions were susceptible to high salinity (30.0 and 40.0 dS m^{-1} NaCl) and discarded from the further research program. The results also revealed that ornamental purslane were more salt tolerant over common purslane. The selected 13 purslane accessions were again subjected to five (0.0, 8.0, 16.0, 24.0 and 32.0 dS m^{-1} NaCl) salinity levels to determine the effect of salinity on detailed morphological, physiological, minerals, antioxidant properties and on stem-root histology of purslane. At the highest (30 dS m^{-1}) salinity significant reduction was observed for morphological traits but for physiology, increase in chlorophyll content was seen in Ac2, Ac4, Ac6 and Ac8 at 16 dS m^{-1} salinity. Whereas, Ac4, Ac9 and Ac12 showed increased photosynthesis activity at the same salinity levels compared to control. Anatomically, stem cortical tissues of Ac5, Ac9 and Ac12 were unaffected at 8 dS m^{-1} salinity but root cortical tissues did not show any significant damages except a very small enlargement in Ac12 and Ac13. In general Ac9, an ornamental purslane was the best salt tolerant accession, while Ac13 a common purslane was the most affected accession. Regarding antioxidant properties on average over all accessions, different levels of salinity treatment resulted in 8-35% increases in TPC; about 35% increase in TFC; and 18-35% increases in FRAP activity. In contrast, at the lower salinity levels (8 and 16 dS m^{-1}) all the measured minerals found to increase but observed to decrease later with increasing of salinity, but performance of different accessions were different depending on salinity levels. Salinity treatment experiments conducted in our study followed by investigation of different morpho-physicochemical and antioxidant attributes of purslane are valuable for the selection of best salt-tolerant accessions with optimum yield and productivity and antioxidant nutrients. Based upon the data generated such accessions can thus be recommended for cultivation in the saline areas of Malaysia to benefitting health of consumers and as well as sustainable and beneficial utilization of the lands.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
Sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

KEPELBAGAIAN GENETIK DAN KESAN KEMASINAN KE ATAS SIFAT MORFO-FISIOKIMIA PADA KOLEKSI GERMPLASMA PURSLANE (*Portulaca oleracea* L.)

Oleh

MD. AMIRUL ALAM

Disember 2014

Pengerusi: Profesor Abdul Shukor Juraimi, PhD
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Pemanasan global telah memberi kesan yang nyata pada iklim, kesihatan, persekitaran dan sistem pertanian secara keseluruhannya. Pemanasan global juga mempercepatkan kadar kenaikan aras laut, meningkatkan risiko banjir bagi penduduk di kawasan tanah rendah, risiko kerosakan harta benda di kawasan pantai yang tinggi, menyebabkan kemasinan yang teruk dan menjadikan tanah pertanian tidak sesuai untuk pengeluaran tanaman. Oleh itu teknologi mesra alam sekitar dengan spesies tanaman yang dapat bertoleransi dengan tekanan persekitaran merupakan penyelesaian terbaik untuk pembangunan pertanian lestari. Purslane (*Portulaca oleracea* L.) adalah tumbuhan perintis yang mempunyai keupayaan bertoleransi terhadap kemasinan dari yang sederhana kepada tinggi, dan mengandungi vitamin dan mineral serta kaya dengan ciri-ciri antioksidan berbanding dengan tanaman sayuran lain yang pernah dilaporkan. Kajian ini bertujuan untuk memilih aksesi purslane yang toleran pada tahap kemasinian yang tinggi dan variasi dalam morfo-fisiologikal yang disebabkan oleh tekanan kemasinian dan kesannya ke atas ciri-ciri antioksidan. Satu survei telah dijalankan untuk mengumpul aksesi purslane daripada tujuh negeri (Selangor, Melaka, Nigeri Sembilan, Kedah, Perak, Pulau Pinang dan Perlis) di Barat Semenanjung Malaysia. Sebanyak 45 sampel purslane dikumpulkan dan dibahagikan kepada tujuh kumpulan berdasarkan variasi morfologi yang berbeza. Variasi morpho-fisiologi dan mineral juga dinilai antara aksesi purslane yang dikumpulkan. Variasi yang sangat ketara telah deapat diperhatikan di antara aksesi serta parameter yang diukur. Variasi juga dicatatkan di antara kumpulan yang sama yang mungkin disebabkan oleh pengumpulan daripada lokasi yang berbeza. Sebanyak dua puluh lapan penanda ISSR dan sepuluh penanda EST-SSR berasaskan analisis kepelbagaian genetik telah digunakan di antara germplasms purslane yang dikumpulkan. Melalui analisis varians molekul (AMOVA) penggunaan penanda ISSR dan EST-SSR menunjukkan 89% dan 71.87% variasi dalam populasi manakala variasi di antara populasi menyumbangkan 11% dan 28.13% masing-masing. Satu dendrogram dibuat dengan menggunkan Kaedah *Unweighted* Kumpulan Pasangan dengan Purata Aritmetik (UPGMA) berdasarkan jarak genetik Nei yang menunjukkan seluruh germplasma dikumpulkan kepada 7 dan 8 kelompok yang berbeza, masing-masing dari penanda ISSR dan EST-SSR. Berdasarkan maklumat dari dendrogram ini, aksesi yang jauh antara satu sama lain menurut sifat asal genetik yang baik dan indeks

kepelbagaian, disarankan untuk digunakan sebagai ibu dan bapa untuk meningkatkan pembangunan varieti. Selain itu, satu eksperimen rumah kaca telah dijalankan dengan 25 aksesi purslane terpilih dan tertakluk lima rawatan tanap kemasinan (0.0, 10.0, 20.0, 30.0 dan 40.0 dS m^{-1} NaCl) untuk menyaring aksesi purslane yang toleran kepada kemasinan yang tinggi. Kesan kemasinan kepada morfologi dan penurunan kandungan bahan kering pada dua aksesi dapat digredkan sebagai toleran (Ac7 dan Ac9), enam aksesi sebagai sederhana toleran (Ac3, Ac5, Ac6, Ac10, Ac11 dan Ac12) dan lima aksesi sebagai sederhana rentan (Ac1, Ac2, Ac4, Ac8 dan Ac13) untuk pengeluaran biomas dan 12 aksesi selebihnya adalah rentan kepada kemasinan yang tinggi (30.0 dan 40.0 dS m^{-1} NaCl) dan disingkirkan dari program penyelidikan selanjutnya. Kajian ini juga mendapati bahawa purslane hiasan lebih toleran kepada tahap kemasinan yang tinggi berbanding purslane biasa. Tigabelas (13) aksesi purslane ini sekali lagi didedahkan kepada lima tahap kemasinan (0.0, 8.0, 16.0, 24.0 dan 32.0 dS m^{-1} NaCl) untuk menentukan kesan kemasinan pada morfologi secara terperinci, fisiologi, mineral, ciri-ciri antioksidan dan histologi batang-akar purslane. Pada tahap kemasinan paling tinggi (30 dS m^{-1}) penurunan ketara diperhatikan pada ciri-ciri morfologi tetapi untuk fisiologi, peningkatan dalam kandungan klorofil dapat dilihat dalam Ac2, Ac4, Ac6 dan Ac8 pada tahap kemasinan16 dS m^{-1} kemasinan. Manakala, Ac4, Ac9 dan Ac12 menunjukkan peningkatan aktiviti fotosintesis di tahap kemasinan yang sama berbanding kawalan. Secara anatomi, tisu kortikal batang Ac5, Ac9 dan Ac12 tidak terjejas pada tahap kemasian 8 dS m^{-1} dan tisu kortikal akar tidak menunjukkan apa-apa kerosakan yang ketara kecuali sedikit pembesaran pada Ac12 dan Ac13. Secara umumnya Ac9 iaitu purslane hiasan adalah aksesi terbaik yang toleran kepada kemasinan, manakala Ac13 yang merupakan purslane biasa adalah aksesi yang paling terjejas oleh kemasinan. Bagi ciri-ciri antioksidan pada purata keseluruhan aksesi, tahap rawatan kemasinan yang berbeza menyebabkan 8-35% peningkatan bagi TPC; kira-kira 35% peningkatan bagi TFC; dan 18-35% peningkatan bagi aktiviti FRAP. Sebaliknya, di tahap kemasinan yang lebih rendah (8 dan 16 dS m^{-1}) semua mineral yang diukur didapati meningkat tetapi kemudiannya menurun dengan peningkatan kemasinan, dengan pebezaan prestasi bagi aksesi yang berbeza yang bergantung kepada tahap-tahap kemasinan. Eksperimen rawatan kemasinan yang dijalankan dalam kajian kita merangkumi penyiasatan sifat-sifat morfologi dan fizikokimia yang berbeza dan antioksidan krokot yang berharga bagi pemilihan aksesi garam-toleran terbaik dengan hasil yang optimum dan produktiviti dan nutrient antioksida. Berdasarkan data yang dihasilkan itu aksesi seperti ini boleh disyorkan untuk penanaman dalam kawasan tanah masin di Malaysia jaya serta faedah kepada pengguna dan kesihatan serta penggunaan lestari dan keberhasilan guna tanah.

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Very unfortunately just after passing my PhD viva, on 13/01/2015 my mother has left us forever from where she will never come. Since admission (08/09/2011) in UPM I didn’t be able to go to my country and didn’t see my parents over 3 years. My mother didn’t get chance to see his youngest son as Doctor (PhD). I feel very guilty that to date I didn’t be able to do anything for anyone especially for my parents. Ya Allah (ST) forgive my weakness and keep my mother’s departed soul in the never ending peach of Jannah!

Md. Amirul Alam
UPM Serdang, Malaysia
December 2014

I certify that a Thesis Examination Committee has met on 12 December 2014 to conduct the final examination of Md. Amirul Alam on his thesis entitled "Genetic Diversity and Effect of Salinity on Morpho-Physiochemical Properties of Purslane (*Portulaca oleracea* L.) Germplasm Collections" 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 fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
AMOVA	Analysis of molecular variance
ANOVA	Analysis of Variance
BCE	β-carotene equivalent
BHA	Butylated hydroxyanisole
cm	centimeter
DAT	Days after transplanting
DM	Dry matter
DPPH	1,1-Diphenyl-2-Picrylhydrazyl
DNA	Deoxyribo Nucleic Acid
$dS\ m^{-1}$	Decisiemens per meter
DW	Dry weight
FRAP	Ferric Reducing Antioxidant Power
FW	Fresh weight
g	gram
GAE	Gallic acid equivalent
L	Liter
mm	millimeter
mg	milligram
RCBD	Randomized Complete Block Design
RE	Rutin equivalent
RNA	Ribo Nucleic Acid
SAS	Statistical Analyses System
SEM	Scanning Electron Microscopy
TCC	Total Carotenoid Content
TE	Trolox equivalent
TFC	Total Flavonoid Content
TPC	Total Phenolic Content

CHAPTER 1

INTRODUCTION

The purslane (*Portulaca oleracea* L.), also known as pigweed, fatweed, pusle, and little hogweed, is an annual succulent herb in the family Portulacaceae that is found in most corners of the globe from sea level to 3835 m above sea level (Mitich, 1997). Purslane comprises about 150 species, of which about 40 species of tropical and warm climate species (El-Jack, 2004). It is most common in tropical and temperate environments, though it is reported from 58° north latitude to 40° south latitude (Matthews *et al.*, 1993). From the ancient age's purslane has been treated as a major weed of vegetables as well as other crops especially in turfgrass and field crops areas (Uddin *et al.*, 2009; 2010). Although it does like plenty of sunlight, captures carbon via the C4 pathway and can revert to CAM under severe stress, is not sensitive to soil conditions and colonizes all kinds of soils (Kamil *et al.*, 2000). Purslane has identified as so prolific due to its wonderful ability to produce seeds, even when the plant is in a very poor condition (Liu *et al*., 2000). The common purslane (*P. oleracea*) produces single or bunch of little yellow flowers at every nodes and ends of its stems but the ornamental one produces flowers of different colors. The blossom of common one remains open only for a while, but ensures the filled up capsule with numerous tiny black seeds that can remain alive as dormant for decades and even up to 40 years (Egley, 1974).

Presently *P. oleracea* is gaining special attention by agriculturalists and nutritionists. It is listed in the World Health Organization (WHO) as one of the most used medicinal plants and has been given the term 'Global Panacea' (Dweck, 2001; Samy *et al*., 2004). Research findings have been reported that *P. oleracea* comprises more nutritive value than other vegetables due to its valuable components such as omega-3 fatty acid, α-tocopherol, ascorbic acid, *β*-carotene and glutathione-rich (Uddin *et al*., 2014). Recent studies have shown that the *P. oleracea* is a rich source of omega-3 and 6 fatty acids, which is important in preventing heart attacks and strengthening the immune system (Simopoulos, 2004). The water extracts of *P. oleracea* shows no cytotoxicity or genotoxi-city, and have been certified safe for daily consumption as a vegetable (Yen *et al.*, 2001). Such beneficial effects of this vegetable weed might be ascribed to the presence of various bioactives and phenolics antioxidants. In spite of a number of studies made on the bioactivities of this plant, the antioxidant properties of *P. oleracea* have rarely been studied.

Currently, the development of modern plant breeding techniques have greatly facilitated wider use of a wealth of diversity from many sources including landraces, and especially, has allowed food production to keep up with population growth (Wood and Lanné, 1999). Currently, the genetic diversity of plants has been assessed more efficiently after the introduction of methods that reveal polymorphism directly from the biochemical and DNA levels. The characterization of plant germplasm using molecular marker techniques is playing an important role in the management and utilization of plant genetic resources worldwide (Hodgkin and Rao, 2002). Molecular characterization not only allows for a more objective quantification of genetic variation than do traditional morphological assessments, but also facilitates identification of duplicates, cataloguing of germplasm, assessment of genetic structure and establishment of core collections (Bretting and Widrlechner, 1995).

Importantly, genotypic characterization is now more attainable than it has been before with the advent of many molecular techniques over the last two decades (Karp, 2002). These include restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), simple sequence repeats (SSRs), expressed sequence tag (EST)-SSR, inter simple sequence repeat (ISSR) and single nucleotide polymorphisms (SNPs).

Soil salinity is the major abiotic stress that drastically affects plant growth and crop productivity worldwide. Due to continuous buildup of salinity in the soil, millions of hectares of arable land have now become unfit for cultivation (Ahmad *et al.,* 2007). It is well established that salinity can affect the plant growth by changing their morphological, physiological and biochemical as well as anatomical characteristics (Tester and Davenport, 2003). Changes in the lipid composition and oil quality characteristics and antioxidant properties of some oilseed crops as results of abiotic stresses such as salinity and drought have been reported recently (Ali *et al.,* 2010). Salinity is also a problem being faced by Malaysia. The extent of saline soils in Peninsular Malaysia has been reported to be 186,523.4 ha, in Sarawak 571,078.0 ha and in Sabah 358,434.0 ha (Dr. Lim Jit Sai, 2000, personal comm.). Due to anthropogenic contributions to global warming, the rate of sea level rise is expected to increase and thus causing salt water intrusion into the coastal areas and the major commodities threatened by salinity are agricultural crops (Aksoy *et al.,* 2003). As a result; the yield of crops are expected to decreases and it may no longer be possible to grow salt-sensitive species in salt affected areas (Singh and Chatrath, 2001). A plausible approach to cope with the salinity problem is the use of salt-tolerant crops (Shannon *et al.,* 2000).
Halophytes purslane has been rated as moderately tolerant with a salinity threshold of 18-24 dS m^{-1} (EC). However, after the first cutting, the halophytic nature of purslane is expressed and the salt tolerance of purslane increases with subsequent harvests (Grieve and Suarez, 1997). Purslane has been studied in detail as a prolific weed, but very little is known about its production as a food crop. The effects of cultural conditions on its nutritional value are rarely studied, although there some authors investigated the best cultural conditions to obtain higher levels of fatty acids (Palaniswamy *et al.,* 2001) and lower levels of oxalic acid (Palaniswamy *et al.,* 2002) in purslane leaves. Due to its high nutritive value and antioxidant properties purslane has considerable potential as human food, animal feed and medicinal applications (Yazici *et al*., 2007). Now it is believed that the regular consumption of dietary antioxidants may reduce the risk of several serious diseases. The diets rich in fruits and vegetables have been associated with health benefits, and this might be attributed to the presence of antioxidant compounds and salinity stress used to enhance the production of some major antioxidant compounds; an important characteristics of salinity tolerant crop species (Chu *et al.,* 2002).

Purslane (*P. oleracea)* is a promising crop to be grown under saline stressed areas of Malaysia. As salinity affects the morphological and physiochemical attributes of agricultural crops, need exists to appraise the influences of salinity on the proximate composition and antioxidant attributes of this valuable weed in order to explore its potential utilization as a functional food commodity. Thus, salt tolerant crop varieties are very essential to fulfill the yield gaps due to different biotic and abiotic stresses for the coastal agriculture in Malaysia and other Southeast Asian countries as well as additional foods demand of global increased population.

With this view, a series of research works have been conducted to identify morphological and genetic diversity among collections and to determine potential and more salt tolerant purslane accessions and their possible morpho-physiochemical changes in respect of salinity stress in Malaysia. Considering the above points, the studies were conducted with the following specific objectives:

i) To identify different kinds of purslane found in different areas of Western Peninsular Malaysia
ii) To evaluate yield potential and performance and to assess their morpho-physiological and genetic diversity among collected purslane accessions
ii) To screen out the salt tolerant purslane accessions
iii) To quantify the effect of salinity on growth, morphological and physiological development of *P. oleracea*
iv) To appraise the minerals nutritional profile and antioxidant properties of *P. oleracea* and their changes due to salinity stress.

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