



UNIVERSITI PUTRA MALAYSIA

***GAMETOPHYTE DEVELOPMENT, ANTIOXIDANT PROPERTIES AND
RESPONSE TO SALINITY OF *Brassica rapa* L. var. *parachinensis****

MONA GHANAD

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By

MONA GHANAD

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
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DEDICATION

In the name of the creator of Science

This thesis is dedicated to:

My Parents



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

GAMETOPHYTE DEVELOPMENT, ANTIOXIDANT PROPERTIES AND RESPONSE TO SALINITY OF *Brassica rapa* L. var. *parachinensis*

By

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October 2015

Chairman: Rosimah Nulit, PhD
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Brassica vegetables has been studied extensively due to high nutritional and medicinal values, as it is naturally low in fat, cholesterol, calories and sodium but rich in potassium, fiber, folic acid, and vitamins A, C, and K. This study was conducted to discover gametophyte development of *B. rapa* var. *parachinensis*, to compare antioxidant properties between leaves and seed of *B. rapa* var. *parachinensis* and among *B. rapa* var. *parachinensis*, *B. oleracea* cv. *alboglabra* and *B. rapa* cv. *chinensis*. The effects of individual salt that is NaCl, KCl and MgCl₂ on germination and seedling growth of *B. rapa* var. *parachinensis* were studied. Seeds (15 seeds) of *B. rapa* var. *parachinensis* plants were grown in a pot containing a mixture of top soil and river soil with ratio 3:1 at University Agricultural Park, Universiti Putra Malaysia (UPM) from July - December 2012. The inflorescences of *B. rapa* var. *parachinensis* in different stages which are from youngest buds to mature flowers were tagged, collected and fixed in FAA 70 (20% formalin, 10% acetic acid, 70% ethanol, v/v) and followed by histological works to discovered male and female gametophyte development. Gametophyte development of *B. rapa* var. *parachinensis* found similar with other angiosperms and *Brassica*. New embryological characteristics had been explored and not reported yet in any Brassicaceae which are the reduced type of anther wall development and the ovule type is Hemitropous. Pollen grain is rounded to ovate, triporate and tricellular. The female gametophyte is monosporic with polygonum type pattern. The composition and antioxidant capacity between leaves and seeds of *B. rapa* var. *parachinensis* and of three *Brassica* vegetables which are *B. rapa* var. *parachinensis*, *B. oleracea* cv. *alboglabra* and *B. rapa* cv. *chinensis* was studied. Seeds of *B. rapa* var. *parachinensis*, *B. oleracea* cv. *alboglabra* and *B. rapa* cv. *chinensis* were grown in the mixture soil with ratio 3 top soil and 1 river soil at Taman Pertanian Universiti, UPM from January – August 2014. Total chlorophylls, carotenoid, phenolics, flavonoids and ascorbic acid were measured spectrophotometrically. DPPH radical scavenging activity and reducing power assay was conducted to measure the antioxidative activity. Leaves and seeds of *B. rapa* var. *parachinensis* have significantly distinct antioxidative components (t-test, p<0.05) and the extract of seed has significantly higher capacity as scavenger of DPPH radical and reducing power than leaves (t-test, p<0.05). The antioxidative profiling of *B. rapa* var. *parachinensis*,

B. oleracea cv. *alboglabra* and *B. rapa* cv. *chinensis* are significantly different (ANOVA, $p < 0.05$). *B. rapa* var. *parachinensis* leaves contains significantly higher of chlorophyll ($49.9 \text{ mg}/0.1 \text{ g} \pm 2.3$), carotenoid ($31.1 \text{ mg}/0.1 \text{ g} \pm 1.3$) and flavonoid ($0.03 \text{ mg}/0.1 \text{ g} \pm 0.0005$) than *B. oleracea* cv. *alboglabra* and *B. rapa* cv. *chinensis*. On the other hand, *B. rapa* cv. *chinensis* leaves contain higher ascorbic acid than *B. rapa* var. *parachinensis* and *B. oleracea* cv. *alboglabra*. The effects of individual salts, NaCl, KCl and MgCl_2 were studied on germination and early seedling growth of *B. rapa* var. *parachinensis*. Four concentrations 50, 100, 150 and 200 mM were used for each salinity. The number of germinated seeds was recorded daily for 9 days. Hypocotyl and radical length and biomass of each seedling was measured at day 9. Germination percentage (% GP), salt tolerant and seed vigor were measured. *Brassica rapa* var. *parachinensis* seed had more viability with NaCl and KCl, but sensitive to MgCl_2 . The seed vigor and tolerance are significantly ($p < 0.05$) higher in KCl than NaCl in which the highest tolerance in 100 mM KCl. The patterns of hypocotyl and radical length found different in which NaCl retard the hypocotyls length but increased the radical length and biomass. On the other hand, KCl at any concentration increased the seed vigor, the tolerance, the length of both hypocotyls, radical and biomass. In conclusion, gametophyte development in *B. rapa* var. *parachinensis* was found similar to other flowering plants. Seeds of *B. rapa* var. *parachinensis* rich on antioxidant compared with leaves. The present findings also discovered that *B. rapa* var. *parachinensis* is a NaCl- tolerant and KCl- tolerant cultivars.

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

**PERKEMBANGAN GAMETOFIT, KANDUNGAN ANTIOKSIDAN DAN
RESPON KE ATAS KEMASINAN OLEH
Brassica rapa L. var. *parachinensis***

Oleh

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Kajian ke atas sayur *Brassica* iaitu kobis, brokoli, bunga kobis, 'brussel sprouts' dan kailan telah banyak dilakukan kerana kandungan nutrisi dan nilai perubatan yang tinggi di mana rendah lemak, kolesterol, kalori dan garam tetapi kaya dengan potassium, serat, asid folik dan vitamin A, C dan K. Kajian dijalankan bertujuan mengkaji perkembangan gametofit jantan dan betina sawi, untuk membandingkan profil dan aktiviti antioksidan di antara daun dan biji benih sawi dan di antara sayur sawi, kailan dan pakchoi. Kesan garam NaCl, KCl, dan MgCl₂ pada kepekatan yang berbeza ke atas percambahan biji benih sawi juga dilakukan. Sebanyak 15 biji benih sawi, ditanam di dalam tanah campuran (3 tanah atas: 1 tanah sungai) di Taman Pertanian Universiti, Universiti Putra Malaysia (UPM) dari Julai-Disember 2012. Kudup bunga dari peringkat muda hingga bunga yang matang diambil dan diawetkan untuk mengkaji perkembangan gametofit jantan dan betina. Kajian mendapati perkembangan gametofit pada sawi adalah sama dengan tumbuhan berbunga yang lain dan juga dengan sayur *Brassica* yang lain. Ciri-ciri baru embriologi telah ditemui iaitu jenis perkembangan dinding anter adalah "reduced" dan jenis ovul adalah hemitropus. Debunga sawi berbentuk bulat, ovat, triporat dan triselular. Gametofit betina adalah monospora dengan corak poligonum. Kajian perbandingan komposisi dan kapasiti antioksidan di antara daun dan biji benih sawi dan juga ke atas ketiga-tiga sayur *Brassica* dilakukan. Sawi, kailan and pakchoi ditanam di dalam tanah campuran (3 tanah atas: 1 tanah sungai) di Taman Pertanian Universiti, UPM dari Januari- Ogos, 2014 dan di atur secara rekabentuk blok rawak dengan lima replikasi. Kandungan klorofil, karotenoid, fenolik, flavonoid dan asid askorbik diukur secara spektrofotometrik. Aktiviti antioksidan diukur secara DPPH dan essay kuasa penurunan. Kedua-dua daun dan biji benih sawi menunjukkan komponen dan aktiviti antioksidan adalah sangat berbeza (ujian t, $p < 0.05$) dimana kandugan klorofil ($49.9 \text{ mg}/0.1 \text{ g} \pm 2.3$), karotenoid ($31.1 \text{ mg}/0.1 \text{ g} \pm 1.3$) dan fenolik ($0.03 \text{ mg}/0.1 \text{ g} \pm 0.0005$) lebih tinggi dari biji benih (t-test, $p < 0.05$). Walaubagaimanapun. biji benih sawi mengandungi 10 kali lebih asid askorbik ($0.22 \text{ mg}/0.1 \text{ g} \pm 0.019$) dibandingkan dengan daun sawi. Kapasiti aktiviti DPPH ($> 80\%$ pada $10 \text{ mg}/\text{essay}$) dan kuasa penurunan lebih tinggi (1.03 pada $10 \text{ mg}/\text{ml}$) dalam ekstrak biji benih dibandingkan ekstrak daun.

Ketiga-tiga sayur *Brassica* menunjukkan profil antioksidan yang sangat berbeza. Sawi mengandungi kandungan klorofil ($49.9 \text{ mg}/0.1 \text{ g} \pm 2.26$), karotenoid ($31.1 \text{ mg}/0.1 \text{ g} \pm 1.26$) dan flavonoid ($0.03 \text{ mg}/0.1 \text{ g} \pm 0.0005$) yang lebih tinggi dari kailan dan pakchois (ANOVA, $p < 0.05$). Manakala, kandungan asid askorbik paling tinggi pada pakchoi ($0.12 \text{ mg}/0.1 \text{ g} \pm 0.0017$) diikuti oleh sawi ($0.022 \text{ mg}/0.1 \text{ g} \pm 0.004$) dan kailan ($0.087 \text{ mg}/0.1 \text{ g} \pm 0.0012$). Ketiga-tiga ekstrak sayur *Brassica* pada kepekatan yang diuji berupaya bertindakbalas dengan radikal-radikal DPPH. Ekstrak sawi menunjukkan kapasiti tertinggi untuk bertindakbalas dengan DPPH diikuti dengan kailan dan pakchoi. Manakala, kailan mempunyai kuasa penurunan yang tertinggi (1.26 pada $10 \text{ mg}/\text{ml}$ ekstrak) diikuti oleh sawi (0.46 pada $10 \text{ mg}/\text{ml}$ ekstrak) dan pakchoi (0.43 pada $10 \text{ mg}/\text{ml}$ ekstrak). Kajian NaCl, KCl dan MgCl_2 ke atas percambahan dan anak pokok sawi pada kepekatan 50, 100, 150, dan 200 mM dan air dinyahion sebagai kawalan dilakukan. Sebanyak 10 biji benih sawi diletakkan di dalam piring petri yang mengandungi 5 ml air ternyahion atau setiap kepekatan garam. Eksperimen ini dilakukan secara rekabentuk blok rawak dengan tiga replikasi. Bilangan biji benih yang bercambah dikira setiap hari selama 9 hari. Pada hari ke 9, sebanyak lima anak benih diambil secara rawak dan panjang hipokotil, radikal dan biomas setiap anak benih dikira. Peratus percambahan, ketahanan terhadap kemasinan dan vigor biji benih dikira. Ketiga-tiga garam menunjukkan kesan yang berbeza terhadap percambahan benih sawi dimana biji benih lebih bertoleransi dengan NaCl and KCl tetapi sensitif terhadap MgCl_2 . Tahap toleransi dan vgor bijibenih adalah lebih tinggi di dalam KCl dibandingkan dengan NaCl (ANOVA $p < 0.05$). Corak percambahan biji benih sawi ke atas NaCl and KCl didapati berbeza. NaCl didapati merencat pemanjangan hipokotil tetapi merangsang pemanjangan radikal dan biomas. Manakala, KCl pada mana-mana kepekatan menambah pemanjangan hipokotil, radikal dan juga biomas. Kesimpulannya, perkembangan gametofit pada Sawiadalah hampir sama dengan tumbuhan berbunga yang lain, selain itu, biji benih sawi lebih kaya dengan sumber antioksidan berbanding daunnya. Kajian juga merumuskan bahawa biji benih sawi lebih toleransi dengan NaCl dan KCl.

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APPROVAL



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

Cl	Chloride ion
DAF	Day after Flowering/Fertilization
DCIP	2,6-Dichlorophenol Indophenol Sodium
DPPH	2,2-diphenyl-1-picrylhydrazyl
DW	Dry Weight
ES	Embryo Sac
FW	Fresh Weight
GAE	Gallic Acid Equivalent
K ⁺	Potassium ion
Mg ²⁺	Magnesium ion
μg	Microgram
μL	Microliter
QE	Quercetin Equivalent
SE	Standard Error
TAE	Tannic Acid Equivalent
w/v	Weight/Volume
v/v	Volume/Volume

CHAPTER 1

INTRODUCTION

1.1 Introduction

The Brassicaceae or Cruciferae is a large family that has more than 338 genera and 3709 species. This family comprises many economically significant production plants (e.g. cabbage and mustard), ornamentals (e.g., *Matthiola*, *Iberis*, *Arabis*, and *Draba*), and weeds (e.g., *Capsella bursa-pastoris* and *Thlaspi arvense*). Most species of this family are being used as vegetables, fodders and medicinal target for centuries and the family members usually show a wide distribution in temperate and cold regions. This family is known as the mustard family and easy to recognize by a cruciform (crossshaped) corolla, six stamens (the four inner stamens are higher than two outer ones) and a capsule often with a septum (Franzke *et al.*, 2011; Liu *et al.*, 2011).

Cruciferous vegetables including broccoli, cabbage, cauliflower, kale, mustard and cress are among prominent food crops throughout the world which are highly rich in vitamins C, D, E, soluble fiber and several nutrients. These vegetables contain a variety of anti-cancer healthy value that have been demonstrated to fight cancer cells and avoid them from forming. Phytochemicals from cruciferous vegetables induce detoxification enzymes, scavenge free radicals, alleviate inflammation, stimulate immune functions, decrease the risk for cancers, inhibit malignant transformation, and regulate the growth of cancer cells (Herr and Büchler, 2010).

Fruits and vegetables contain many antioxidants, including phenolics, carotenoids, anthocyanins and tocopherols (Naczka and Shahidi, 2006) whose intake been verified to be oppositely associated with the cardiovascular diseases and some cancers (Duthie *et al.*, 2000).

Some studies have been proven that the frequent consumption of cruciferous vegetables such as broccoli, cauliflower, cabbages, radishes, turnips, and kale, among others, is helpful to reduce the risk of developing chronic disorders, including cardiovascular diseases (Frei, 1995; Visioli *et al.*, 2000), stroke (Gillman *et al.*, 1995), diabetes (Craig, 1997), and even cancer (Ray, 2005; Stan *et al.*, 2008). *Brassica* comprises a large number of plant species, including many familiar vegetables. Due to their economical and agricultural importance, the *Brassica* plants have been the subject of much scientific interests particularly the *B. oleracea* L., *B. napus* L., *B. juncea* L., cauliflower and cabbage. However, limited studies have been conducted on choy sum, *B. rapa* var. *parachinensis* until to date. Therefore, this species was chosen in this study. Hong Kong Choy Sum (Malay called sawi) is a very popular vegetable in Asia especially in Malaysia. Hong Kong Choy Sum is herbaceous and easy to grow. The leaves are elliptical, with the ends usually blunt and hairless. The flowers are small, yellow colour, and four flowers crown with six stamens surrounds the pistil. The fruit resembles in a pod and a fruit called siliqua. *B. rapa* var. *parachinensis* contains a lot of vitamins A, B, C, E, K, carbohydrates, protein, calcium, potassium, iron and magnesium. This plant is very rich in fiber that can help the digestive process. There are also many diseases that can be avoided by eating this vegetable such as anemia,

reduce the risk of ulcer, reduce cholesterol levels in the blood and accelerates blood clotting during wound.

1.2 Problems Statements and Justification

Although embryological evaluation on the ontogenesis and megasporogenesis of angiosperms are long admitted and embryological characteristics are well recorded in botanical literatures for many species of angiosperms (Sundaresan and Alandete-Saez, 2010; Dubas *et al.*, 2012), however, study about members of Brassicaceae specifically is still limited. Therefore, the aim of this work is to study the male and female gametophyte developments in *B. rapa* var. *parachinensis* not only for systematic comparisons, but also the micro and mega gametophyte developmental processes.

ii) Scientists have indicated great interest about the function of free radical-mediated in the etiology of human illnesses. The use of artificial antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) has been restricted because of their toxic effects and carcinogenicity (Shahidi, 1997; Jeong *et al.*, 2004; Marmesat *et al.*, 2010), and toxicity effects like liver swelling have been reported (Siddhuraju and Becker, 2003) with synthetic antioxidants. In addition, synthetic antioxidants display low solubility and moderate antioxidant activity (Marmesat *et al.*, 2010).

Natural herbs and spices are known as the sources of natural antioxidants and thus play a crucial impress on the chemoprevention of diseases and ageing. An extensive list of studies have been published on the antioxidants and phenolic substances of spices (Shan *et al.*, 2005; Lu *et al.*, 2011), herbs (Silva *et al.*, 2007; Surveswaran *et al.*, 2007; Krishnaiah *et al.*, 2011), vegetables and fruits (Vasco *et al.*, 2008; Konczak and Rouille, 2011) and vegetables (Isabelle *et al.*, 2010; Halvorsen and Blomhoff, 2011) from different areas of the world, Malaysia somehow has not obtained such interest and research targeted on one of the essential vegetables that is commonly used as the main food. Therefore, there is a demand for scientific data on antioxidant potential of Brassicaceae family and their functions in the diet and health related issues of the native population of Malaysia.

iii) Some studies have been done extensively on the effects of salt stress on seed germination (Akbarimoghaddam *et al.*, 2011; Ologundudu *et al.*, 2014), but little information is available on the performance of economically important oilseed and vegetable crops on saline zone in which one of them is the Brassicaceae family. The current research paid attention to understand the seed germination requirements of *B. rapa* var. *parachinensis*. The effect of different accessibility of salinity levels on percentage of germination, rate of germination, and recovery responses of *B. rapa* seeds will be studied to reveal their individual effect and the interaction between these factors on the seed germination.

1.3 Objectives of Study

Hence, the objectives of this study are:

1. To study of male and female gametophyte development of Hong Kong Choy Sum (*B. rapa* var. *parachinensis*) flowers.
2. To compare the antioxidant properties between *B. rapa* var. *parachinensis* leaves and seeds.
3. To compare the antioxidant properties of *B. rapa* var. *parachinensis*, *B. oleracea* cv. *alboglabra* and *B. rapa* cv. *chinensis* leaves.
4. To study the effect of NaCl, KCl and MgCl₂ on germination of *B. rapa* var. *parachinensis* seeds.



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LIST OF PUBLICATIONS

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