Grafting to improve bitter melon (*Mormodica charantia* L.) productivity and fruit quality

By

Thanh Son Le

Master of Forestry in Silviculture

Supervisors:

Dr Sophie E. Parks

Dr Paul D. Roach

Dr Len Tesoriero

Dr Suzie Newman

August 2018

School of Environmental and Life Sciences

Faculty of Science

University of Newcastle

Australia

Thesis submitted for the degree of

DOCTOR OF PHILOSOPHY IN FOOD SCIENCE

STATEMENT OF ORIGINALITY

This thesis contains no material which previously has been accepted for the award of any other degree or diploma in any universities or tertiary institution. Further, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference has been made in the text.

Thanh Son Le

Date: 30th August 2018.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my special gratitude to my supervisor Dr Sophie E. Parks, for her guidance, encouragement, consultancy, patience, trust and understanding. She has been really conscientious in helping me to overcome the obstacles and make the completion for this thesis.

I greatly appreciate my co-supervisors Dr. Paul D. Roach, Dr. Len Tesoriero, and Dr. Suzie Newman for their support and invaluable advice during my study.

I would like to specially thank the Australia Awards and ACIAR – Australian Centre for International Agricultural Research for awarding me a full scholarship to study in Australia.

I would like to specially thank Joshua Jarvis, who supported and assisted me in the operation of the greenhouse systems and helped in conducting my experiments.

I would like to thank Associate Professor Dr. Minh Nguyen, who was a co-supervisor, and Mrs. Penny O'rock, a language teacher who helped me during my initial years studying at the University of Newcastle.

I would like to express appreciation to Dr. Quan Vuong, Associate Professor Dr. Chris Scarlett and Professor Dr. Martin Francis, for helping and encouraging me during my study. I would like to thank Mrs. Lynne William, my scholarship manager and Ms. Pam Steenkamp and Ms. Nicole Day for their administrative support.

Thanks are gratefully given to all of the staff within the School of Environmental and Life Sciences, the University of Newcastle, Ourimbah Campus, Australia and the National Institute of Medicinal Materials, Ministry of Health, Vietnam, for their huge assistance during my research program.

I take this opportunity to convey my sincere thanks to the Central Coast Primary Industries Centre, NSW Department Primary Industries for their excellent technical assistance with this study.

I would like to thank my friends, Mrs. Thuy Nguyen, Mrs. Xuan Tran, Mr. Tang Nguyen, Mr. Chuyen Hoang, Mr. Thanh Dang, Mrs. Quynh Pham and others, who provided support, time and advice.

Finally, I wish to acknowledge my parents, my wife, lovely son Minh Hieu and my beloved daughter Bao Ngan for their support, encouragement and sacrifice during my PhD research program.

ABSTRACT

Bitter melon (*Momordica charantia* L.) is a tropical and sub-tropical plant, which is widely cultivated in Asia and Africa. Bitter melon fruit has a remarkably long history of use as food and traditional medicine because it has high nutritional value and bioactive compounds. The demand for bitter melon is increasing but its cultivation is facing some challenges, such as low yielding varieties, soil-borne diseases and limited growth in harsh conditions. Traditional cultivation and/or the use of indigenous varieties are the main causes of low productivity compared to the commercial high-yielding varieties. Moreover, soil-borne diseases can also lead to yield loss. Pythium root rot and Fusarium wilt are common diseases that cause the death of seedlings and mature plants. Bitter melon performs poorly in unfavourable conditions, such as saline soil and cold temperatures. Unfortunately, bitter melon is increasingly being produced in sub-optimal conditions, including high salinity, and this is particularly the case in Vietnam. Therefore, it is important to improve the productivity of local varieties that can be tolerant to salinity and resistance to diseases.

The aim of this study was to improve the productivity and performance of a Vietnamese bitter melon variety (VINO 12) by grafting it on different rootstocks that may improve productivity, increase soil-borne disease resistance and enable it to be grown under saline conditions. In this study, rootstock seedlings were exposed to salinity and *Pythium aphanidermatum* treatments to evaluate their resilience to these stresses. The three rootstocks used in this study were pumpkin (*Cucurbita maxima*) varieties including Queensland Blue (Qb), Sampson (Sp) and Ringer (Rg). These were chosen because they are less affected by soil-borne diseases in Australia. Initially, the survival rate of the three rootstock and bitter melon scion seedlings was determined based on resistance to *Pythium aphanidermatum* and salinity. Then, the three rootstocks were used for grafting bitter melon and grown in subsequent experiments. Two grafting methods were applied, the single leaf splice (SLS) method and the tongue approach (TA) method. The most successful grafting method (SLS) was used in subsequent experiments. The grafted bitter melon plants were grown indoors and outdoors for two subsequent seasons (off season in 2016 and main season in 2017) under saline and non-saline conditions. The growth, fruit yield and fruit quality of the grafted plants grown under the different conditions were assessed to compare with controls (ungrafted and self-grafted) grown under the same conditions.

When tested with *Pythium aphanidermatum*, the Sp rootstock had the lowest rate of seedling death (29%) while Rg was second best (44%), bitter melon was the second worst (63%) and Qb was the worst (96%). All three rootstock and the scion seedlings could grow under saline conditions (16 dSm^{-1}) with survival rates of 60% and above. However, at 26 dSm^{-1}, the Sp rootstock seedlings had the highest survival rate (76%) and the Qb rootstock was the second best (52%) while the Rg rootstock and the bitter melon seedlings did not survive (0%).

The SLS grafting method was more successful than the TA method. The SLS method had a success rate of 81-91% for all three rootstocks, whereas the TA method only achieved a 60-76% success rate. The SLS method was then applied for grafting with the three rootstocks for growing in the subsequent experiments.

All three rootstocks and saline conditions at 16 dSm^{-1} did not significantly affect the development of the grafted plants grown indoors and outdoors for both main seasons and off seasons. However, the number of female flowers, fruits and fruit yield was influenced by the three rootstocks. In general, the grafted plants had more female flowers and fruits as well as a higher fruit yield than those of the control. Among the three rootstocks, the Rg and Sp rootstocks were found to have the highest fruit yield, which were from 45-53% and 39-64% higher for Rg and from 33-71% and 10-31% higher for Sp than that of the control plants under saline and non-saline conditions, respectively.

In terms of fruit quality, there was no consistent effect of the rootstocks and salinity. However, the Qb rootstock gave the best fruit quality under some limited and specific growing conditions. The main observation was that bitter melon fruit grown during the main season 2017 had higher TSC, TPC and antioxidant capacity than the fruits grown during the off season 2016. Of these, the fruits grown outdoor during the main season 2017 also had the highest TSC, TPC and antioxidant capacity. The values were 2-3 times higher for TSC, 9-10 times higher for TPC and 5-20 times higher for antioxidant activities for the plants grown outdoor during the main season 2017 than for those grown indoor.

In conclusion, the Sp rootstock seedlings had the highest resistance to *Pythium aphanidermatum* and salinity. The SLS method was superior for grafting bitter melon to rootstocks and all three rootstocks were suitable for grafting with the Vietnamese VINO 12 bitter melon scion. Among the three rootstocks, the Rg and Sp rootstocks were found to give the highest bitter melon fruit yield under both saline and non-saline conditions. However, there was no consistent effect of the rootstocks and salinity on the fruit quality although the Qb

rootstock gave the best fruit quality under some limited and specific growing conditions. Furthermore, growing the bitter melons outside during the summer season caused the biggest increase by far in the fruit TSC, TPC and antioxidant capacity. Therefore, the Sp rootstock is recommended to be used as rootstock for resistance to Pythium and salinity, while Rg and Qb are suggested to be used as rootstock for fruit yield and fruit quality, respectively, under select conditions.

Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS, SCIENTIFIC SYMBOLS AND UNITS OF MEASUREMENT

Abbreviations

a*	Red/green coordinate
b*	Yellow/blue coordinate
C	Chroma
CO_2	Carbon dioxide
CRD	Complete randomized design
DAP	Days after pollinating
DAS	Days after sowing
DW	Dry weight
EC	Electrical conductivity
ECe	Electrical conductivity of the soil saturation extract
Fig	Figure
FW	Fresh weight
GA3	Gibbrellic acid
HCl	Hydrochloric acid
Ho	Hue angle
H_2SO_4	Sulphuric acid
Kgf	Kilograms force
$K_2S_2O_8$	Potassium persulfate
L	Lightness
MeOH	Methanol
mL	Millilitre
mm	Millimetre
mPDA	Modified Potato Dextrose Agar
NAA	α- Naphthalene acetic acid
NaCl	Natri clorua
NaOCl	Javel or Hypochlorite
NaOH	Sodium hydroxide
Na_2CO_3	Soda or Sodium carbonate

NPK	Nitrogen (N), phosphorus (P), and potassium (K)
PA	*Pythium aphanidermatum*
PCA	Potato carrot agar
RHD	Rootstock hypocotyls diameter
RH	Relative humidity
SE	Standard error
SD	Standard deviation
SHD	Scion hypocotyls diameter
SLS	Single leaf splice
TA	Tongue approach grafting method
WA	Water agar

Units of measurement

%	Percentage
°C	Degree Celsius
dS/m (dSm^{-1})	Siemens per meter
g	Gram
g/L	Gram per litre
kg	Kilogram
cm	Centimeter
$g\ g^{-1}$	Gram per gram
$mg\ g^{-1}$	Milligram per gram
mm	Millimeter
nm	Nanometer
µl	Microliter
µm	Micromole
v/v	Volume per volume

COMMON AND SCIENTIFIC NAMES OF CROPS USED AND MENTIONED IN THIS STUDY

Common name	**Scientific name**
Bitter melon, bitter gourd	*Momordica charantia* L.
Bitter melon (small fruit)	*Momordica charantia* L. var. *minima* Williams et Ng.
Bitter melon (large fruit)	*M. charantia* L. var. *maxima* Williams et Ng.
Bottle gourd	*Lagenaria siceraria*
Cucumber	*Cucumis melo* L. var. *cantaloupensis*
Figleaf gourd	*Cucurbita ficifolia*
Luffa	*Luffa cylindrica*
Luffa	*Luffa aegyptiaca*
Melon	*Cucumis melo* L.
Muskmelon	*Cucumis melo* L. var. *reticulatus*
Rockmelon	*Cucumis melo* L.
Oriental melon	*Benincasa hispida*
Pumpkin	*Cucurbita maxima*
Pumpkin	*Cucurbita moschata*
Pumpkin, summer squash	*Cucurbita pepo*
Squash	*Cucurbita spp.*
Watermelon	*Citrullus lanatus*
Winter melon	*Cucumis melo* var. *inodorus*
Common bean	*Phaseolus vulgaris* L.
Eggplant	*Solanum melongena* L.
Red-pepper	*Capsicum annuum* L.
Peach fruits	*Prunus persica* (L.) Batsch
Quinoa	*Chenopodium quinoa* Willd.
Tomato	*Lycopersicon esculentum* Mill.
Tomato	*Solanum lycopersicum* L.

CHAPTER 1

LITERATURE REVIEW

Bitter melon or bitter gourd (*Momordica charantia* Linn), an important cucurbit species, is one of the major vegetables grown in the tropical regions of Asia, the Amazon, East Africa and the Caribbean and is cultivated throughout the world (Taylor 2002). Bitter melon has been regularly consumed as part of Asian and African traditional cuisines for centuries. It is a common cucurbit in the wild flora of Africa, occurring throughout most of tropical Africa and occasionally collected from the wild as a vegetable or medicinal plant. All parts of the plant have been used in indigenous medical systems. Leaves and especially fruit are used in folk medicine to treat diabetes in Asia (Chang et al. 2006) and the New World (Behera et al. 2011). The demand for the medicinal materials of bitter melon is increasing while bitter melon production can be constrained by some factors such as low yielding varieties, traditional cultivations, soil-borne diseases and saline soils.

Bitter melon fruit is a nutritious vegetable, rich in vitamins, iron, minerals, phosphorous and dietary fibre. The fruit can be cooked with other vegetables, stuffed, stir-fried or added in small quantities to beans and soups to provide a slightly bitter flavour (Behera et al. 2010). Bitter melon salad is a very popular food for the hot seasons in Vietnam (Vo 2012). In addition, the fruit can be dehydrated, pickled or canned. However, the most common food preparation style is for fruit to be blanched, parboiled or soaked in salt water before cooking to reduce the bitter taste. Flowers, young shoots and leaves are also cooked and eaten as leafy vegetables and are used in the preparation of tea (Bich et al. 2006, Behera et al. 2010).

Bitter melon has potential as a natural medicine. It can be used to produce medicines and functional foods shown to improve health and reduce the effects of diabetes. Many studies have shown that some medicinal products using bitter melon are beneficial for health. These products are not only effective in treating some diseases, such as type 2 diabetes, but also have anti-cancer, anti-virus, anti-inflammatory and cholesterol lowering effects (Budrat and Shotipruk 2009, Tan et al. 2014) as well as positive effects on cardio and cerebro-vascular diseases (Semiz and Sen 2007). Medicinal properties of the plant include anti-microbial, anti-cancerous, anti-mutagenic, anti-tumour, anti-infertility, anti-diabetic (Raman and Lau 1996, Klomann et al. 2010) and anti-rhematic properties (Thiruvengadam et al. 2012). Some *in vivo*

studies have shown that bitter melon fruit and fresh juice reduce adiposity, lower serum insulin and normalise glucose tolerance in animals (Semiz and Sen 2007).

Despite the increasing popularity of bitter melon and its potential as a natural medicine, a number of production issues need to be addressed. Growers do not have many interventions to increase the productivity of bitter melon under stressful conditions. For example, soil-borne diseases can have negative effects on fruit yield and quality (Abawi and Widmer 2000, Nisini et al. 2002). The problem of soil salinity is increasing, which has also lead to a reduction in the area of cultivated land (Abrol et al. 1988, Metternicht and Zinck 2003). Australia has 17.2–17.4 and Vietnam 1.0–2.0 million hectares of saline land (Rengasame 2006, Tien 2010). In addition, salinity is harmful to plant growth and decreasing crop yield (Yamaguchi and Blumwald 2005). Moreover, the use of local or indigenous varieties and traditional cultivation methods particularly has few interventions to increase the productivity of bitter melon in Vietnam. As a result, fruit yield is low; the average weight of fruit reaches 50–60 grams and the fruit yield usually achieves 10-13 tons per hectare (Hoi et al. 2013).

The main aim of this project was to evaluate the performance of bitter melon, in terms of fruit production (yield) and fruit quality in response stressful conditions (disease, pressure and increased salinity). Specifically, the intervention of grafting has been studied to address performance under these conditions.

1.1 An overview of *Momordica charantia* L. and its characteristics

Bitter melon belongs to Cucurbitaceae, a large family with 130 genera (Okoli 1984) and 950–980 species, including the mainly herbaceous climbers and woody lianas. The exact number of species in the genus *Momordica* is unclear. It has approximately 100-150 species, depending on the information source. According to Behera (2011), botanists have described over 150 species of *Momordica*, around 60-80 species in Africa (Dhillon et al. 2005, M. Rai 2008, Schaefer and Renner 2011) and 12 in Asia and Australia (Bharathi and John 2013). All of those have unisexual flowers, and of the African species, 24 are dioecious and 23 monoecious. All Asian species are dioecious (Schaefer and Renner 2010, Bharathi and John 2013). The bitter melon has nearly 40 varieties (Walters and Decker-Walters 1988).

1.1.1 Biological characteristics

Momordica charantia L. is known by many common names. There are nearly a hundred different names for bitter melon (Morgan and Midmore 2002). Some of these common names are listed in Table 1.1.

However, the names “bitter melon” or “bitter gourd” are the most popular English names and they have been used to describe most bitter melon varieties throughout the world.

Table 1.1 The name of *Momordica charantia* L. in some main areas and continents

Continent	Country/language	Name/local name
Asia	Japan/Japanese	Tsuru reishi, niga-uri
	Laos/Laotian	Phak, ha, haix, saix
	Malaysia/Malaysian	Peria, paippa, peiok, daun peria (leaves); daun peria katak (leaves)
	Philippines/Filipino	Ampalaya, palia, amargoso,
	Thailand/Thai	Mara, paya-aki, phakha, maha
	Australia/English	Bitter melon, bitter gourd
	Vietnam/Vietnamese	Muop dang (North), kho qua, o qua (South), muop mu, chua hao (Muong ethnic group – Thanh Hoa province; Ma hoi khom (Tay ethnic group – Cao Bang, Lang Son provinces)
	China/Mandarin	Foo gwa yip (leaves), foo gwa, fu kua, fu kwa, jin li zhi, kor-kuey, kugua, lao pu tao
	India/Hindu	Kareli, karela
Europe	England/English	Bitter gourd, African cucumber, alligator pear, balsam pear, bitter cucumber, carilla gourd, Chinese melon, Karella
	France/French	Margose, ame`re, paroka, momordique a feuilles de vigne, assorossie
	Italy/Italian	Pomo balsam
	Spain/Spanish	Balsamina, calabaza Africana, estropajo
Africa	Nigeria	Ejirin wewe, African cucumber
Latin America	Brazil/Portugese	Melão de São Caetano
	Latin	Balsam apple, balsam pear, bitter cucumber, bitter pear, carilla cundeamor, karolla.

Adapted from (Bich et al. 2006, Lombello and Pinto-Maglio 2007, Schaefer and Renner 2010, Soladoye et al. 2012, Rizvi and Mishra 2013)

Botanical description

Bitter melon is a scandent and monoecious annual plant. The plant has many stems and branches. The puberulent tendrils are 20cm long and the petioles are slender, 4-6cm long with white pubescent hairs (Figure 1.1). It flowers and fruits from May to October (Lu and Jeffre. 2011) in Vietnam and in Australia from December to July.

Figure 1.1 *Momordica charantia* (Bich et al. 2006)

1.1.1.1 Leaves

Bitter melon leaves are ovate-reniform or suborbicular (Figure 1.2). The approximate diameter of the leaf is 4-12cm, and the length of leaf is 4-12cm, membranous. The leaves are puberulent on veins and with 5-7 partite lobes (Hien and Widodo 1999, Do 2000). The form of lobes are ovate-oblong with the veins palmate. The margin of the leaf is crenate or irregularly lobed and the apex is obtuse or acute (Hien and Widodo 1999, Bich et al. 2006)

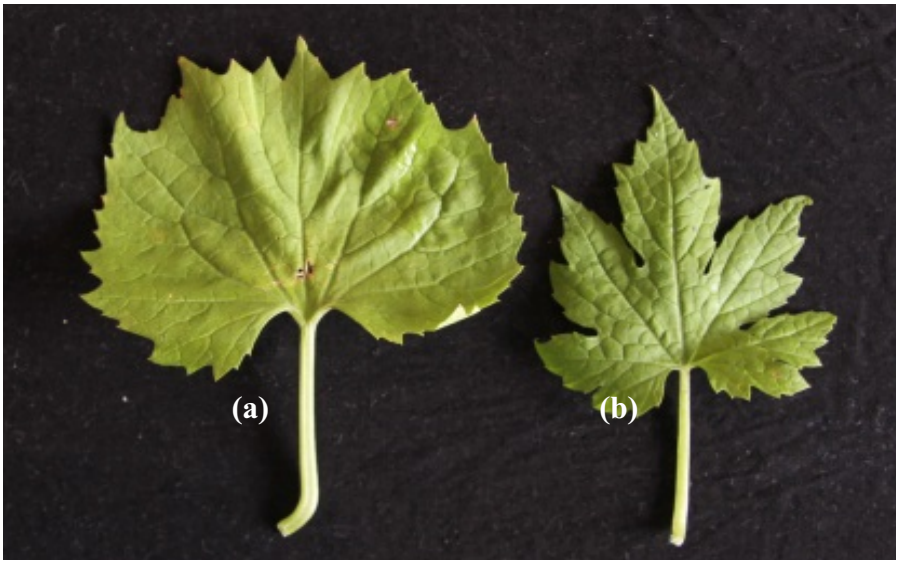


Figure 1.2 Bitter melon leaf: first leaf (a) and mature leaf (b)

1.1.1.2 Flowers

The bitter melon male flowers are solitary in axils of leaves with pedicel slender (Figure 1.3). The pedicel is 3-7cm long with puberulents. The flower has a median reinform bract or orbicular, 5-15mm long, and both surfaces have more puberulents. The flower's calyx is segmented, ovate-lanceolate, 4 – 6 × 2-3mm long with white pubescent and acute apex. The corollas are yellow with obovate segments that are 15-20mm and 8-12mm diameter and length respectively. The form of corolla is obtuse or retuse. The stamens are 3 and free. The anther cells are conduplicate.

The bitter melon female flowers are solitary with a pedicel that is 10-12cm long. The flower has a bract at its base. The ovary is fusiform and densely verrucose. The stigmas are expanded with 2 lobes (Hien and Widodo 1999).

Figure 1.3 Bitter melon flower: Female (a) and male flower (b)

1.1.1.3 Stem

Similar to other species in the Cucurbitaceae family, bitter melon has a basic stem structure, as shown in Figure 1.4. The schematic diagram shows a transverse section of a Cucurbita maxima stem, showing bicollateral vascular bundles with isolated strands of extra fascicular phloem in the periphery of the fascicular phloem, and entocyclic and ectocyclic extra fascicular phloem strands in the cortex. Also seen are extra fascicular commissural sieve tubes form the lateral connections between the longitudinal strands (Kempers et al. 1993).

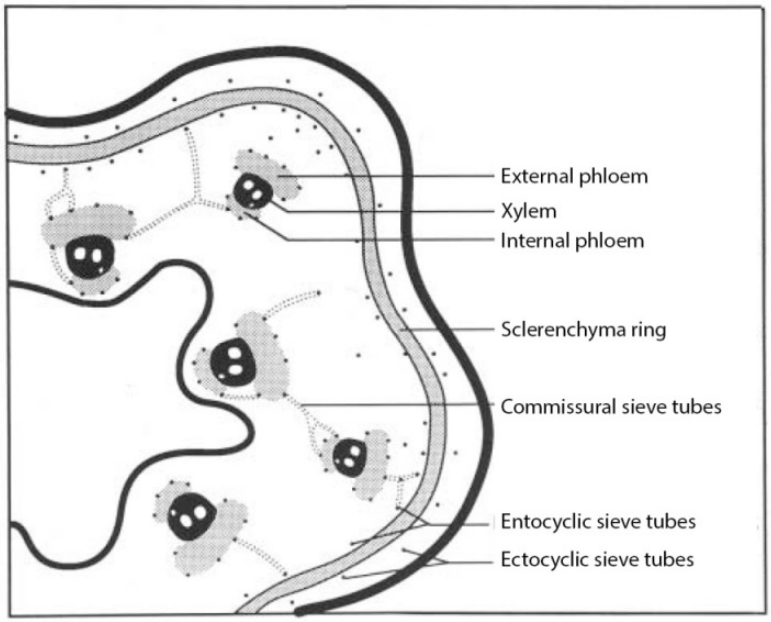


Figure 1.4 *Cucurbita* stem (Kempers et al. 1993)

1.1.1.4 Fruits and their components

Bitter melon fruits vary in shape and size; they can be ovoid, ellipsoid or spindle shaped. The approximate diameter of the fruit is 2-8cm, and the length is 11-45cm. The fruit is regularly spiny and warty or ridged, dehiscent with 3 fleshy valves (Hien and Widodo 1999).

The fruit is pale yellow-green to very dark green when young and orange when mature, hollow in cross-section, with a relatively thin layer of flesh surrounding a central seed cavity filled with large flat seeds and pith (Figure 1.5).

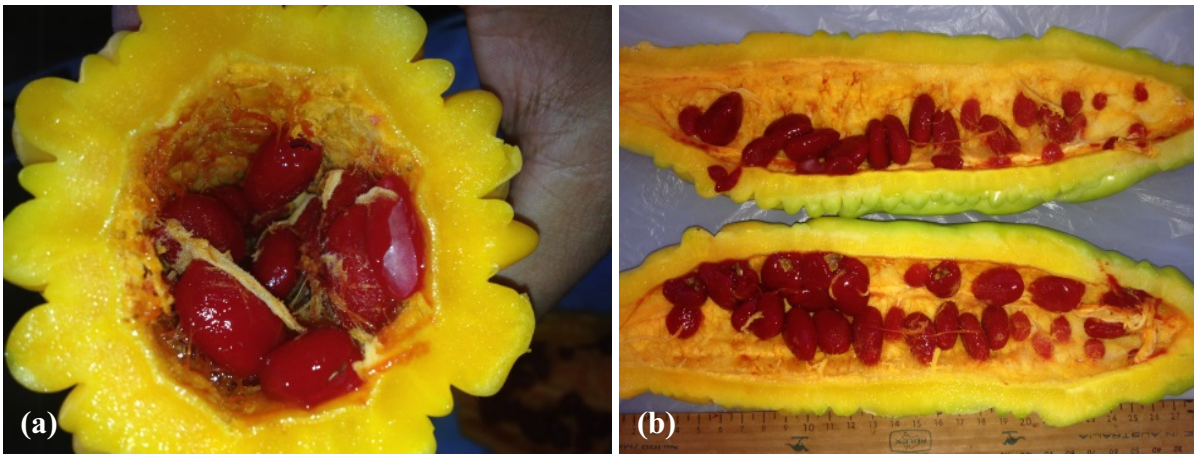


Figure 1.5 The ripe bitter melon fruit: cross-section (a), and long-section (b)

1.1.1.5 Aril and seeds

The bitter melon aril (pith) appears white in unripe fruit and red when the fruit ripens. The flesh is crunchy and watery in texture (Figure 1.5). The number of seeds per fruit ranges from 10-60. The seeds are oval-shaped and brown, 8-16mm in length, 4-10mm wide and 2.5-3.5mm thick (Figure 1.6).

Figure 1.6 Bitter melon seeds

1.1.1.6 Fruit types

Bitter melon fruit types are highly variable. For example, Table 1.2 describes the particular traits of bitter melon varieties that have been evaluated in Australia.

Many different bitter melon varieties, including indigenous and imported varieties are used in Vietnam (Pham and Nguyen 1999) and Australia (Morgan and Midmore 2002, Tan et al. 2014).

The choice of bitter melon variety grown by farmers depends on market preference and on their suitability for the conditions in which they are being grown. Growers are encouraged to compare the performance of several varieties during different seasons to identify superior types (Palada and Chang 2003).

In Vietnam, bitter melon is divided into two main groups or two sub-species based on the size, colour and shape of fruit (Pham and Nguyen 1999).

Table 1.2 The characteristic of fruit types evaluated in Australian research

Name of variety	Characteristic at harvest
Kiew Yoke 59	• Smooth light green fruit • Fruit weight 500 - 600g
Kiew Yoke 68	• Broad shouldered, glossy fruit • Fruit weight 500 - 600g
Known You Green	• Originated from Taiwan • Smooth, shiny, beautiful green skin • Fruit has ribbed stripes and weighs 400-700g • Flesh is green and mildly bitter
Verdure	• Fruit is short but eye-catching with a maximum weight of 500g • Green skin and light green flesh
Moonrise	• Fruit are long shaped, with light green skin and flesh • Weigh up to 700g
Moonlight	• Green skin, fruit weigh up to 650g • Light green skin and flesh
Moon Beauty	• Fruit are oblong shaped and have shiny white skin with a wart like surface • Fruit are 30cm long and 9cm width and weigh 700g • Thick and crispy flesh with great taste
New South Wales Local OP selection	• A cross between a locally grown Vietnamese type (of unknown origin) and Okinawa
Big Top	• Triangular shape with broad shoulder, weigh up to 300g • Green skin preferred by the Chinese communities
Jade	• Long, weigh up to 700g

Name of variety	Characteristic at harvest
	• Dark green skin • Preferred by the Indian communities, very bitter taste
Hanuman	• Medium-long, weigh up to 650g • Light green skin and flesh • A similar variety called "moonlight" is already grown in Australia and is used by most Asians in Australia
White	• Medium-long, weigh up to 650g • Pale green skin • Good producer and flavour preferred by the Chinese communities
Indra	• Short, but longer than Niddhi, weigh up to 50g • Dark green skin • Preferred by the Indian communities, very bitter
Niddhi	• Short, weigh up to 50g • Dark green skin • Preferred by the Indian communities, very bitter

Adapted from Morgan and Midmore (2002) and Tan et al. (2014).

Group 1: *Momordica charantia* L. var. *minima* Williams et Ng. (Figure 1.7). The group has three varieties, including long fruit, medium fruit and short fruit.

Figure 1.7 Some fruit styles of *Momordica charantia* L. *var. minima* Williams et Ng.

Group 2: *M. charantia L*. var. *maxima* Williams et Ng. (Figure 1.8). This group has two varieties, including white colour with long fruit and green or white-green colour with long fruit (more than 20cm).

Figure 1.8 Some fruit styles of *Momordica charantia* L. *var. maxima* Williams et Ng.

A table below describes the particular traits of bitter melon varieties growing in Vietnam.

Table 1.3 The characteristics of Vietnamese bitter melon varieties

Group	Characteristic
Momordica charantia L. *var. minima* Williams et Ng. (Figure 1.7)	• Fruit has green colour • Fruit diameter < 5cm, seed size 13-13.45mm x 6.8-8.5mm • Long fruit (12-22cm) • Medium fruit (8-12cm) • Short fruit (6-7.5cm)
M. charantia L. var. *maxima* Williams et Ng. (Figure 1.8)	• Fruit has a white and white-green colour • Fruit diameter > 5cm, seed size 14.8mm x 8.5mm • Long fruit (12-17cm) with white colour • Long fruit (more than 20cm) with green or white-green colour

Adapted from Pham and Nguyen (1999). However, others divided bitter melon into three main groups based on fruit shape and colour (Palada and Chang 2003):

Group 1: small, 10-20cm long, 100-300g, usually dark green, very bitter.

Group 2: long, 30-60cm long, 200-600g, light green in colour with medium size, protuberances, and only slightly bitter.

Group 3: triangular fruit type, cone-shaped, 9-12cm long, 300-600g, light to dark green with prominent tubercles, moderately to strongly bitter.

The distinction of bitter melon fruit morphology is not related to yield and fruit quality. It only helps the growers to judge the differences between bitter melon species or varieties (Fig 1.9). In fact, the selection of bitter melon varieties for growing can be changed after several growing seasons. However, the indigenous bitter melon varieties have higher saponin content in fruit, although their fruit types (diameter, length and weight) are smaller than commercial varieties (Pham and Nguyen 1999).

Figure 1.9 Some commercial bitter melon varieties supplied in 2015–2016 in Vietnam

1.1.2 Nutritional and medicinal properties

1.1.2.1 Nutrient composition

The fruits of bitter melon contain rich amount of vitamins, iron and phosphorous (Thiruvengadam et al. 2012). Fresh bitter melon is used as a nourishing food, as it contains 93.8% water, 0.9% protein, 0.1% lipid, 3.3% dietary fibre, 0.6% ash, and 0.05% vitamin C, as well as 20 kJ energy per 100g, (Zhu et al. 2012). The fruit is also rich in minerals including potassium, calcium, zinc, and magnesium and is a good source of dietary fibre (Joseph and Jini 2013).

The quantity and active ingredient of bitter melon fruit depends on the different varieties and the planted regions. Some bitter melon varieties cultivated in Bangladesh contain more than 72% fatty acids, 86.83-91.09% neutral lipids, 4.37-7.34% glycolipid, 3.22-4.62% phospholipids, 33.93-36.21% lipid, 18.23-21.36% protein, 383.45-440.96 µg/g calcium, 41.10-45.03 µg/g ion, and plus other essential minerals (Ali et al. 2008).

According to Joseph and Jini (2013), the fruit also contains high amounts of vitamin A, vitamin E, vitamin B1, B2 and B3, as well as vitamin B9 (folate) which are listed in Table 1.4. The

caloric values for leaf, fruit and seed are 213.26, 241.66 and 176.61 Kcal/100g, respectively (Morgan and Midmore 2002, Joseph and Jini 2013).

Table 1.4 Proximate principles and nutrient composition of bitter melon fruit

Nutrient/vitamin	Unit	Content
Carbohydrate	g/100g	2.83-10.6
Potassium	mg/100g	171-265
Sodium	mg/100g	2.4
Calcium	mg/100g	23.4-38.0
Iron	mg/100g	1.55-2.0
Copper	mg/100g	0.19
Manganese	mg/100g	0.08
Zinc	mg/100g	0.46
Vitamin B2	mg/100g	0.45
Vitamin B3	mg/kg	0.89
β- carotene	mg/kg	1.95
Moisture	g/100g	83.2-92.5
Crude saponin	g/100g	7.80

Adapted from Behera et al. (2010) and Hoi et al. (2013).

Research has found that the leaves are nutritious sources of calcium, magnesium, potassium, phosphorus and iron. Both the edible fruit and the leaves are great sources of the B vitamins (Sathish Kumar et al. 2010). The seeds contain galactose binding lectins, vicine, amino acids, fatty acids, terpenoids, and momordicosides (A, B, C, D and E) (Raman and Lau 1996, Behera et al. 2011).

1.1.2.2 Biological activities and health benefits

Bitter melon is "sweet" for our health (Fang and Ng 2013). The Orient's traditional medicine shows that different parts of the bitter melon plant have been used for different purposes. The leaves and flowers are commonly used in tea for stomach pain, for bathing and to reduce prickly heat disease (skin inflammation) in children (Do 2000, Tien 2005, Vo 2012). The entire bitter melon plant has been used for diabetes and dysentery. The roots have been used for tumours, wounds and rheumatism and are reputed to have an aphrodisiac property (Taylor 2002). More importantly, bitter melon plants are becoming more popular as a medicine to regulate blood

sugar and other diseases related to insulin in the human body today (Pham 2001, Keller et al. 2011). The fruit also has been demonstrated to contain charantin, steroidal saponin, momordium, carbohydrates, mineral matters, ascorbic acid, alkaloids and glucosides. Studies have shown some bioactive compounds in the fruit, such as total phenolic compounds, total saponin compounds and total antioxidant activity (Tan et al. 2014). The ethanolic extract of the fruit showed the presence of alkaloids, tannins, glycosides, steroids, proteins and carbohydrates (Patel et al. 2010). Therefore, the extracted flesh, juice and dried fruit have beneficial effects on health (Tan et al. 2014). Other studies have reported that the fruit products have been linked with a wide range of therapeutic effects, such as anti-cancer, anti-viral, anti-inflammatory and anti-diabetic properties (Pham et al. 2011, Vo 2012).

The use of different parts of bitter melon plant, including stem, leaf and root, depends on the experiences of different ethnic communities. In the Amazon, the plant has a long history of use by indigenous peoples. The leaves are used like a tea for diabetics and as an antiviral for measles and hepatitis. In addition, it is also used to treat skin diseases, such as sores, wounds and infections. In Brazil, bitter melon is used for tumours, wounds, rheumatism, malaria, leucorrhoea, inflammation, menstrual problems, diabetes, colic, fevers, worms, to induce abortions, and as an aphrodisiac. It is also employed topically for skin problems, such as eczema and leprosy. In Mexico, the entire plant is used for diabetes and dysentery and the root is a reputed aphrodisiac. In Peruvian herbal medicine, the leaves or aerial parts of the plant are used to treat measles, malaria and all types of inflammation. In Nicaragua, the leaf is commonly used for stomach pain, diabetes, fevers, colds, coughs, headaches, malaria, skin complaints, menstrual disorders, aches and pains, hypertension, infections and as an aid in childbirth (Pham et al. 2011, Vo 2012, Joseph and Jini 2013).

Bitter melon has been used as a traditional antidiabetic remedy in Eastern countries for many years. The medicinal value of bitter melon has been attributed to its high antioxidant properties due to in part to phenols, flavonoids, isoflavones, terpenes, anthroquinones and glucosinolates, all of which confer a bitter taste (Joseph and Jini 2013). Table 1.5 shows some information of the worldwide ethno-botanical uses.

1.1.2.3 Phytochemistry

Phytochemists have recently isolated a number of potential medical components from this plant such as the ribosome inactivating protein (RIP), MAP30 (Momordica anti-HIV protein), which suppresses HIV (human immunodeficiency virus) activity, M. charantia lectin (MCL), M.

charantia inhibitor (MCI) and momordicosid A and B, both of which can inhibit tumour growth (Thiruvengadam et al. 2012). The total saponins, phenolic compounds and antioxidant properties in bitter melon fruits have recently been reported as valuable compounds for the antioxidant activities in foods (Tan et al. 2014). In fact, some bitter melon products for people with diabetes are being sold in chemist warehouses in Australia in 2017 (Figure 1.10).

Table 1.5 Worldwide ethnobotanical uses of bitter melon

Country	Use
Brazil	Burns, colic, diabetes, eczema, fever, hemorrhoids, hepatitis, leprosy, leucorrhoea, malaria, menstrual colic, pain, pruritus, rheumatism, scabies, skin, tumors, vaginitis and wounds.
China	Aphrodisiac, cancer (breast), diabetes, food, glucosuria, halitosis, hematuria, polyuria and refrigerant.
Colombia	Bites (snake) and malaria.
Cuba	Anemia, colitis, emmenagogue, fever, hepatosis, hypoglycemic, kidney (stone), sterility (female) and vermifuge.
Ghana	Aphrodisiac, dysentery, fever and gonorrhoea.
Nigeria	Anti-diabetic
Haiti	Anemia, appetite stimulant, dermatosis, eye, fever, insecticide, laxative, liver, skin, rage and rhinitis.
India	Contraceptive, diabetes mellitus, dysmenorrhea, gout, jaundice, kidney (stone), laxative, leprosy, liver, pneumonia, rheumatism, scabies, skin, tonic and vegetable.
Mexico	Aphrodisiac, burns, diabetes, dysentery, purgative, scabies and vermifuge.
Malaya	Abdomen, asthma, burn, dermatosis, diarrhea, headache, scalds and stomach ache.
Nicaragua	Headache, anemia, blood, childbirth, cold, cough, diabetes, fever, hypertension, infection, malaria, pain (stomach), pain (menstrual), rash and lung.
Panama	Cold, diabetes, fever, gallbladder, hypertension, insecticide, malaria and pruritus.
Peru	Diabetes, diarrhoea, inflammation, lung, malaria, measles, skin (sores) and wounds.

Country	Use
Thailand	Antidiabetic, anti-tumorous, anti-cancer, cholesterol lowering.
Trinidad	Diabetes, dysentery, fever, hypertension, malaria and rheumatism.
Elsewhere	Allergy, arthritis, asthma, antibiotic, aphrodisiac, dyspepsia, dysentery, dysmenorrhea, earache, hypertension, insecticide, jaundice, leprosy, liver, night blindness, pain (intestine), phlegm, psoriasis, menstrual abnormalities, ringworm, roundworms, soap, splenitis, styptic, throat (sore), thrush, tiredness and wounds.

Adapted from Taylor (2002), Soladoye et al. (2012) and Budrat (2009)

Figure 1.10 Some products from bitter melon

The main constituents of bitter melon responsible for its anti-diabetic properties, are glycosides, saponins, alkaloids, phenolic constituents, fixed oil and free acids (Liu et al. 2009). Bitter melon also consists of alkaloids, charantin, chorine and momordicosides (G, F1, F2, I, K, L) (Harinantenaina et al. 2006, Behera et al. 2011). In addition, it contains acids such as myristic acid, oleanolic acid, oleic acid, axalic acid, proteins, lutein and lycopene. The fruit pulp also has soluble pectin. Moreover, other studies show that the main constituents of bitter melon which are responsible for the antidiabetic effects are triterpene, proteid, steroid, alkaloid, inorganic, lipid and phenolic compounds. Several glycosides have been isolated from the *M.charantia* stem and fruit. These glycosides are grouped under the genera of cucurbitane type triterpenoids. Specifically, four triterpenoids have AMP-activated protein kinase activity which is a plausible hypoglycaemic mechanism of *M.charantia* (Tan et al. 2008).

In numerous studies, at least three different groups of constituents were found in all parts of bitter melon having clinically demonstrated hypoglycemic properties (blood sugar lowering) or other actions of potential benefit against diabetes mellitus. These hypoglycemic chemicals include a mixture of steroidal saponins known as charantins, insulin-like peptides and alkaloids. The hypoglycemic effect is more pronounced in the fruit of bitter melon where these chemicals are in greater abundance. To date, close to 100 in vivo studies have demonstrated the blood glucose-lowering effect of bitter melon fruit (Taylor 2002, Vo 2012).

1.2 Challenges for bitter melon production

The cultivation of bitter melon has challenges including soil-borne diseases, soil salinity and climate stress. Therefore, studies on the grafting of bitter melons are desirable to demonstrate rootstocks that have tolerance or resistance to these constraints.

1.2.1 Diseases

Like other cucurbit species, bitter melons are susceptible to approximately 200 diseases (Chandra et al. 2010). The root rot caused by Rhizoctonia sp, damages seedlings (Hoi et al. 2013). Some diseases, such as Fusarium wilt and Pythium root rot, can cause widespread stem death in short time, thus significantly reducing yield and fruit quality. These are the most damaging diseases to the yield of bitter melon. The effects of Fusarium wilt on bitter melon vigour are shown in Figure 1.11 (Singh et al. 2012).

Plant parasitic nematodes are also an important cause of root disease in bitter melon. Other pathogens or environmental stress may cause similar symptoms either alone or in combination with nematodes. However, nematodes are often overlooked in searching for the cause of these symptoms (Singh et al. 2012). The effect of root-knot nematodes on bitter melon root is shown in Figure 1.12.

Figure 1.11 Wilting and drying out of bitter melon caused by *Fusarium oxysporum* f.sp *momordicae* (Singh et al. 2012)

Pythium species cause seedling blights and death, and cause feeder rootlet rot of mature plants. The most important species is *Pythium aphanidermatum* which has a mainly tropical distribution and is pathogenic to a wide host range. It infects mainly roots of seedlings or the root tips of older plants and that consistently inhibits root growth (Heine et al. 2006). This disease can also infect the feeder rootlets, causing stunting and yellowing of the leaves of older plants, causing stem rot and eventually post-emergence damping-off (Burgess et al. 2008).

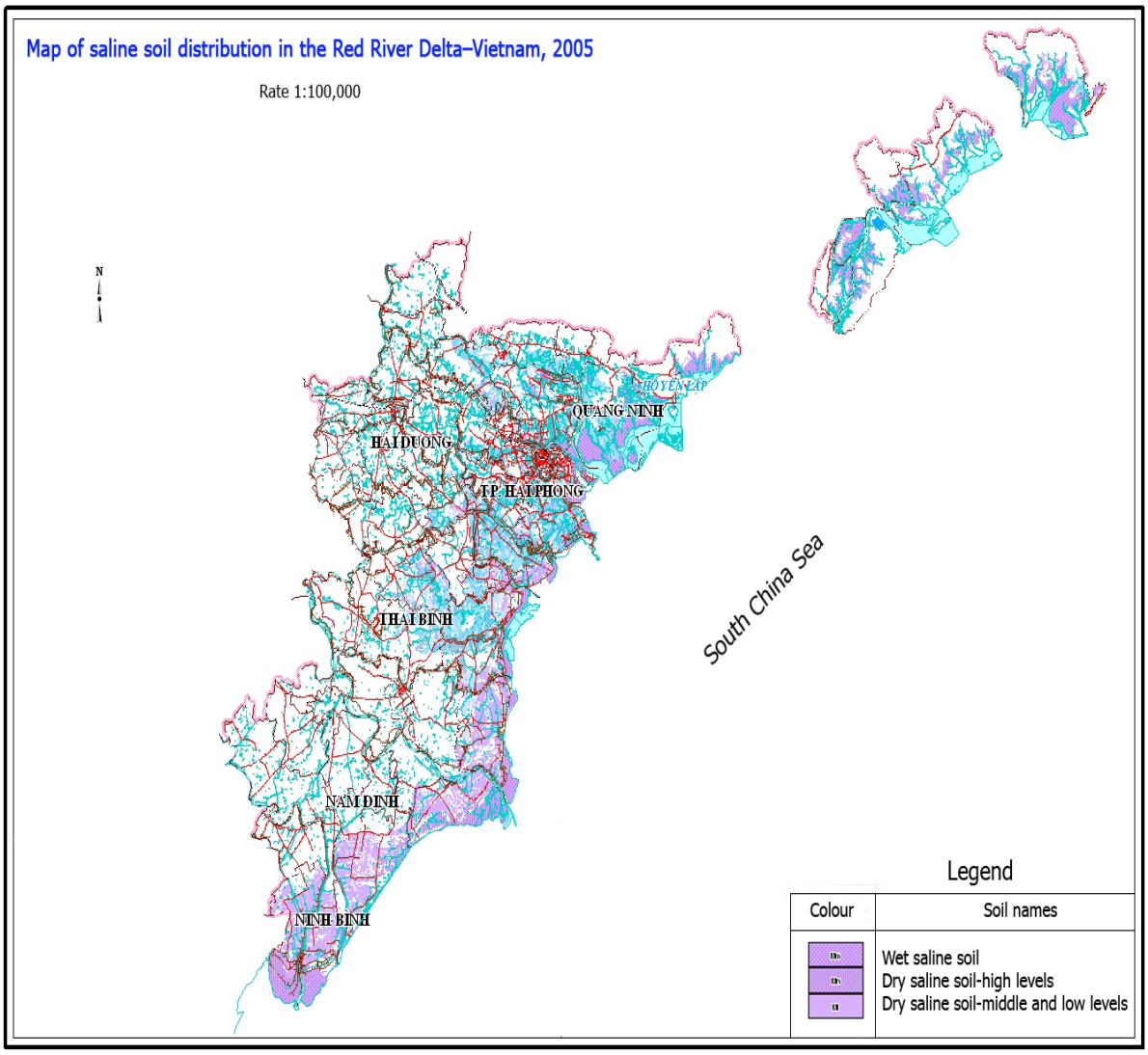
Figure 1.12 The effect of Root-Knot Nematodes on bitter melon root (Singh et al. 2012)

According to Davis et al., (2008) the main problem with bitter melon production in Asia is Fusarium wilt caused by *F. oxysporum* f.sp. *momordicae*. In Vietnam *F. oxysporum* formae speciales also affect the yield of pumpkin, watermelon and cucumber while *Pythium* species cause widespread damage, while also significantly reducing the yield of bitter melon (Dau et al. 2009, Hoi et al. 2013).

1.2.2 Salinity

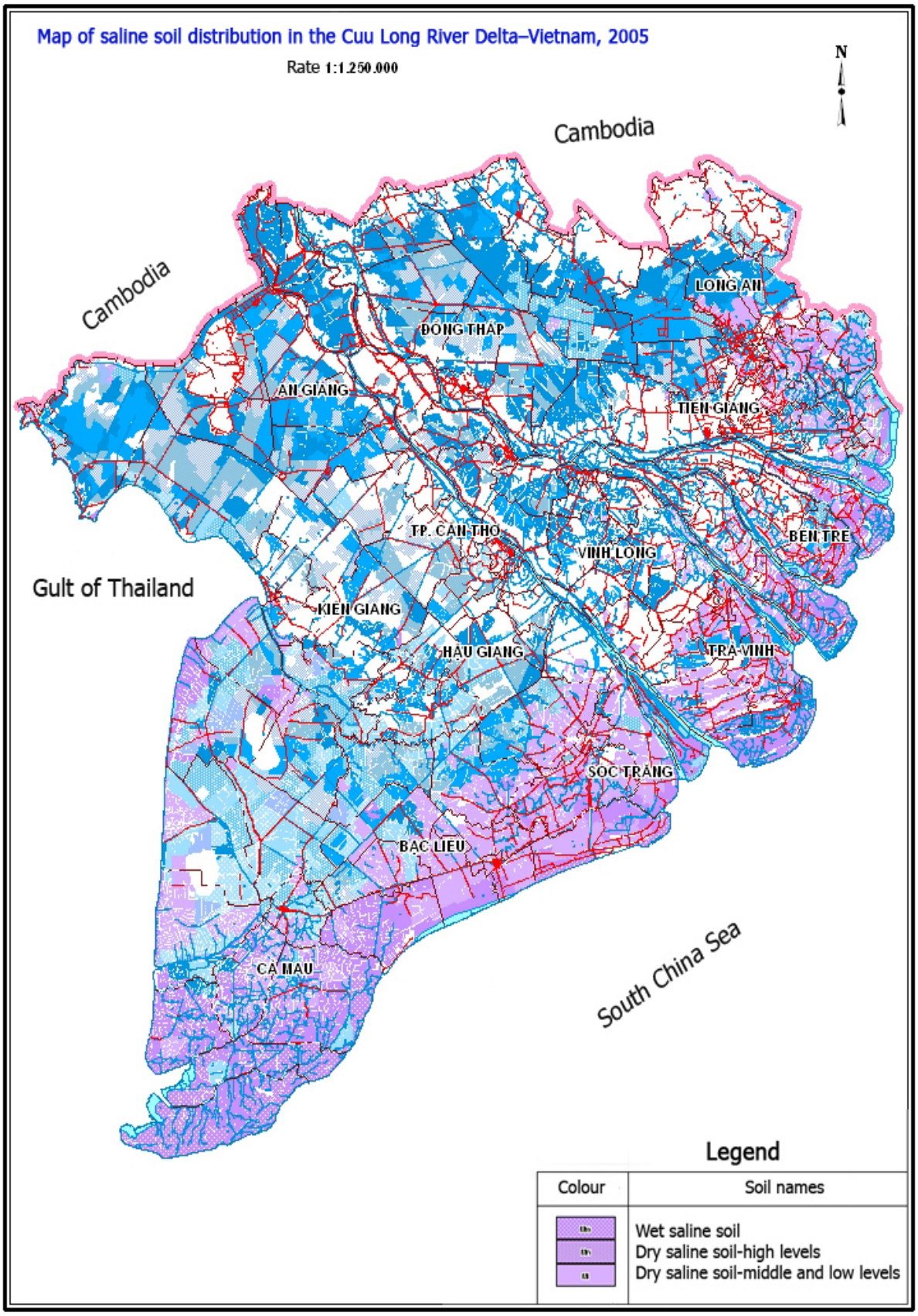
Salt-affected soils occupy nearly 7% of the world's land area (Chhabra 1996). The extent of salt-affected soils in different parts of the world increases annually and directly threatens agricultural production. Pichu (2006) estimated that approximately 17.4 million hectares of Australia's agricultural and pastoral zone have high salinity levels from 4.0 dSm^{-1} to 16.0 dSm^{-1}, while the size of land with these salinity levels in Vietnam is roughly 1.0 million hectares (Đức et al. 2009, Đức and Đạo 2011). It has been widely recognised that saltwater intrusions into agricultural land can come from aquacultural shrimp ponds and brackish water (Tho et al. 2008).Worldwide, more than 800 million hectares of soils are salt-affected, therefore, choosing salt-tolerant crops and managing soil salinity are important strategies to boost agricultural economy (Rengasamy 2010).

The two major production areas in Vietnam (Figure 1.13 and Figure 1.14), including the Red river delta in the North (Đức et al. 2009) and the Cuu Long river delta in the South (Đức and Đạo 2011) are affected by saline soils. Crops cannot be grown in these dry saline soil areas (Table 1.6). In addition, another agricultural production area in the South of Vietnam, the Mekong delta has soil salinity range of 29.25 dSm^{-1} to 33.44 dSm^{-1}. Farmers in these areas have switched *en masse* from rice cropping to shrimp culture in wet areas. Due to the recent failures in shrimp farming, many farmers wish to revert to a rotational system with rice in the wet season and shrimp in the dry season. So far, all their attempts to grow rice have failed (Tho et al. 2008).



Adapted from Đức et al. (2009).

Figure 1.13 Saline soil areas in the Red river delta, Vietnam 2005 (Rate 1:100,000)



Adapted from Đức and Đạo (2011).

Figure 1.14 Saline soil areas in the Cuu Long river delta, Vietnam 2005 (Rate 1:250,000)

Plants affected by salinity are stunted and grow more slowly than those unaffected (Läuchli and Grattan 2007, Shrivastava and Kumar 2015). Table 1.6 indicates the yield reductions that could be expected when various vegetable crops are irrigated with saline water and grown in moderate-to-slow-draining soils in some experimental studies. The growth of many plants, including cucurbit species is restricted at salinity levels between 4.0 to 8.0 dSm^{-1} (Tien 2010).

Table 1.6 The area of saline soil in two largest agricultural productions in Vietnam

Soil names	**Salinity levels (dSm^{-1})**	**Acreage (ha)**	
		Red river delta	**Cuu Long river delta**
Wet saline soil	>16.0	36607.39	119910.55
Dry saline soil-high levels	8.0-16.0	30140.75	283574.79
Dry saline soil-middle and low levels	2.0-8.0	65504.99	480714.31
Total		**132253.13**	**884199.65**

Adapted from Hồ Quang Đức (2009), Hồ Quang Đức and Nguyễn Văn Đạo (2011).

Some cucurbit species are moderately tolerant to tolerant of salinity, such as cucumber 2.5 dSm^{-1} and pumpkin 3.9 dSm^{-1} (Kotuby-Amacher et al. 2000). Although there has been no study on the effects of salinity on bitter melon, based on the experience with other cucurbits we could predict that the level of tolerance of bitter melon to salinity would not exceed 8.0 dSm^{-1} (Table 1.7).

Table 1.7 Effects of salinity levels on the fruit yield of some cucurbit species

Cucurbit crop	**Threshold value**	**Yield loss**			**References**
		10%	**25%**	**50%**	
	dSm-1				
Cucumber	2.5	3.3	4.4	6.3	
Pumpkin	3.9	4.9	5.9	7.9	(Kotuby-Amacher et al. 2000)
Watermelon	2.0	2.5	3.5	4.5	

1.2.3 Bitter melon crop production

1.2.3.1 Climatic requirements

Bitter melon grows well under conditions similar to those preferred by other cucurbits, such as cucumber, gourd, pumpkin and luffa (Palada and Chang 2003). It is normally grown as an annual crop, and is mainly cultivated during the spring, summer, and rainy seasons, with some winter production in subtropical climates. In tropical climate regions, it is cultivated throughout the year (Bich et al. 2006). However, there are different opinions on optimum temperature for good plant growth, which are related to fruit yield (Table 1.8).

In Australia, winter production occurs in the Northern Territory, Queensland and in the north of Western Australia. Summer production occurs in New South Wales and Victoria. Bitter melon growing in Sydney usually commences between September and October and continues until May when minimum temperatures are warmer. Corresponding to temperature, humidity requirements for bitter melon are likely to be similar to those for other cucurbit species (Morgan and Midmore 2002).

In Vietnam, bitter melon is usually planted at the end of March in the north, when the weather is warmer, and finished in September. However, in the South of Vietnam this plant can grow all year around because the average temperature consistently ranging from 27.9ºC to 30.5ºC, which falls within the suitable temperature range for growing bitter melon (Hoi et al. 2013). Bitter melon is often planted on hillsides or at the front of houses for shading, or on fences or shrubs (Bich et al. 2006, Vo 2012). In Africa and most countries in Asia, bitter melon is also grown in the field on trellises such as bamboo poles, wood stakes, PVC pipes or other sturdy materials to provide support and to keep the fruit and foliage off the ground (Palada and Chang 2003).

1.2.3.2 Outdoor production

Bitter melon is cultivated and marketed by smallholder farmers, and is an important crop in home gardens throughout southern and south-eastern Asia (Dhillon et al. 2016). Depending on the investment, bitter melon can be produced in the field or without the use of automatic fertigation system. Nutrition is provided to the plants through drippers in these systems and designed the same as plants growing indoors.

Table 1.8 The climatic classification of bitter melon and some other cucurbit crops

Crop location	Temperature (°C)			Yield (tonnes/ha)	References
	Acceptable for germination	Optimum for yield	Acceptable for growth		
India	18-27	25-30	18-30	NA	(Davis et al. 2008, Behera et al. 2010)
Australia	10-50	24-27	16-35	23-81	(Morgan and Midmore 2002)
Vietnam	10-25	20-35	18-35	10-13	(Hoi et al. 2013)

1.2.3.3 Indoor production (under cover production)

Greenhouse production of bitter melon is not common. A greenhouse can be used for bitter melon production to extend the growing season. However, in a closed greenhouse without insects, hand pollination is required (Tan et al. 2014) since the fruit yield is highly dependent on the proportion of flowers that are pollinated. In one study, the greenhouse temperature was maintained between 18°C and 30°C and humidity between 60 and 80% (Tan et al., 2014).

Indoor production for fresh vegetables offers advantages compared to outdoor production with regard to quality principally because the products are not exposed directly to the rapid changes of climate conditions. On the other hand, vegetable cultivation in a greenhouse under artificially created conditions may affect the internal quality of the product. This is reflected in a different taste and flavour between indoor and field vegetables (Gruda 2005).

1.2.3.4 Soil and fertiliser requirements

Bitter melon tolerates a wide range of soils but prefers a well–drained sandy loam soil rich in organic matter. There are many different opinions about the most suitable pH for the growth and development of bitter melon (Table 1.9).

In Australia, if soil is highly fertile and prepared with enough organic matter, further feeding may not be necessary. Typically, 2L of mineral fertilizers per hectare is applied monthly and can be added after planting until the plant reaches trellis height, then potassium nitrate at 50 kg/ha up to flowering (Morgan and Midmore 2002). Nitrogen application can be reduced during fruit set as nitrate is known to suppress flowering in many species (Lovatt 1999). The addition of calcium nitrate at 50 kg/ha until flowering improves shelf life (Dordas 2009).

The optimal proportion of N, P and K used for growing bitter melon is around 100:50:50 kg/ha respectively (Morgan and Midmore 2002). Vietnamese farmers usually use a fertiliser mix, including composted manure, NPK (5:10:3), urea and potassium at different times. For example, composted manure and fertiliser should be applied as basal dressings with urea and potassium as top dressings throughout the growth cycle (Hoi et al. 2013).

Table 1.9 Soil pH conditions used to grow bitter melon

Soil pH conditions	Growing system	Reference
6.0-6.7 (max 8.0)	Field	(Palada and Chang 2003)
6.0-6.7	Field	(Morgan and Midmore 2002)
6.0 – 6.7 (max 8.0)	Field	(Behera et al. 2010)
5-6.5	Greenhouse	(Tan et al. 2014)
6-6.5 (max 8.0)	Field	(Bharathi and John 2013)
5.5-6.5	Field	(Hoi et al. 2013)

1.2.3.5 Trellising

Bitter melon grows very fast and vines elongate rapidly within two weeks after planting. Thereafter, the plant sends out lateral stems. Trellising the plant off the ground will increase fruit yield and size, reduce fruit rot and make spraying and harvesting easier. The trellis is typically arranged either in a lean-to or tunnel structure. The trellis should be 1.8–2.0m high, constructed from stakes 1.2–1.8m apart, which is similar to the plant row spacing (Palada and Chang 2003). Higher yields are obtained with 2m rather than 1m high trellises and the crop is more accessible. Overhead or T-trellising (Figure 1.15) may increase the proportion of marketable fruit (Morgan and Midmore 2002).

Building trellis systems, along with grafting and pruning have contributed to the increase of both tomato and cucumber productivity (Kimura and Sinha 2008, Gao et al. 2009, Zhang et al. 2010). Consequently, similar studies on these cultivation techniques for bitter melon are indispensable.

Figure 1.15 Bitter melon trellises: A-shape structure (a); Overhead trellising (b); Straight trellising (c)

1.2.3.6 Harvesting

Bitter melon fruit takes 15–20 days after fruit set or 90 days from planting, for fruit to reach marketable age (Morgan and Midmore 2002, Palada and Chang 2003). Normally, harvesting starts about 50–60 days after sowing and is done two or three times a week, but harvesting time depends on the local markets. Bitter melon can be harvested at an earlier stage depending on the purpose of use. Therefore, the size of fruit harvested can depend upon consumer (Palada and Chang 2003, Bharathi and John 2013). However, such marketable fruits may not be optimal for being used as medicinal materials.

1.2.3.7 Manipulation of yield and fruit quality

Cultivation techniques such as foliar application of hormones greatly affect fruit quality and productivity. A study undertaken to determine the effects of various applications of ethrel (2-Chloroethylphosphonic acid) and gibberellic acid (GA3) on sex modification in *M. charantia* revealed the highest frequency (29.5%) of pistillate flowers observed in plants treated with 500ppm ethrel at germination. Similarly, spraying of adult plants with 100ppm GA3 increased

the proportion of pistillate flowers to 26% relative to 15% in untreated controls. Both ethrel and GA3 induced significantly higher numbers of pistillate flowers than control plants (Thomas 2008).

1.3 Grafting to improve vegetable production and quality

Grafting has impacted on product quality of fruits and vegetables through using resistant rootstocks to control soil-borne diseases and environmental stress, such as cooler or hotter or saline conditions. As a result, grafting increases yield and raises the quality of fruits and vegetables, improving flavour, firmness and health-related compounds (Davis et al. 2008, Rouphael et al. 2010). These impacts can be affected by grafting methods and the type of rootstock used (Davis et al. 2008).

1.3.1 An overview of vegetable grafting

Grafting is a technique in which two or more pieces of living plant tissue from different plants are joined together and grow as a complete plant. It can be done by hand or by automated methods using grafting machines, semi-automatic machines or robots (Lee et al. 2010).

Vegetable production of grafted seedlings originated in Japan and Korea to avoid serious crop loss caused by infection of soil-borne diseases, aggravated by successive cropping since the 1920s (Davis et al. 2008, Lee et al. 2010). It was first commonly used in Japan by grafting watermelon (*Citrullus lanatus*) onto pumpkin (*Cucurbita moschata*) and bottle gourd (*Lagenaria siceraria*) rootstocks. The main purpose of these studies was to control soil-borne diseases using resistant rootstock (Sakata et al. 2005, Davis et al. 2008). This technology was then introduced into some countries in Europe, such as France and Spain, with the grafting of cucumber and melon scions onto fig-leaf gourd (*Cucurbita ficifolia*). The aim was to control Fusarium wilt and black root rot diseases. Melon plants were then grafted onto *Benincasa* spp. to offset the effects of low soil temperatures in early greenhouse-grown melons (Edelstein 2004, Davis et al. 2008). Grafting was introduced into North America from Europe and it has attracted many growers in Canada, the USA and Mexico (Davis et al. 2008). The type of rootstock affects scion growth, yield, and fruit quality (King et al. 2010).

A number of grafting methods have been employed including insertion graft, inarching and using of inter-stock, approach graft, cleft graft, pin graft and side graft (Fig 1.16). The grafted union consists of two parts: rootstock and scion. The result of the grafted combinations is that the cells of scion and rootstock interlock for mechanical strength. Grafting involves the following steps:

1) Rootstock and scion selection
2) Making the graft union
3) Graft healing
4) Grafted plant acclimation

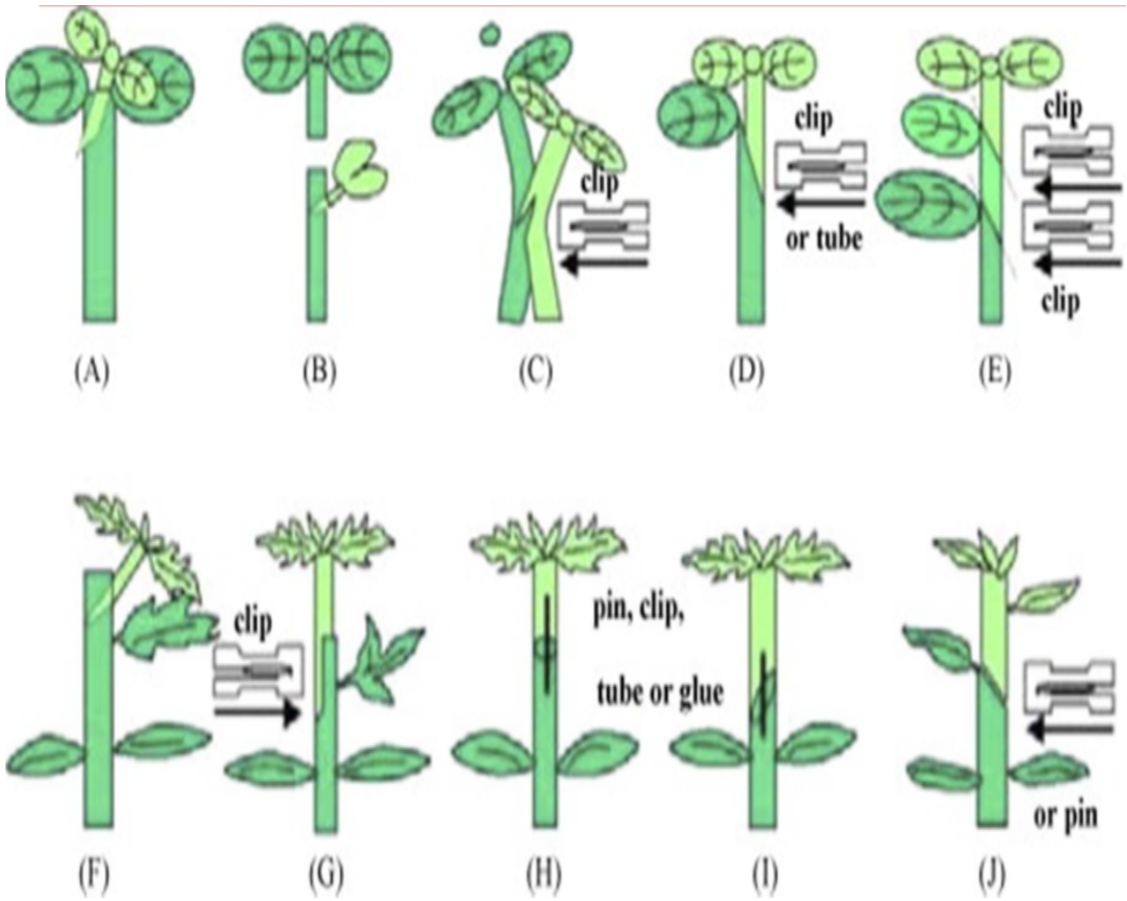


Figure 1.16 Diagram of grafting methods (Lee et al. 2010)

(A and B) hole insertion grafting; (C) tongue approach grafting;

(D, E and J) splice grafting; (F and G) cleft grafting;

(H and I) pin grafting.

A successful graft requires good connection of vascular bundles between the rootstock and the scion (Traka-Mavrona et al. 2000), the healthy growth and development of the grafted plants and the growth of grafted plants under optimum environmental conditions. Under suboptimal temperature and low relative humidity conditions, callusing and healing at the graft union are delayed, resulting in a lower grafting success rate (Lee 2006). Promotion of cell division from the graft union is optimum under environmental conditions of 25-30°C and 95% or higher relative humidity, which protects the wounded tissues from desiccation (Lee 2006).

1.3.2 Utilisation of grafting in plant production

The utilisation of grafting in plant production is widely applied to fruit trees, such as mango, longan and orange, and some types of vines, such as watermelon and grape. The use of grafted plants does not apply extensively to all vegetables. The focus has been on various fruiting vegetables, which belong in the two botanical families Solanaceae and Cucurbitaceae, such as tomatoes, cucumbers and melons (Rouphael et al. 2010).

Vegetable grafting has been safely adapted for the production of organic as well as environmentally-friendly produce and minimises uptake of undesirable agrochemical residues. In many countries the number and size of commercial vegetable seedling producers that graft vegetable seedlings has increased markedly reflecting the increase in farmers' preferences for high-quality and better performing plants (Lee et al. 2010). Using grafted seedlings has attracted the interest of greenhouse hydroponic (soil-less culture) tomato growers. Over 40 million grafted tomato seedlings are estimated to be used annually in North American greenhouses (Kubota et al. 2008). In Japan, the proportion of the areas using grafted plants of watermelon, cucumber, melon, tomato and eggplant fluctuated from 57% to 59% of the total production area in the period 1980–1998 (Oda 2006). China produces more than half the world's watermelons and cucumbers (*Cucumis sativus* L.), and approximately 20% of these are grafted (Davis et al. 2008).

1.4 Benefits of grafting vegetables

1.4.1 The purposes of grafting vegetables

The cultivation of grafted plants has gradually increased throughout the world and serves a spectrum of purposes:

1) To boost plant growth and development.
2) To control wilt and root rot diseases caused by plant pathogens.
3) To strengthen tolerance to thermal or saline stress.
4) To increase nutrient and mineral uptake to the shoot (Rivero et al. 2003).
5) To provide new fruit with different characteristics, such as colour, shape and size (Rouphael et al. 2010).

Examples of these purposes of using grafted cucurbits are described in Table 1.10.

Table 1.10 The purposes of using grafted cucurbits

Scions	Rootstocks	Purposes	Reference
Watermelon	Long gourd, Early Max (Golden west)	To improve fruit yield and quality	(Alexopoulos et al. 2007)
Watermelon	Black seed pumpkin	To increase sugar content, quality and taste	(Gao and Liao 2006)
Watermelon	`Macis' (*Lagenaria siceraria*) and `Ercole' (*C. maxima* × *C. moschata*)	Saline treatments	(Colla et al. 2006)
Watermelon	9 local bottle gourds and two commercial varieties	To improve plant growth, yield, and fruit quality	(Karaca et al. 2012)
Cucumis melo	*Cucurbita maxima* x *C. moschata*	To change fresh or effluent water to tolerate excess boron	(Edelstein et al. 2007)
Watermelon	*Cushaw pumpkin (C. argynosperma*) *Hybrid squash* (*C. pepo*)	To improve fruit quality	(Davis et al. 2005)
Melons	*Cucurbita* sp.	To increase the management of sudden wilt	(Edelstein et al. 1999)

Grafting is not only to improve the development of plants but also to increase the vegetable yield and quality. In addition, grafted plants can improve the resistance to soil-borne disease and saline conditions.

1.4.2 Grafting plants to reduce the impact of diseases and improve disease management

Vegetable diseases reduce yield and quality out-turn. As such they are of great importance to the vegetable industry. Managing disease control is a critical aspect of vegetable production (Koike et al. 2006).

Grafting is an important integrated pest management strategy to manage soil-borne pathogens and other pests of solanaceous and cucurbitaceous crops. Major diseases managed by grafting are caused by fungal pathogens such as *Verticillium, Fusarium, Pyrenochaeta* and *Monosporascus,* pathogens such as *Phytophthora*, bacterial pathogens, particularly *Ralstonia*,

root knot nematodes and several soil-borne virus pathogens (Louws et al. 2010). Grafting is used to reduce infections by soil-borne pathogens and to enhance tolerance against biotic stresses (Schwarz et al. 2010). Grafting plants allows for a more rapid response to the appearance of new races of pathogens, and provides a less expensive and more flexible solution for controlling soil-borne diseases than breeding new resistant cultivars (Cohen et al. 2007).

Grafting is used routinely for continuous cropping systems to reduce susceptibility to soil-borne diseases (Louws et al. 2010). In Vietnam, grafting of watermelon onto resistant cucurbit rootstocks was used for control of Fusarium wilt (Dau et al. 2009). Another study showing an improved survival rate of grafted bitter melon plants when exposed to *Phytophthora capsici* the cause of phytophthora blight disease. One of four rootstocks grafted with Xiangzaoyou (a Chinese bitter melon variety) had a survival rate of 98% with blight morbidity only 1.7% (Chang-hua et al. 2011).

According to Bletsos (2005), grafting on resistant squash rootstocks 'Mamouth' and 'Nun 9075 RT' had positive effects on Fusarium wilt control in melon plants. Another study showed that, the grafted watermelon plants not only had a reduced number of plants affected by Fusarium wilt but also has higher yields compared to the ungrafted plants (Cohen et al. 2002). Ten years later, Cohen et al. (2012) demonstrated that two Ananas-type melons (*Cucumis melo* L.), cvs. 6405 and Eyal, that were grafted onto interspecific F1 *Cucurbita* rootstock TZ-148 and transplanted at spacings of 60, 90, 120 and 180cm within rows in soil infested with the fungus *Macrophomina phaseolina*, that grafted plants did not wilt, compared to 80% and 70% wilting of non-grafted melon plants in experiments conducted in 2006 and 2008, respectively.

Grafted plants can avoid problems caused by Fusarium wilt. The grafting of desirable but susceptible scions onto resistant rootstocks is a valuable method for preventing diseases caused by pathogens surviving in soil (Burgess et al. 2008). Studies at the World Vegetable Centre in Taiwan have found that the grafting of bitter gourd and cucumber scions onto pumpkin, luffa, and fig-leg gourd rootstocks can improve resistance to disease caused by Fusarium wilt (Ko 1999). Successful studies on the grafting of some cucurbit species scions onto other cucurbit species rootstocks are shown in Table 1.12.

Table 1.11 Grafting for disease resistance

Scions	Rootstocks	Disease Resistance	Reference
Watermelon (*Citrullus lanatus*)	Pumpkin (*Cucurbita* sp.)	Soil pathogens	(Davis et al. 2008)
Watermelon (*C. lanatus*)	Bottle gourd (*Lagenaria siceraria*)	Soil-borne diseases	(Davis et al. 2008)
Melon (*Cucumis melo*)	*Cucurbita* sp	Sudden wilt *Monosporascus cannonballus*	(Edelstein et al. 1999)
Cucumber hybrids (Dutch)	31 *Cucurbita* sp (resistant rootstocks)	*Fusarium oxysporum*	(Pavlou et al. 2002)
Watermelon (*C. lanatus*) Cucumber (*Cucumis sativus*) Oriental melon (*Benincasa hispida*)	Shintozwa (*Cucurbita maxima* x *Cucurbita moschate*) Hongtozwa (*Cucurbita moschata*) Fig-leaf gourd (*Cucurbita ficifolia*) Bottle gourd (*Lagenaria siceraria*)	*Fusarium oxysporum f. sp. niveum F. oxysporum f. sp.cucumerium F. oxysporum f. sp. melonis F. oxysporum f. sp.lagenariae*	(Ko 1999)
Cucumber (*Cucumis sativus*)	Fig-leaf gourd (black seeded gourd) (*Cucurbita ficifolia*)	Fusarium wilt-Black Root Rot (*Phomopsis sclerotioides*)	Global Technology Dissemination AVRDC- The World Vegetable Centre; http://avrdc.org/
Bitter gourd (*M. charantia*)	Pumpkin (*Cucubita* spp.)	Fusarium wilt	AVRDC http://avrdc.org/
Bitter gourd (*M. charantia*)	Luffa (sponge gourd) (*Luffa aegyptiaca*)	Fusarium wilt	AVRDC http://avrdc.org/

1.4.3 Grafting plants to improve quality and productivity

Grafting can improve the plant growth and yield. Grafted watermelon plants flowered about 10 days earlier and showed more vigorous vegetative growth than the control plants. As a result, grafted plants had up to 148% higher fresh weights than the control plants. The plants showed 42–180% higher dry weight, 58–100% more leaves and larger leaf area compared to the control plants. In total yield, watermelon grafted plants produced 27–106% higher yield than the control (Yetisir and Sari 2003) Similarly, when a bitter melon variety (Lanshan) was grafted onto local Luffa rootstock, improvements were noted in the growth of the new grafted plant, including plant height, stem diameter and weight of the grafted seedlings. In addition, the yield of grafted plants significantly improved by 131.6%-258.5% compared to the control (Xingxue et al. 2012).

In some crops, grafting can improve the fruit quality, such as sugar, carotene, lycopene content and firmness of fruit. For example, grafting watermelon increased total carotenoids by 20% and amino acids by 35% (Davis et al. 2008). Grafting tomato and cucumber plants, with increased productivity may also be a viable alternative (Kimura and Sinha 2008, Gao et al. 2009, Zhang et al. 2010). Studies conducted in South Korea (Lee 2006) demonstrated better yield and quality of several fruit-bearing vegetables such as watermelon, cucumber, muskmelon, tomato, eggplant and red-pepper. Other studies reported that newly grafted plants with different luffa rootstocks can improve fruit quality by 38% to 258.5% (Jiebao and Tianlun 1997, Xingxue et al. 2012). The rootstock affected the form of the crop, the size of the placental cavity and the thickness of both the monocarp and the escarp (Leoni et al. 1989). Grafting of mini-watermelon under irrigation deficit increased productivity and induced small positive changes in plant quality and nutritional value. However, lycopene and total vitamin C contents for grafted plants were higher by 40.5% and 7.3% respectively than those from ungrafted plants. Spermidine and putrescine concentrations were reduced by grafting with 24% and 59% respectively (Proietti et al. 2008).

Specifically, grafting can increase crop productivity and quality because of using resistant rootstocks. Grafted melon (*Cucumis melo* L.) seedlings on to the *Fusarium oxysporum* f. sp. *melonis* (Fom) commercial resistant squash rootstocks ‘Mamouth’ and ‘Nun 9075 RT’ were more vigorous than the controls as assessed on plant height, stem diameter and root biomass. When compared with the controls, this resulted in an increased (over three years) early production (326.3%, 265.8% and 489.1%) and late production (371.0%, 357.0% and 404.2%). Fruit size was also larger in early production (29.2%, 50.9% and 32.3%) and late production

(4.3%, 15.2% and 26.0%). The total soluble solids increased in early production (27.4%, 39.6% and 47.9%) and late production (7.59%, 10.07% and 5.6%) when compared with the controls. Thus, grafting on resistant squash rootstocks 'Mamouth' and 'Nun 9075 RT' had positive effects on growth and production in melon (Bletsos 2005).

Grafting plants may change the characteristics of physical properties in fruits. It is a result of the translocation of metabolites associated with fruit quality to the scion through the xylem and/or modification of the physiological processes of the scion (Alan et al. 2007, Alexopoulos et al. 2007). Possible quality characteristics showing these effects are fruit appearance, including size, shape, colour, and absence of defects and decay. Moreover, grafted plants can change firmness, texture, flavour (sugar, acids and aroma volatiles) and health-related compounds, including desired compounds such as minerals, vitamins and carotenoids, as well as undesired compounds such as heavy metals, pesticides and nitrates (Rouphael et al. 2010).

In some cases, however, rootstocks can reduce crop yield and quality. Grafting watermelon scions onto gourd rootstocks did not affect the length, circumference or diameter of seedless fruit but grafting did reduce the weight of the fruit (Davis et al. 2005). *Cucurbita* type rootstocks had 127–240% less yield than the control in watermelon grafted plants. This could be attributed to incompatibility of *Cucurbita* rootstocks because some of the plants died before harvesting. Grafting eggplant onto either of the two wild species had a positive effect on the growth and production (Bletsos et al. 2003, Davis et al. 2008). Research on bitter melon fruit grafted onto Luffa IL9 and IL16 as rootstocks improved the fruit quality of bitter gourd more than other rootstocks (Zhen Dong et al. 2013).

To sum up, there are many reasons why rootstocks improve scion fruit quality. The rootstock/scion compatibility maintains the synchronized and sustainable development of both scion and rootstock. The compatibility of scion/rootstock is resulted in the similarity of tissue and structure, physiological and biochemical characteristics, growing stage of rootstock and scion, phytohormones, and the environment (Davis et al. 2008). As a result, water and nutrient flow smoothly through the grafted union, grafted plants are fully qualified for plant development. In contrast, incompatibility can be affected by tissue and structure difference, physiological and biochemical characteristics, growing stage of rootstock and scion, phytohormones, and the environment. Incompatibility may also occur as a result of lack of cellular recognition, wounding responses, presence of growth regulators, or incompatibility toxins (Davis et al. 2005, Rouphael et al. 2010). Consequently, research on the effects of rootstocks on the quality and quantity of bitter melon fruit would be useful.

1.4.4 Grafting plants to improve tolerance to unfavourable environments

The use of rootstocks can enhance plant vigour through attainment of soil nutrients, tolerance of low soil temperatures, salinity and flooded-soil conditions (Davis et al. 2005). Due to the limited availability of arable land and the high market demand for vegetables around the world, cucurbit crops are frequently cultivated under unfavourable soil and environmental conditions. Therefore, there is potential for grafted plants used to improve tolerance to unfavourable environments (Schwarz et al. 2010).

Grafting plant can improve flood tolerance. A study on young seedlings of bitter melon (cv. New Known You #3) grafted onto luffa (*Luffa cylindrica* Roem. cv. cylinder #2) rootstocks investigated the adaptation of photosynthetic activities under flooding conditions. The results demonstrated that grafted bitter melon seedlings had reduced leaf photosynthetic rate, stomatal conductance, transpiration, soluble protein, and activity of ribulose-1,5-bisphosphate arboxylase/oxygenase (rubisco) in comparison with control seedlings. Bitter melon is not a flood resistant plant, however, the grafted bitter melon plant can survive and growth under flood conditions after grafting onto a flood-tolerant luffa roostock (Liao and Lin 1996).

Grafted plants can enhance salinity tolerance. The watermelon cultivar 'Crimson Tide' was grafted onto three different rootstocks and grown under saline conditions to investigate effects of salinity on grafted and non-grafted watermelon. One *Cucurbita maxima* and two *Lagenaria siceraria* were used as rootstock. Plants were irrigated with two different saline solutions (0.5 [control] and 8.0 dSm^{-1}) every two days during the first 15 days of the experiment and every day during the last 15 days. Grafted plants had faster growth parameters than non-grafted plants under saline conditions. There was a reduction in shoot dry weight of 41% in non-grafted plants while this reduction varied from 22.0% to 0.8% in grafted plants under saline conditions. The ratios of survival were lower in non-grafted plants than grafted plants under saline conditions (Yetisir and Uygur 2010). Similarly Huang et al. (2010) demonstrated that an appropriate rootstock could improve cucumber tolerance to salinity induced by major nutrients. A greenhouse experiment was conducted to determine plant growth, leaf physiological responses and mineral content of cucumber plants (*Cucumis sativus* L. cv. Jinchun No.2), either self-grafted or grafted onto the rootstock 'Black Seeded' fig-leaf gourd (*Cucurbita ficifolia* Bouché) and 'Chaofeng Kangshengwang' (*Lagenaria siceraria* Standl.). Plants were grown in nutrient solutions with 1, 4 and 8 times the concentration of macronutrients in the base solution for 10 days. In short, grafting cucumber onto 'Black Seeded' fig-leaf gourd increased plant tolerance to salinity induced by major nutrients.

Grafting watermelon, cucumber and oriental melon onto Shintozwa (*Cucurbita maxima* x *Cucurbita moschata*), Hongtozwa (*Cucurbita moschata*), Fig-leaf gourd (*Cucurbita ficifolia*) and Bottle gourd (*Lagenaria siceraria*) produced new grafted plants with increased resistance to low temperatures and high salt concentrations (Ko 1999). In fact, the salinity tolerance of some Cucurbit varieties, such as cucumber, pumpkin and rockmelon were the most salt-tolerant varieties known in the Cucurbitaceae, tolerant up to salinity in the range of 2.0-8.0 dSm^{-1} (Table 1.11).

Table 1.12 General guidelines for some Cucurbit species response to soil salinity

Salinity (dSm^{-1})	**Plant response**	**Name of Cucurbit varieties**
0 to 2	Mostly negligible	No information
2 to 4	Growth of sensitive plants may be restricted	Cucumber (*Cucumis sativus* L.) Luffa (*Cucumis pepo* L. var *melopepo* Alef.)
4 to 8	Growth of many plants is restricted	Pumpkin (*Cucurbita maxima* L.) Rockmelon (*Cucumis melo* L.)
8 to 16	Only tolerant plants grow satisfactorily	No information
Above 16	Only a few, very tolerant plants grow satisfactorily	No information

Adapted from Handreck and Black (1994) and Kotuby (2000).

In addition to the widely recognised benefits of disease tolerance and high crop yields, grafting technology is also highly effective in ameliorating crop losses caused by adverse environmental conditions such as low soil temperature and high soil salts, especially under protected cultivation where successive cropping or continuous farming is routinely practiced (Lee et al. 2010). Young seedlings of bitter melon (*M. charantia*) grafted onto luffa (*L. cylindrica*) rootstocks can also grow better under flooding conditions (Koutsika-Sotiriou and Traka-Mavrona 2002).

1.5 Assessing rootstocks

Unlike other cucurbit species such as watermelon and cucumber, research on the grafting of bitter melon onto rootstocks is limited. Some experimental studies and comparisions have

shown that rootstocks increase fruit yield and improve bitter melon fruit quality, such as increased vitamin C, sugar and total saponin content (Xingxue et al. 2013). Research so far is generally restricted to tests of grafting survivals of some bitter melon varieties with different rootstocks. Differences in stem diameter between scion and rootstock can reduce the chance of scion vascular bundles meeting stock bundles, thus resulting in fewer sites with vascular and phloem connections (Traka-Mavrona et al. 2000). Other studies have shown the effects of rootstocks on fruit quality, leaf chlorophyll content and photosynthetic characteristics of bitter gourd (Liao and Lin 1996, ZhenDong et al. 2013). In addition, there is only limited research on grafted bitter melon under harsh environmental conditions, which includes addressing flooding stress and resistance to phytophthora blight disease (Liao and Lin 1996, Chang-hua et al. 2011). Table 1.13 lists some experimental studies and preliminary assessments of grafted bitter melon by using two main grafting methods: Tongue approach and Single leaf splice. Thus, using bitter melon grafted plant with salinity resistance is a critical research and practivality.

The survival ratio of new grafted bitter melon depends on the grafting methods and type of rootstocks. Furthermore, the use of different rootstocks is focused on other purposes, for example, improving yield and active elements in fruits, and disease resistance. As yet, there are not many studies in Australia and Vietnam regarding growing grafted bitter melon under harsh environmental conditions. The use of grafted bitter melon plants for growing in saline soils and increasing disease resistance is a relatively new idea. It is potentially successful because the research is based on the results of previous studies on other cucurbit species in case of using saline tolerant and disease resistant rootstocks.

Genetic compatibility of scion-rootstock plays an important role in the success rate of grafting. This rate depends primarily on the compatibility of the graft union in terms of fast formation of the vascular connections between the rootstock and the scion and fast renewal of root and canopy growth (Cohen et al. 2007, Aloni et al. 2010).

The survival ratio of grafting melon (*Cucumis melo* L) scions onto squash (*Cucurbita* spp) rootstocks was only 10%-15% because of graft-rootstock incompatibility, due to poor vascular connections possessing connecting sieve tubes, cambium and xylem in the heterograft *Cucumis/Cucurbita* (Traka-Mavrona et al. 2000, Pina and Errea 2005).

Table 1.13 Experimental studies and preliminary assessments of grafted bitter melon

Scions	Rootstocks	Purposes	References
Bitter melon	*Luffa acutangula* (Shuangyi)	Giving high quality of fruit and increasing resistance to nematode	(Xiangbo et al. 1998)
Large white and green bitter gourd	*Luffa acutangula* (Shuangyi) *Luffa acutangula* (Gongrong) *Luffa cylindrical*	Changing protein and sugar contents	(Chun-feng 2009)
Bitter melon	Dishcloth gourd	Improving yield and resistance against root knot nematodes	(Jiebao and Tianlun 1997)
Bitter gourd (Bixiu)	(Local) Luffa	Finding appropriate methods for grafting bitter melon	(Chun-feng 2009)
Bitter gourd (Lanshan)	3 Local luffa	Finding appropriate methods for grafting bitter melon	(Xingxue et al. 2012)
Bitter melon	*Luffa cylindrical*	Investigation for adaptation of photosynthetic activities under flooding conditions	(Liao and Lin 1996)
Bitter melon *Momordica charantia* L.cv.Lanshan	*Luffa cylindrical (L.)* Roem *Luffa ylindrical*. Lanshan	Increasing the vitamin C content bitter taste, fruit weight and fruit diameter; Decreasing soluble sugar and saponin content	(Xingxue et al. 2013)
Bitter melon v. "Guinongke 2"	Wild-luffa rootstocks *(Luffa cylindrical Roem.)*	Improving fruit quality and luminous energy utilisation; leaf chlorophyll content and photosynthetic characteristics	(ZhenDong et al. 2013)
Bitter melon	*Luffa* (Xiangzaoyou*)*	Controlling effect on phytophthora blight	(Chang-hua et al. 2011)

The genetic compatibility of scion-rootstock plays an important role in the success rate of grafting. This rate depends primarily on the compatibility of the graft union in terms of fast

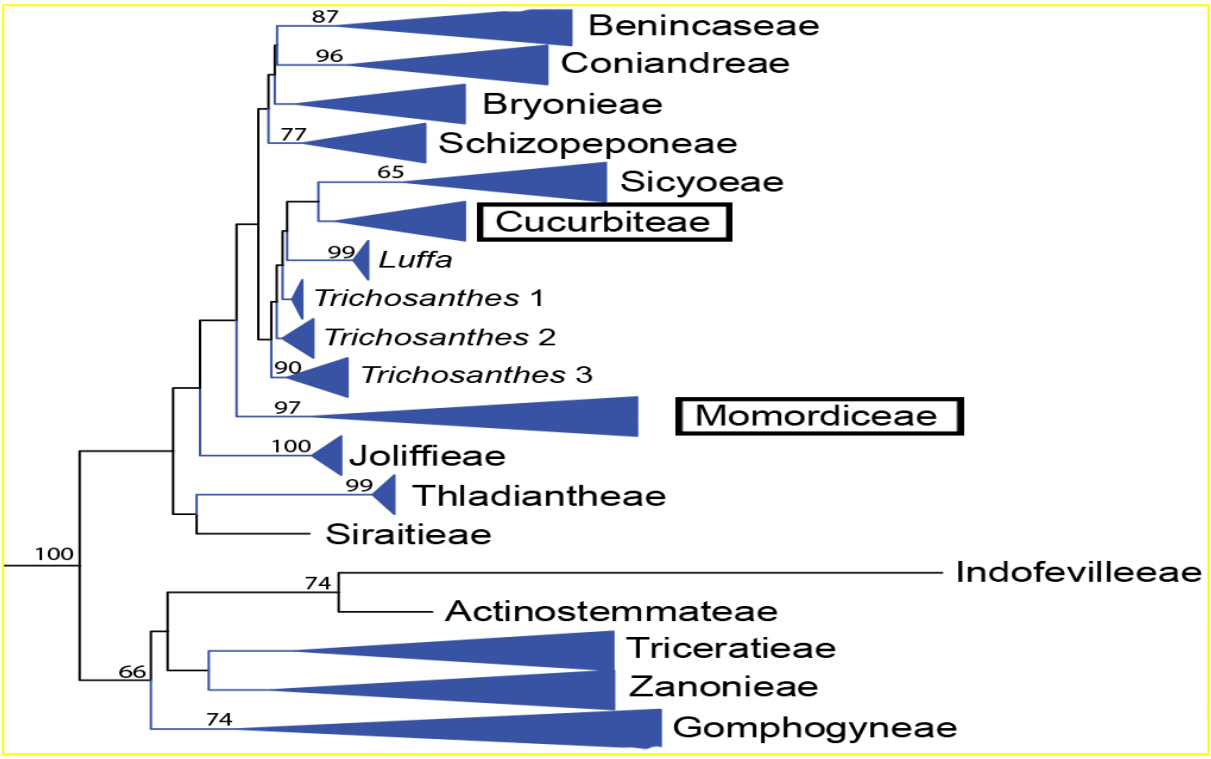
formation of the vascular connections between the rootstock and the scion and fast renewal of root and canopy growth (Cohen et al. 2007, Aloni et al. 2010). The survival ratio of grafting melon (*Cucumis melo* L) scions onto squash (*Cucurbita* spp) rootstocks was only 10%-15% because of graft-rootstock incompatibility, due to poor vascular connections possessing connecting sieve tubes, cambium and xylem in the heterograft *Cucumis/Cucurbita* (Traka-Mavrona et al. 2000, Pina and Errea 2005).

Grafting scions (from a plant) onto rootstocks (from other species or varieties) can change survival ratios. This could possibly be attributed to the differences in stem diameter between *Cucurbita* and *Cucumis*, which reduces the chance of scion vascular bundles meeting stock bundles, thus resulting in fewer sites with vascular and phloem connections. The dissimilar numbers of connecting sieve tubes between *Cucumis/Cucurbita* and *Cucumis/Cucumis* caused differences in stem anatomy, *Cucurbita* having a large pith cavity (Pina and Errea 2005). So, scion and rootstock diameter similarity is important to graft survival.

Generally, the success of grafting is linked to the genetic similarity of scions and rootstocks, decreasing from species to tribes and families. The species used for rootstocks in this project belong to different tribes of Cucurbitaceae family. The Vietnamese bitter melon scion used is *Momordica charantia* L., (Tribe – Momordiceae). The rootstock used is Pumpkin (*Cucurbita maxima* L.), including 3 varieties, all belonging to the same tribe (Cucurbiteae): Queensland blue (Pumpkin), Sampson (Sampson), and Ringer (Ringer). Their genetic relationship is highlighted in Figure 1.17.

1.6 Conclusion

The grafting of bitter melon is a limited area of study but research on other cucurbit species has been successful and has shown improved plant growth, fruit yield and quality. In addition, the use of grafted cucurbit plants has increased disease resistance and also enhanced resistance to harsh environmental conditions, including saline soils and extreme temperatures. These results are the scientific basis for this study on grafting bitter melon. Therefore, this research hopes to contribute significantly to the improvement of a scion (Vietnamese bitter melon varieties) when grafted onto some rootstocks (Australian varieties). Specifically, this study will assess the effectiveness of a grafted new Vietnamese bitter melon variety with unknown disease resistance and tolerance to soil salinity.



Adapted from Schaefer & Renner, 2011.

Figure 1.17 Relationships of *Momordica* L. with other genus in Cucurbitaceae family

1.7 Hypothesis, Aims and Objectives

1.7.1 Hypothesis

Grafting onto an appropriate rootstock can significantly improve Vietnamese bitter melon production. Selected rootstocks with resistance to soil-borne diseases and saline conditions can mitigate poor conditions in term of plant growth, fruit yield and fruit quality.

1.7.2 The Main Aim and Objectives

The **aim** of this study was to improve a Vietnamese bitter melon variety (VINO 12) by grafting with different rootstocks that may improve productivity, increase soil-borne disease resistance and can grow under saline conditions.

To achieve the overall aim, the following **objectives** were targeted:

(1)To test survival ability of rootstocks under different saline conditions, their resistance to soil-borne diseases, particularly *Pythium* root rot, and to determine the most suitable grafting method for bitter melon scion with rootstocks.

(2) To determine the development of grafted plants, their fruit production (number of fruits, fruit size and total fruit weight), and fruit yield under saline conditions grown indoors and outdoors for both main season and off season in comparison with control.

(3) To determine quality of grafted fruits (physical properties: fruit colour, moisture content and firmness; chemical content: total saponins, phenolic compounds; and antioxidant properties) under saline conditions grown indoors and outdoors for both main season and off season in comparison with control.

1.7.3 Summarised diagram of the proposed research

All experiments were conducted at Gosford, Australia. Off season refers to cold and low temperatures, from May to August, and main season refers to hot and warm temperatures, from October to February (Table 1.14).

Table 1.14 Timetable for growing bitter melon in some parts of Australia
(E: early, L: late, +: all)

Location	**Planting date**											
	J	F	M	A	M	J	J	A	S	O	N	D
Victoria	+	.	.	.	.	.	.	.	.	+	+	+
Gosford	.	.	.	.	.	.	.	.	.	+	+	+
Murwillumbah	.	.	.	.	.	.	.	.	**E**	+	+	+
Darwin	.	.	**E**	+	+	+	+	**L**	.	.	.	.
Kununurra	.	.	.	+	+	+	+	.	.	.	.	.

Adapted from Morgan and Midmore (2002)

Based on the above research objectives, the structure of the dissertation was formulated as follows:

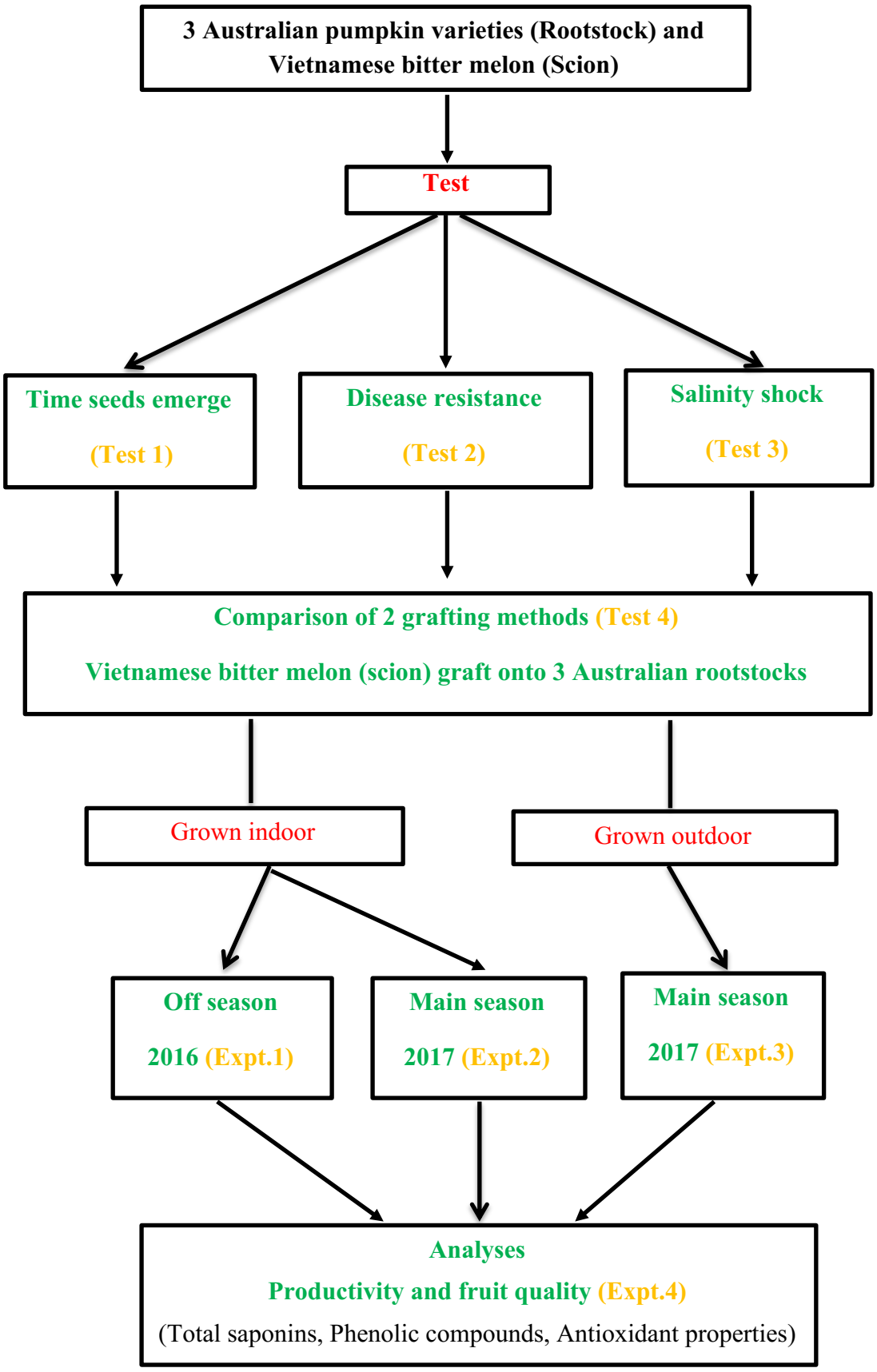


Figure 1.18 Summarised diagram of the research conducted

1.7.4 The list of experiments

To achieve these research objectives, the experiments were conducted in the order shown in the table 1.15.

Table 1.15 Experiments conducted

No	Test and experiments	Time	Purpose
1	Timing of seed germination to obtain graft-compatible seedlings.	9/2015 - 3/2016	To optimise the timing of germination seeds so that rootstocks and scions are at an appropriate size for grafting.
2	Effects of Pythium root rot disease on the scion and rootstocks seedlings.	10/2015 - 2/2016	To study the incidence of Pythium root knot nematode in cucurbitaceous rootstocks and bitter melon scions.
3	Effect of salinity on the development of scions and rootstocks seedlings.	1/2016 - 3/2016	To select rootstocks which can grow in saline conditions and determine the levels of salinity in which they can be planted.
4	Grafting methods effect on the rate of grafting success.	2/2016 - 5/2016	To evaluate a simple grafting method for bitter melon with some cultivated cucurbitaceous rootstocks.
5	Effect of rootstocks on the development of grafted bitter melon plants and fruit yield growing indoors in off season and under saline conditions.	5/2016 - 10/2016	To identify compatible rootstocks for bitter melon from the cucurbitaceous species. To study the performance of bitter melon grafts with various rootstocks on yield. To evaluate the effect of rootstocks grafted onto bitter melon as affected by salinity in terms of production compared with non-saline conditions. To evaluate the potential benefit of rootstocks grown under more stressful environmental conditions, including those in production under-cover during the off season compared with outdoors in the main season.
6	Effect of rootstocks on the development of grafted bitter melon plants and fruit yield growing indoors in main season and under saline conditions.	11/2016 - 3/2017	
7	Effect of rootstocks on the development of grafted bitter melon plants and fruit yield growing outdoors in	11/2016 - 3/2017	

No	Test and experiments	Time	Purpose
	main season and under saline conditions.		
8	Effect of rootstocks and saline conditions on fruit quality.	4/2017 - 7/2017	To evaluate the effects of rootstocks and saline conditions on bitter melon fruit quality compared with non-saline conditions.

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 Plant materials

For the purposes of this research, studying germination and grafting bitter melon, Vietnamese bitter melon variety – VINO 12 (*Mormordica charantia* L) seeds were used for scions and three Australian pumpkin varieties (*Cucurbita maxima* L.) were used as rootstocks, including Queensland blue (Qb), Sampson (Sp) and Ringer (Rg).

The seeds of three rootstock varieties (Qb, Sp and Rg) were purchased from Ace Ohlsson Pty Ltd., (Flemington NSW 2140, Australia) and the seeds of a variety of scion – VINO 12 were obtained from Vietnong Liability Company (Xuan Bac commune, Xuan Loc district, Dong Nai Province, Vietnam). The information related to seed sources is shown in Table 2.1

Table 2.1 Seed sources used in this study as scion and rootstocks

No	Variety	Other names	Botanical name	Company supply
1	Bitter melon	VINO 12	*Momordica charantia* L.	Vietnong Liability Company (Vietnam)
2	Queensland blue	Pumpkin	*Cucurbita maxima* L.	ACE OHLSSON Pty Ltd (Australia)
3	Sampson	Hybrid pumpkin, Jarrahadale	*Cucurbita maxima* L.	
4	Ringer	Hybrid Kent pumpkin, Jaf	*Cucurbita maxima* L.	

2.2 Seedling and grafting

The seeds were surface sterilised (0.1% NaOCl for one minute) and sown directly into small trays (5cm diameter and 7cm height) containing a potting mix made of coir/coconut fibre, peat moss, vermiculate and perlite. These materials and others, such as grafting clips and bamboo sticks were purchased from a Bunnings Warehouse on the Central Coast, NSW, Australia.

The seeds were planted 1-2cm below the surface of the tray before placing all trays in incubators at temperature of 25°C for 12 hours under light, followed by 12 hours in darkness

at 80% humidity and kept in this incubator for 3-7 days, depending on varieties. These conditions were maintained until the seeds emerged. New seedlings were kept in a small greenhouse with an automatic spray through a sprinkler system.

The result of each variety and treatment were analysed by follow equation:

$$\text{The germination } = \frac{The\ number\ of\ geminated\ seeds}{The\ number\ of\ seed\ initiated} \times 100$$

The grafting of cucurbit species is usually conducted at the seedling stage. For this research the tongue approach and splice methods were used. As with grafting other cucurbit species, these techniques were conducted by hand, using a grafting knife. After the scion was placed onto the rootstocks, grafting clips or tube splices were used to fix the graft in position.

All the seedlings were grafted after developing two true leaves. Two types of grafting methods were used: tongue approach (TA) and single leaf splice (SLS).

The newly grafted plants for each batch were placed in a propagation house. The air temperature and the relative humidity of the greenhouses were maintained within bands of 18°C to 26°C and 60% to 80%, respectively. The success of grafting process analysed using the following equation:

$$\text{The success of grafted plant } = \frac{The\ new\ grafted\ plants}{The\ number\ of\ scions/rootstocks} \times 100$$

2.3 Production

2.3.1 Growing conditions and fertilising

For the experiment of improving fruit yield and quality, the new grafted plants were fertigated with a complete nutrient solution with a target electrical conductivity (EC) of 1.1–1.6 dSm^{-1} and a pH of 5.0-6.5. The greenhouse was climate-controlled with natural conditions, with the temperature maintained between 18 and 23°C and the relative humidity maintained within bands of 78.06% to 92.10% (Table 2.2). There was no supplemental light system. An automatic fertigation system with two main parts was used for growing bitter melon indoors and outdoors.

For the experiment on salinity shock, the plants used as rootstocks were shocked with saline conditions. NaCl was added into the water nutrient supply then was poured slowly onto the seedlings until water emerged from the base of the bag. The salinity of water nutrient supply for plants was increased by 2.0 dSm^{-1} per day from 2.0 dSm^{-1} to 26.0 dSm^{-1} and applied once per day, at 4:00pm.

Table 2.2 The differences in temperature and humidity indoors and outdoors in off seasons and main seasons during the experiments

Conditions (Time)	**Temperature (ºC)**			**Humidity (%)**		
	Average	Maximum	Minimum	Average	Maximum	Minimum
Outdoors (5-10.2016)	13.33±2.00	24.23±3.43	4.22±2.09	61.17±3.34	78.81±7.85	30.79±3.68
Indoors (5-10.2016)	18.46± 0.78	26.59±0.71	12.99±0.46	78.06±5.37	99.45±1.79	37.80±5.36
Outdoors (11.2016-3.2017)	22.59±1.84	35.91±4.98	14.82±2.88	77.10±3.63	94.83±4.40	48.35±10.48
Indoors (11.2016-3.2017)	23.23±0.94	29.28±1.20	17.06±2.10	92.10±4.18	100±0.00	58.76±13.95

For the experiment on improving tolerance to salinity condition, the nutrient solution and greenhouse conditions were the same as the experiment on improving fruit yield and quality. Table 2.3 lists the composition of the fertiliser.

Table 2.3 Nutrient formula for growing bitter melon

No	**Stock Solution**	**60L Stock**
I.	**Part A**	
1	Calcium Nitrate	4500g
2	Iron EDTA	180g
II.	**Part B**	
1	Potassium	6000g
2	Mono-potassium Phosphate (MPK)	1200g
3	Magnesium Sulphate	3600g
4	Manganese Chelate	48g
5	Zinc Chelate	15g
6	Boric Acid	15g
7	Copper Chelate	33g
8	Ammonium Molybdite	7.2g

For the experiment of salinity tolerance, nutrients with added NaCl were supplied once per week for all treatment plants growing inside and outside the greenhouse, while control plants

were provided with a similar volume of nutrient solution of normal salinity levels at the same time. The automatic nutrient supply was stopped two hours before the saline nutrients were applied by hand (the volume of water applied to the salinity treatment was the same volume applied to the 'normal salinity' control plants. It was poured slowly onto the plant substrate until water emerged from the base of the bag). The automatic nutrient supply was restarted after a further two hours.

The volume of saline nutrient supply was increased depending on plant development. The grafted and ungrafted bitter melon plants were supplied from 500ml to 1250ml of nutrient solution with the level of salinity from 4.0dSm^{-1} to 16.0dSm^{-1} (respectively) twice per week (Tuesdays and Fridays), whereas the control plants were provided a corresponding volume of nutrients. The volume of nutrient with salinity levels supplied per plant is described in Table 2.4. Although the salt concentration in soils with transient salinity may not be as high as that in soils affected by seepage salinity, subsoil salinity usually ranged between ECe (electrical conductivity of the soil saturation extract) of 4.0 dSm^{-1} and 16.0 dSm^{-1} (Rengasame 2006).

Table 2.4 Saline conditions used to grow grafted bitter melon

Time (week)	**1**	**2**	**3**	**4**	**5**	**6**	**>6**
Salinity level (dSm^{-1})	4.0	6.0	8.0	10.0	12.0	14.0	16.0
Volume of salinity nutrient supply/day (ml)	500	750	750	1000	1000	1250	1250

The specific diagrams for the grafted bitter melon plants design, based on a computerised randomisation, are described below:

Table 2.5 The locations of plants grown inside greenhouse

Row 1	CONTROL	Bm/Rg (salinity)	Bm/Qb (salinity)	CONTROL (salinity)	Bm/Sp (salinity)
Row 2	Bm/Sp (salinity)	CONTROL (salinity)	Bm/Sp	Bm/Qb	Bm/Rg
Row 3	Bm/Rg	Bm/Qb (salinity)	CONTROL	Bm/Rg (salinity)	Bm/Sp
Row 4	CONTROL (salinity)	Bm/Rg	Bm/Sp (salinity)	Bm/Qb	CONTROL (salinity)
Row 5	Bm/Qb	Bm/Sp	CONTROL	Bm/Rg (salinity)	Bm/Qb (salinity)

Bm: Bitter melon; Qb: Queensland Blue; Sp: Sampson; Rg: Ringer; Control: ungrafted bitter melon.

Table 2.6 The location of plants grown outside greenhouse (main season 2017)

Row 1	Bm/Rg (salinity)	Bm/Qb (salinity)	CONTROL (salinity)	Bm/Sp	Bm/Rg
Row 2	Bm/Rg	Bm/Sp (salinity)	Bm/Rg (salinity)	Bm/Qb	CONTROL
Row 3	Bm/Qb (salinity)	CONTROL	Bm/Sp	CONTROL (salinity)	Bm/Sp (salinity)
Row 4	Bm/Sp (salinity)	Bm/Rg	Bm/Qb (salinity)	CONTROL	Bm/Qb
Row 5	CONTROL (salinity)	Bm/Qb	CONTROL	Bm/Sp	Bm/Rg (salinity)

Bm: Bitter melon; Qb: Queensland Blue; Sp: Sampson; Rg: Ringer; Control: Bitter melon scion was grafted onto Bitter melon rootstock.

2.3.2 Production techniques

2.3.2.1 Transplanting, trellising and pruning

The grafted plants were transplanted into coir bags 4-6 weeks after grafting, when new leaves and lateral stems appeared. Nutrient was supplied using four drippers and using an automatic fertigation system.

Straight trellising (high fences), made of iron poles, steel wires and plastic wires, was used for growing bitter melon indoors and outdoors.

Old leaves, lateral branches and stems were removed once per week with scissors.

All equipment (drippers, manifolds, coir bags, iron poles, wires and scissors) were purchased from a Bunnings Warehouse, Central Coast, NSW, Australia.

2.3.2.2 Pollinating

In the greenhouse, flowers were hand pollinated in the morning from 7:00am to 12:00pm, to coincide with the time of naturally occurring pollination by insects. For the outdoor plants, the female flowers were pollinated by insects (bees and butterflies). Each individual female flower was labelled with information, including code of the plant (scion/rootstock) and pollination day or flowering day.

2.3.2.3 Controlling pH and salinity determination

The pH value and salinity level were measured with a pH meter (Hanna Instruments, USA). The salinity of the nutrient supply for plants was 1.6 to 2.0 dSm^{-1}. Salt was added into the nutrient supply until the salinity achieved levels of 4.0, 6.0, 8.0 and 16.0 dSm^{-1}.

2.3.2.4 Fruit productivity and physiological properties

In the greenhouse, fruit was harvested at 15 days and 30 days after pollinating by hand in main and off season respectively. The outdoor plants were only grown in the main season and fruit was harvested at 20 days.

The individual weight of bitter melon fruit was measured using an electronic scale (Mettler, Toledo, Switzerland, + 0.01 g). The length and diameter of fruit were measured by a tape measure.

Measurement of colour of bitter melon fruit was conducted according to the method of Manera et al., (2013). Details are described in Chapter 5.

The firmness of fruit was determined with a Penetrometer (Facchini, Alfonsine, Italy) with an 8mm flat plunger. Details are described in Chapter 5.

2.4 Determination of bioactive components and antioxidant properties

These experiments were conducted in the lab at Ourimbah campus, The University of Newcastle, Australia. Bioactive compounds, including total saponins and total phenolic

compounds and antioxidant capacity were determined using spectrophotometric assays. Details are described in Chapter 5.

2.5 Isolation pathogen and pathogenicity testing

For the experiment to determine the disease resistance of rootstocks and scions, 15 seeds were germinated per pot and culled to 10 seedlings per pot, before doing pathogenicity testing. All plants were stem inoculated with either of the pathogens: *Pythium aphanidermatum*.

These experiments were conducted at the NSW Department of Central Coast Primary Industries Centre, Ourimbah, NSW, Australia (151° 22'E, 33° 21'S). The grafted plant and control were arranged in a randomised block design.

2.6 Statistical analysis

All analytical measurements were carried out with 3-5 replications and the results reported as mean values ± standard deviations. The data was analysed by one–way or two–way analysis of variance using SPSS software package version 23.0 (IBM Corp., United States), depending on whether there were one or more influencing factors. The means were compared by Turkey ($p<0.05$) and post–hoc tests were used to determine statistical differences between parameters.

CHAPTER 3

EXAMINATION OF SEED GERMINATION TIME, TESTING SEEDLING RESISTANCE TO SALINITY AND SOIL BORN DISEASES, AND DETERMINATION OF GRAFTING METHODS

3.1 Introduction

Establishing a successfully grafted plant requires high quality rootstock and scion seedlings with uniform size. Generally, the diameter of a scion must be in the following range rootstock's stem diameter ≥ diameter of scion ≥ rootstock's pith cavity diameter (Davis et al. 2008). The timing of sowing to achieve the desired maturity of the seedlings to control the size of scions and rootstocks is one of the many factors that affect the success of the grafting (Yetisir and Sari 2003). This study investigated timing of sowing to maturity. The newly grafted plants also needed to meet two criteria: (i) increased disease resistance and (ii) salinity resistance.

The pre-grafting seedling period from sowing to seedling maturity plays a critical role in the establishment of grafted plants. This process basically goes through stages, including seed germination (days to radicle emergence), seedling growth until two true leaves appear and the seedlings are ready for either grafting or planting (Davis et al. 2008). This is not a straightforward process, given each species, has a different seed germination rates under the same conditions, due to different seed properties, that vary in characteristics such as size or thickness of the seed coat (Seiwa 2000), factors that affect the growth of seedlings. Using splice graft, slide graft and approach grafting methods, seedlings are usually grafted after developing 1-2 true leaves (Lee et al. 2010). Therefore, controlling the period of time from sowing, through seedling emergence and formation of 1-2 true leaves for grafting is very important (Howell 1981).

The compatibility of scion–rootstock relates to the diameter of both scion and rootstock and affects the rate of grafting success. The successful combination between thick–stemmed seedlings (bitter melon scion) and hollow stem rootstocks is difficult because of the seedling size of the rootstocks. To overcome this challenge in other species such as watermelon, producers use the hole insertion graft method instead of the grafting methods mentioned above (Davis et al. 2008, Lee et al. 2010). According to Cansev and Ozgur (2010), after comparing the hole-insertion grafting (HIG) and cleft grafting (CG) methods, grafting success rate is

affected by the rootstock and not by the scion or grafting methods. Grafting two cucumber varieties (Marathon F1 and Assos F1) as scions, onto two pumpkin hybrid varieties: P.360 (*Cucurbita maxima* x *Cucurbita moschata*) and Arican-97 (*Cucurbita maxima* Duch.) as rootstocks showed that there was a significant difference between the two rootstocks with respect to grafting success rate (99.2% for P.360 and 80.8% for Arican-97). Based on the results of grafting some Cucurbit species, such as cucumber and watermelon, the tongue approach (TA) and single leaf splice (SLS) grafting method were used for grafting bitter melon in this study because these two methods are easy to apply.

Morgan and Midmore (2002) suggested that Luffa (*Cucumis pepo* L. var *melopepo* Alef.) provides an excellent rootstock for bitter melon due to its ability to increase yield and provide Fusarium wilt control. Yet fusarium has not been reported on bitter melon in Vietnam or Australia, (Chen et al. 2010). As such, resistance to salinity and Pythium root rot diseases are the focus of this study, given it is a common disease associated many crops in Vietnam (Burgess et al. 2008). Hence, Pythium root rot resistance or tolerance was chosen as a criterion for selection of rootstocks and scions. According to Burgess (2008), *Pythium* species cause seedling blights and death (damping-off diseases), and cause feeder rootlet rot of mature plants, but rarely cause death of older plants. This severe feeder rootlet rot disrupts the uptake of nutrients, which causes stunting, slight yellowing and yield loss. *Pythium aphanidermatum* (PA), *P. irregulare,* and *P. ultimum* are major pathogens of cucurbit species, potatoes and carrots (Grünwald et al. 1997, Moorman and Kim 2004). Damping-off disease affecting cucumber seedlings and root and crown rots of mature plants in response to PA infection causes considerable damage to cucumber crops worldwide (El-Tarabily et al. 2009). *Pythium* spp. can affect plants very quickly in one study, long English cucumber (*Cucumis sativus* L. cv. *corona*) plants were rapidly affected by PA, and within 10 days after inoculation 50% of the plants were dead (Chérif et al. 1994).

The effects of salinity on germination, growth and yield of cucumber have been previously studied. Salinity delayed germination but did not reduce final germination percentage significantly even at 10.7 dSm^{-1} and 16.2 dSm^{-1}. Seedling and plant growth were reduced significantly with salinities higher than 1.2 dSm^{-1} (Chartzoulakis 1992). Al-Sadi (2010) demonstrated that increasing irrigation-water salinity from 0.01 to 5.0 dSm^{-1} significantly increased mortality in cucumber seedlings inoculated with PA and reduced dry weight of non-inoculated seedlings. The concentration of NaCl required to reduce growth of PA isolates by

50% varied from 23.0 to 62.0 dSm^{-1}, with an average of 46.0 dSm^{-1}. However, oospore production was more sensitive to salinity and no oospores were produced above 20.0 dSm^{-1}.

Thus, determination of seed germination time, selection of grafting method, pathogenicity testing with PA and shocking with saline conditions to rootstocks are important tests needed to assess the value of rootstocks before grafting. In addition, through the results of these experiments we can determine the level of salinity to be used in later experiments.

3.2 Materials and Methods

The potting mix was created using five ingredients (measured in unit volume/litre) in the following proportions: four bags of coir, two litres of vermiculate, four litres of perlite and seven litres of peat moss mixed coarse river sand (ratio of 1:1).

All the coir bags were soaked in distilled water for two days before making the potting mix, to ensure full saturation. The ingredients were then mixed in an auto-electric mixer and stirred for 60 minutes. Distilled water was added during mixing.

3.2.1 Seed germination tests and determination of the time from sowing to seedling maturity for grafting

This test was conducted two months before the growing seasons in both main season and off season. The experiment had a in a complete randomized design (CRD). In this study, incubators were set at 25ºC for the three pumpkin varieties (rootstocks) and bitter melon (scion) germination (Morgan and Midmore 2002).

Bitter melon seeds were sown directly into coir on sowing trays and planted 1-2cm deep using one factorial design with three replicates (Figure 3.1). Each replicate corresponds to a sowing tray that contained 100 seeds (10 holes x 10 rows). The seeds were sprayed with 200ml distilled water per sowing tray daily to ensure adequate moisture, and examined daily for seven days until no further germination took place.

The seeds of each rootstock variety were sown directly into coconut fibre on a small tray (5cm diameter and 7cm height) and planted 1-2cm below the surface of the medium. Each replicate contained 30 seeds (30 small trays) arranged in five rows in a large tray (batch). A batch was sprayed with 200ml distilled water, daily per tray, to ensure adequate moisture for germination (Figure 3.1). Each variety was designed with five batches corresponding to five replicates.

Figure 3.1 The germination of seeds

a (Bitter melon), b (Queensland Blue), c (Sampson) and d (Ringer)

All seeded trays were placed into an incubator at a temperature of 25ºC and humidity of around 80% for 12 hours under light, followed by 12 hours in dark (approximately 50µmol photons m^{-2} s^{-1}). This photoperiod was provided by cool white fluorescent lights for 14 days. A seed was considered to have germinated, or the seed germination was finalised, as the seed coat split and the radical protruded 5mm.

Germination speed index (GSI) estimates the mean number of seeds germinated per day and was calculated using the formula (1) as described by (Ribeiro and Costa 2015):

GSI= G1/N1 + G2/N2 +…+ G20/N20 (1)

Where G1, G2,.. G20- is the number of seeds germinated every day and N1, N2,.., N20- is the number of days after seed incubation began.

Germination rate of seeds was calculated as:

$$\text{The germination} = \frac{\text{The number of geminated seeds}}{\text{The number of seed initiated}} \times 100$$

Germinated seeds were checked and counted every day and the optimal germination day was determined by the maximum number of seed germinates on any given day.

The time-frame of the pre-grafting seedling period was calculated by adding germination time to the time from seed germination to a complete seedling, when the seedling reached two true leaves and were ready for grafting.

The newly grafted plants were placed in a growth room with temperature maintained at 22(±1)°C, with humidity 90% and a 12–h photoperiod (approximately 100μmol $m^{-2}s^{-1}$). The numbers of surviving grafts were counted weekly for four weeks.

3.2.2 Effects of grafting methods on the rate of success

The aim of these tests was to select a simple grafting method that could be applied by farmers.

The seeds were sown on different dates to ensure they sprouted at the same time based on the results of section 3.2.1 with three rootstock varieties (Qb, Sp and Rg). To compare between the two grafting methods the following experiment was conducted in April 2016.

Healthy seedlings, of the same in size with two true leaves were used for testing two grafting methods: tongue approach (TA) and single leaf splice (SLS) graft (Table 3.2). A completely randomised design was used for eight treatment combinations; each treatment/rootstock contained 30 seedlings with three replicates. The newly grafted plants were placed in trays in a randomised complete block design and were placed in plastic containers in a dark room with the temperature maintained at 16–22°C and 80–90% humidity for 7–10 days (Davis et al. 2008). They were then moved to a growth room with the same temperature and humidity. The room was controlled and light intensity reduced for seven days. Finally, the new seedlings were taken out of the plastic containers and placed in a greenhouse for four weeks, until new leaves or new laterals appeared (Figure 3.2). The numbers of surviving grafts were counted weekly and the experiment was monitored for a total of six weeks.

The control grafted plants were bitter melon scions grafted onto bitter melon rootstocks.

The results were analysed using the following equation:

$$\text{The success of grafted plant} = \frac{The\ new\ grafted\ plants}{The\ number\ of\ combinations} \times 100$$

Figure 3.2 Bitter melon scion grafted onto Qb, Sp and Rg rootstocks by TA grafting method. The rootstock was grafted when cotyledons and the first true leaf start to develop.

Table 3.1 The strategies of grafting methods used in the research

Step	Grafting methods	
	Tongue approach	**Single leaf splice**
1	-Choose a healthy seedling as a rootstock (Qb, Sp and Rg). -Remove the true leaves of the rootstock by bending the stem above the cotyledons.	-Choose a healthy seedling as a rootstock (Qb, Sp and Rg). -Remove the true leaves of the rootstock by bending the stem above the cotyledons.
2	Make a slanting cut downward into the stem of the rootstock 1cm below the cotyledons.	-Cut one cotyledon off.
3	-Choose a healthy bitter melon seedling as a scion. -Cut off the above–ground part of the scion. -Make a slanting cut upward 2/3 of the way into the scion stem, 1–2cm below the cotyledons.	-Choose a healthy bitter melon seedling as a scion. -Cut off the above–ground part of the scion with a slanted cut 1–2cm below the cotyledons on the scion stem.

Step	Grafting methods	
	Tongue approach	**Single leaf splice**
4	-Join scion and rootstock together. -Fix firmly using grafting clip. -Use a bamboo stick as a stake to support the plant.	-Join the cut edges of the scion and rootstock together, and fix firmly using a grafting clip. -Use a bamboo stick as a stake to support the plant.
5	-Move the grafted seedlings immediately into the shaded grafting chamber. -Healing period 7–10 days.	-Move the grafted seedlings immediately into the shaded grafting chamber. -Healing period 7–10 days.
Notice	The leaves of the scion should be grafted so that they are perpendicular to the rootstock cotyledons.	The scion leave should be perpendicular to the rootstock cotyledon.

3.2.3 Effects of saline conditions on the development and survival of rootstock seedlings

The aim of these tests was to select rootstocks that can grow in saline conditions before grafting.

Water and nutrient supply was stopped two days before the tests of salinity shock. The plants used as rootstocks were shocked with saline conditions. An amount of NaCl was added into the water nutrient supply then was poured slowly onto the seedlings until the water emerged from the base of the bag. The salinity of water nutrient supply for plants was increased by 2.0 dSm^{-1} per day from 4.0 to 26.0 dSm^{-1} and supplied once per day (4:00pm) with 25ml/plant (Table 3.3). The salinity of water nutrient supply for plants in each treatment was continued for ten days at the highest threshold of salt concentration (14.0 dSm^{-1}, 16.0 dSm^{-1}….26.0 dSm^{-1}).

Each treatment included ten seedlings (after developing 1-2 true leaves) and all treatments were replicated five times with different salinity levels (seven levels, including 14.0, 16.0, 18.0, 20.0, 22.0, 24.0 and 26.0 dSm^{-1}). Each variety was placed in a randomised complete block design (RCBD) because the size and the development of seedlings varied.

Table 3.2 Salinity levels supplied

	Salinity level supply (dSm^{-1})						
Day	**14**	**16**	**18**	**20**	**22**	**24**	**26**
1	4	4	4	4	4	4	4
2	6	6	6	6	6	6	6
3	8	8	8	8	8	8	8
4	10	10	10	10	10	10	10
5	12	12	12	12	12	12	12
6	14	14	14	14	14	14	14
7	14	16	16	16	16	16	16
8	14	16	18	18	18	18	18
9	14	16	18	20	20	20	20
10	14	16	18	20	22	22	22
11	14	16	18	20	22	24	24
12	14	16	18	20	22	24	26
13	14	16	18	20	22	24	26
14	14	16	18	20	22	24	26
15	14	16	18	20	22	24	26
16	Finished	16	18	20	22	24	26
17		Finished	18	20	22	24	26
18			Finished	20	22	24	26
19				Finished	22	24	26
20					Finished	24	26
21						Finished	26
22							Finished

3.2.4 Effects of Pythium aphanidermatum on the development of rootstock seedlings

The aim of these experiments was to identify levels of resistance or tolerance of rootstocks to *Pythium aphanidermatum* (PA) a common pathogen of Cucurbit plants.

Isolate collection: A pure culture of PA for pathogenicity test was isolated from a root rot affected bitter melon plant sampled from Khoai Chau district, Hung Yen province, Vietnam in 2015.

Diseased root sections were washed thoroughly in tap water, surface sterilised by dipping in 70% ethanol, rinsed in sterile water and then damp-dried on sterile paper tissues. Small segments (~2mm long) were aseptically cut from the root section at the margin of symptomless and diseased tissue. Segments were plated on either Water Agar (WA) or modified Potato Dextrose Agar (mPDA) medium (Burgess et al. 2008). The plates were placed under 12h light: 12h dark (approximately 50 μmol photons $m^{-2}s^{-1}$); ultraviolet and fluorescent light at 25ºC. Colonies of PA were developed from all segments on both media. The colonies were subcultured to WA and purified by hyphal tipping (Burgess et al. 2008), and finally grown on potato carrot agar (PCA) under light as above.

Testing for Pythium root rot: This activity took place in Research Centre for Cultivating and Processing of Medicinal Plants (National Institute of Medicinal Materials), Hanoi, Vietnam. The pathogenicity test was conducted using the soil inoculation method (Burgess et al. 2008). Healthy seeds of the rootstocks and bitter melon were sown in an artificial soil mix consisting of 1:1 (v/v) sand and sterilised rice hulls. The plants were ready for pathogenicity testing 20 days after sowing. Inoculum was prepared by growing an isolate of PA in a medium consisting of sterilised moist millet seed and rice hulls, 1:1 (v/v), in bottles under alternating light and dark conditions at 25ºC for ten days. The millet seed had been immersed in water for 12h at 5ºC before combining with rice hulls. The medium was autoclaved twice on successive days and inoculated using three 1cm squares from a colony of PA on PCA in each bottle. Flasks were shaken 2-3 days after inoculation to ensure an even distribution of the pathogen throughout the substrate.

The soil around each of 75 young plants of each variety was inoculated by incorporating 50mL of inoculum into the top 5cm of the soil mix. For each variety tested, there were 45 control plants with no inoculation. Each treatment was test replicated three - five times with 15 plants per replicate.

In summary, for the pathogen-treated test plants: 3 rootstocks and 1 scion x 5 replications (15 seedlings/pot x 5 pots/treatment) = 20 pots.

For the control plants (no inoculation): 3 rootstocks and 1 scion x 3 replications (15 healthy seedlings/pot x 3 pots/treatment) = 12 pots.

Stem inoculation: This experiment was conducted in both Vietnam and Australia

Fifteen seeds were germinated per pot (diameter: 15cm, height: 20cm) of which ten healthy seedlings were selected.

For the test: 3 rootstocks and 1 scion x 5 pots/treatment x 3 replication (10 healthy seedlings/pot) = 60 pots.

For the control: 3 pots/rootstock or scion (10 healthy seedlings/pot) = 12 pots.

The lower stems of plants were pierced with a sterile inoculating needle or hypodermic needle and a small piece of agar from a pure culture of the pathogen was placed onto the wound site. Similarly the lower stem of control plants were pierced with the sterile inoculating needle, but do not treated with inoculum. Parafilm or plastic wrap was used to cover the wounds or injection sites. The pots were placed in an evaporative-cooled greenhouse at 27 ± 2°C, watered daily to container capacity and fertilised weekly with inorganic liquid fertiliser at the manufacturer's recommended rate. They were examined regularly for 8 weeks, after which the roots were sampled, washed thoroughly in tap water, surface-sterilised and macerated as described above. Each treatment was replicated ten times in a fully randomised complete block design with ten plants in each replicate for each sampling and the experiment was repeated.

3.2.5 Statistical analysis

All analytical measurements were carried out in three to fifteen replications and the results were reported as mean values ± standard deviations. Data were analysed by one – way analysis of variance using SPSS software version 22.0 (IBM, Corp., United States) to determine differences among treatment – dependent characteristics. The least significant difference (Tukey) was set for $p \leq 0.05$.

3.3 Results

3.3.1 Seed germination tests and determination of the time from sowing to seedling maturity, with four varieties used as scion and rootstocks.

Controlling seed germination times for both scions and rootstocks is one of the determinants of grafting success rate in Cucurbit species. Therefore, it is important to determine the germination times of seeds used as scions and rootstocks. The results (Table 3.3) showed that the time taken for seed germination varied with bitter melon scions and the three rootstock species. Even with different sowing times (main season and off season), there were significant differences between germination time and the rate of seedling emergence per day.

Table 3.3 Cumulative germination for rootstock and scion seeds

Dates	Germination rate (%)								
	3rd	4th	5th	6th	7th	8th	9th	10th	11th
Main season									
Qb	26.22±13.21^{cA}	78.22±13.44^{bA}	94.67±3.25^{aA}	97.78±3.25^{aA}	97.78±3.25^{aA}	97.78±3.25^{aA}	97.78±3.25^{aA}	97.78±3.25^{aA}	97.78±3.25^{aA}
Sp	0.00±0.00^{dC}	15.11±7.33^{cB}	79.56±9.25^{bB}	95.55±4.11^{aA}	97.78±3.25^{aA}	97.78±3.25^{aA}	97.78±3.25^{aA}	97.78±3.25^{aA}	97.78 ± 3.25^{aA}
Rg	14.67±8.05^{cB}	79.11±8.68^{bA}	98.67±2.76^{aA}	99.56±1.72^{aA}	99.56±1.72^{aA}	99.56±1.72^{aA}	99.56±1.72^{aA}	99.56±1.72^{aA}	99.56 ± 1.72^{aA}
Bm	0.00± 0.00^{dC}	0.00±0.00^{dC}	0.00±0.00^{dC}	11.56±9.91^{cB}	87.56±9.04^{bB}	96.89 ± 4.95^{aA}	96.89±4.95^{aA}	96.89±4.95^{aA}	96.89 ± 4.95^{aA}
Off season									
Qb	0.00±0.00^{dA}	22.50±10.35^{cA}	58.75±16.42^{bA}	82.50±11.65^{aA}	87.50±10.35^{aA}	88.75±11.26^{aA}	88.75±11.26^{aA}	88.75±11.26^{aAB}	88.75±11.26^{aAB}
Sp	0.00±0.00^{dA}	0.00±0.00^{dB}	8.75±6.41^{dC}	30.00±13.09^{cC}	57.50±20.53^{bB}	82.50±16.69^{aAB}	93.75±7.44^{aA}	93.75±7.44^{aA}	93.75±7.44^{aA}
Rg	0.00±0.00^{eA}	0.00±0.00^{eB}	38.75±20.31^{dB}	65.00±11.95^{cB}	78.75±9.91^{bA}	82.50±8.86^{abAB}	90.00±7.56^{aA}	90.00±7.56^{aAB}	90.00±7.56^{aAB}
Bm	0.00±0.00^{eA}	0.00±0.00^{eB}	7.67±8.58^{eC}	33.00±16.01^{dC}	60.33±22.51^{cB}	70.67±19.46^{bB}	74.67±16.34^{abB}	78.00±15.40^{abB}	80.00±13.65^{aB}

*Data are the means ± standard deviations (n ≥ 8). Data in the same row sharing different superscript letters (a, b, c, d) indicate significant difference in the germination rates of one variety on different dates (P < 0.05). Data in the same column sharing different superscript letters (A, B, C, D) indicate significant difference in the germination rates of four varieties on the same date with the same period (P< 0.05).

**Time: over 11 days of incubation at 25° C (12d:12h) thermos-period.

In the main season, at 25 ± 1°C, the earliest emergence was observed in the Queensland blue (Qb) and Ringer (Rg), three days after sowing (DAS), while that of the Sampson (Sp) and Bitter melon (Bm) were longer with four and six DAS, respectively. The Qb seeds emerged earlier than the Bm seeds from two to three days. The average of germination rate has reached over 96.0 % ($P<0.05$) of all varieties. However, this did not happen in the off season, when all studied rootstocks and scion varieties emerged at the fifth day except for the Qb variety.

Three rootstock varieties, on the other hand, did not show significant differences in emergence rate at the sixth day in main season and ninth day in off season. Days 6, 8, 9 and 11 were the best for first count of the Qb, Sp, Rg and Bm, respectively. In addition, the germination rates in the off season were lower than that in the main season, from around 88.0 to 93.0%, except for bitter melon (80.0%).

In both the main season and off season, there were differences in time from seeds emerging to complete seedlings with two leaves among the varieties. It was ten days for the Qb, eleven days for the Sp, twelve days for the Rg and thirteen days for the Bm. As shown in Table 3.4.

Table 3.4 The time from sowing to grafting

Time trial	Variety	Time (days)		
		Seed emerge*	Seedling**	Grafting***
Main season (2017)	Queensland Blue	5-6	10	15-16
	Sampson	6-7	10-11	17-18
	Ringer	5	11-12	16-17
	Bitter melon	8-9	13	21-22
Off season (2016)	Queensland Blue	6-7	10	16-17
	Sampson	8	10-11	18-19
	Ringer	9	11-12	20-21
	Bitter melon	11	13	24

* The time (days) of seed emerge is the time seeds are in incubators, (from the time seeds are sown until the seeds germinate.

** The time (days) of seedling is the time from seed emergence to the seedling with 2 true leaves, from the time seeds are moved out from incubators until they are seedlings with 2 true leaves.

*** The time (days) of grafting is the total of time from sowing until seedling can be used for grafting. This is the following equation: **Grafting time = Seed emerge time + Seedling time**

The results of Table 3.4 shows the period of time from sowing to complete seedlings with two true leaves before grafting through the seed emergence. The results varied depending on sowing time, growing season and variety. It fluctuated from 3 -5 days.

Similar to other cucurbit species, the germination rate of bitter melon (scion) and 3 rootstocks reached percentage values of 85 to 95%, with the germination time 3-11 days inside incubator at temperatures of 25°C, depending on the varieties. Then they need extra time, from 7–13 days to grow into a complete seedling.

In cucurbit grafting, the differences in seedling size between scion stem and rootstock stem play an important role in the selection of suitable grafting method. The size of seedling used as rootstock and scion shows in the Table 3.5 and Figure 3.3–3.6.

Table 3.5 The diameter and height of seedling for rootstocks and scions

Varieties	Diameter (mm)	RHD-SHD	Height (cm)
Queensland Blue	3.52 ± 0.03	+ 0.21	9.06 ± 0.05
Sampson	3.61 ± 0.06	+ 0.30	8.71 ± 0.07
Ringer	3.25 ± 0.13	- 0.06	9.23 ± 0.06
Bitter melon	3.31 ± 0.08	0.0	11.37 ± 0.72

RHD: rootstock hypocotyls diameter

SHD: scion hypocotyls diameter

Figure 3.3 Bitter melon seedlings

Figure 3.4 Queensland Blue seedlings

Figure 3.5 Sampson seedlings

Figure 3.6 Ringer seedlings

3.3.2 The effects of grafting methods on grafting success rate

The combination between scion and rootstock plays an important role in grafting technique. However, in this study we used only the two most common grafting methods which have been used for the cucurbit species because of the high success rate. Based on the success rate for selection a grafting method can be applied to all experiments in this study. The results (Table 3.6) showed that the grafting success rate was considerably high with both tongue approach (TA) and single leaf splice (SLS) methods using the Rg as rootstocks. The grafting success rates were 88.89% with TA and 91.11% with SLS. However, there was a significant difference between the success rates of two grafting methods using the other two rootstocks. The success rates of grafting bitter melon scions onto the Qb and Sp rootstocks by TA method (76.67% and 60.00%, respectively) were considerably lower than those by SLS methods (90.00% and 81.11%, respectively).

The use of the SLS grafting method for all three rootstocks resulted in the high success rate, after controlling scion and rootstock diameter, by controlling sowing time and potting materials. Therefore, SLS grafting method was used throughout in all related experiments in this study.

A two-way ANOVA was conducted that examined the effect of rootstock and grafting method on success rate. There was no statistically significant interaction between the effects of rootstock and grafting method on success rate of grafting, $F(2,24)= 5.343$, $P=0.12$, $(n=5)$.

Table 3.6 Value representing the effects of Splice and Slide grafting methods on the success rate of new Bitter melon grafted plants

Grafting methods	**The rate of grafting success between Bm/Qb**		**The rate of grafting success between Bm/Sp**		**The rate of grafting success between Bm/Rg**	
Replication	**Tongue approach**	**Single leaf splice**	**Tongue approach**	**Single leaf splice**	**Tongue approach**	**Single leaf splice**
1	72.22	94.44	66.67	83.33	94.44	94.44
2	83.33	88.89	61.11	77.78	83.33	88.89
3	66.67	83.33	50.00	77.78	88.89	83.33
4	83.33	94.44	72.22	88.89	88.89	88.89
5	77.78	88.89	50.00	77.78	88.89	100.00
% Success rate	**76.67 ± 7.24^{b}**	**90.00 ± 4.65^{a}**	**60.00 ± 9.94^{c}**	**81.11 ± 4.97^{ab}**	**88.89 ± 3.93^{ab}**	**91.11 ± 6.33^{a}**

Data sharing different superscript letters (a, b or c) in the same row indicate significant difference ($P<0.05$) using the least significant difference (Turkey) test.

All values are the means ± standard deviations (n=5), (30 seedlings in each replicate).

3.3.3 The effects of salinity on the seedlings

Shocking with saline conditions is an important test in order to select rootstocks before grafting. The increase of salinity levels led to the decrease of seedling survival rate, and each variety has different salt tolerance. The results showed that both the Qb and Sp were the best salinity tolerant varieties among the four tested varieties (3 rootstocks and scion). Table 3.8 illustrates the results of salinity shock experiments of seedlings.

Saline conditions did not affect the survival rate of the Sp seedlings at concentration less than or equal to 18.0 dSm^{-1}. This rate fell from 98.0% to 76.0% when the levels of salinity increased from 20.0 to 26.0 dSm^{-1}, respectively. However, the survival rate of the Qb seedlings decreased from 96.00% at 18.0 dSm^{-1} to 52.0% at salinity level of 26.0 dSm^{-1} (Figure 3.7).

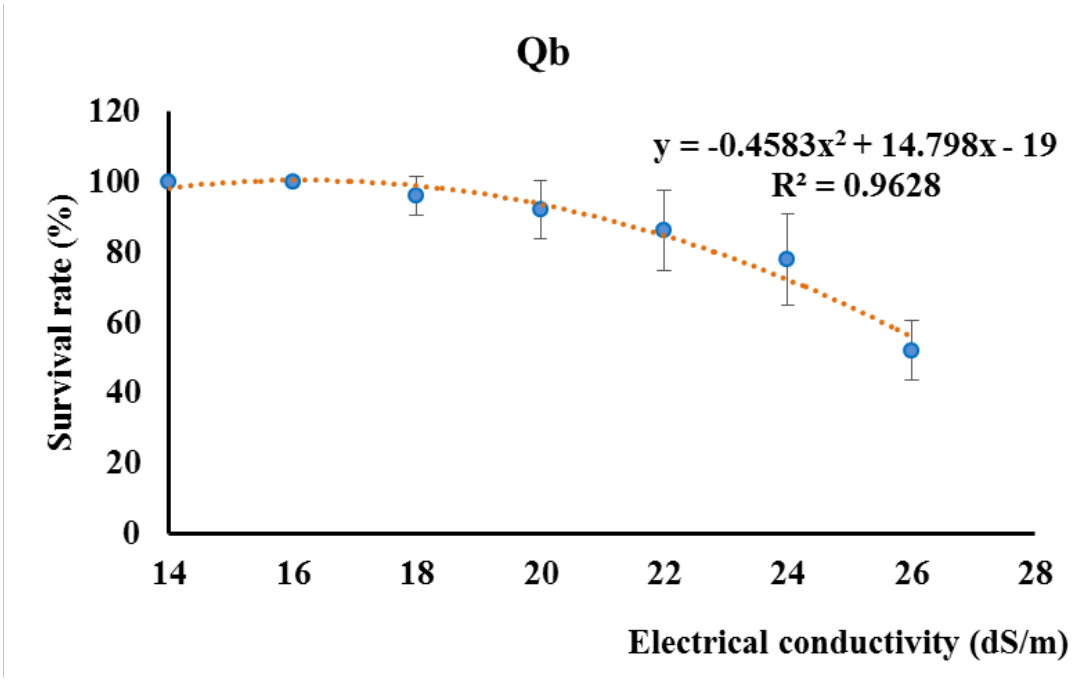


Figure 3.7 Data are the means (n = 5), representing the negative effects of salinity of the growing medium solution on the survival rate of the Qb seedlings

The linear correlation between seedling survival and salinity condition was observed at 7 levels (treatments), $y= -0.4583 x^2 + 14.798 x - 19$, $R^2= 0.9628$ for the Qb and $y= -0.2619 x^2 + 8.462 x + 32.286$, $R^2= 0.9951$ for Sp (Figure 3.8).

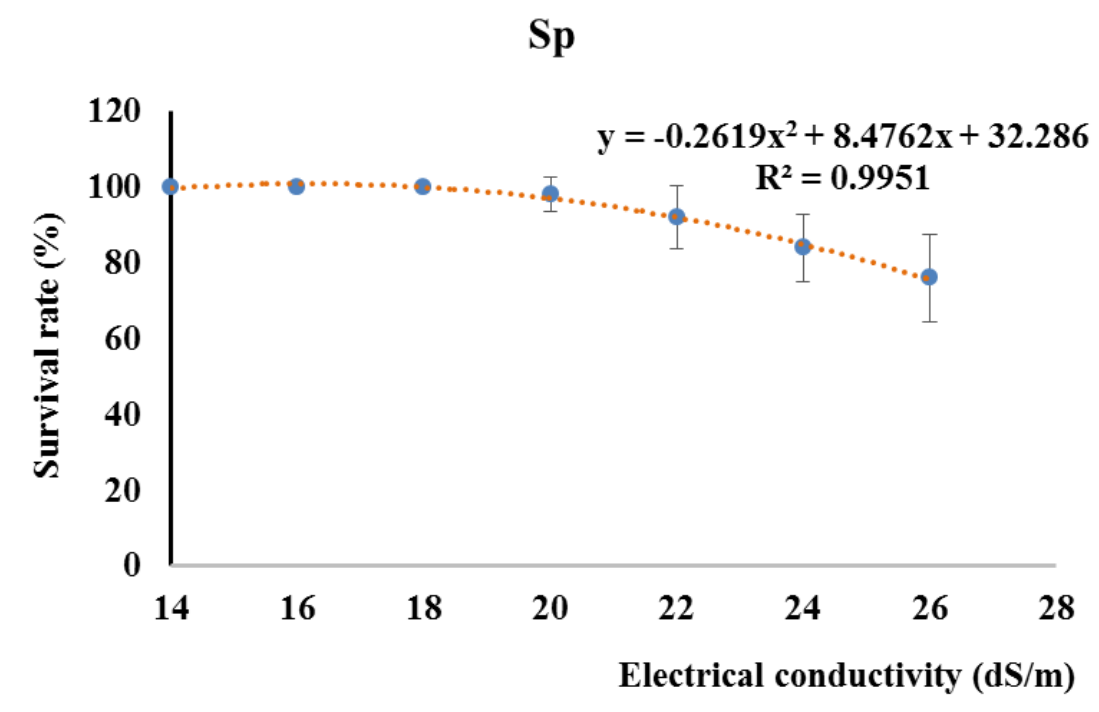


Figure 3.8 Data are the means (n = 5), representing the negative effects of salinity of the growing medium solution on the survival rate of the Sp seedlings

In a similar trend, the survival rate of the Ringer (Rg) and the Bitter melon (Bm) seedlings decreased from 90.0% and 68.0% at salinity level of 14.0 dSm^{-1} to 36.0% and 12.0%, respectively, at the level of salinity 20.0 dSm^{-1}. The linear correlation between seedling survival and salinity condition were observed at seven levels (treatments), $y= 0.1964\ x^2 - 15.893\ x + 276.71$, $R^2= 0.9889$ for the Rg (Figure 3.9) and $y= 0.5119\ x^2 – 26.976\ x + 352.29$, $R^2= 0.9522$ for the Bm (Figure 3.10).

However, the Bm seedlings died completely at a concentration of 22.0 dSm^{-1}, while the survival rate of the Rg seedlings decreased rapidly and completely died at the concentration of salt 26.0 dSm^{-1} (Figure 3.10).The comparison between the survival rates of the four varieties at the different salinity levels is shown in Table 3.8. The Qb and Sp seedlings can grow under saline conditions at levels from 20.0 dSm^{-1} to 26.0 dSm^{-1} with a survival rate ≥ 52% while the Rg and Bm seedlings can grow at the levels of salinity 16.0 dSm^{-1} with a survival rate of 74.0% and 60.0%, respectively. This result is very important in selecting the appropriate salt concentration for all four cultivars in the further experiments.

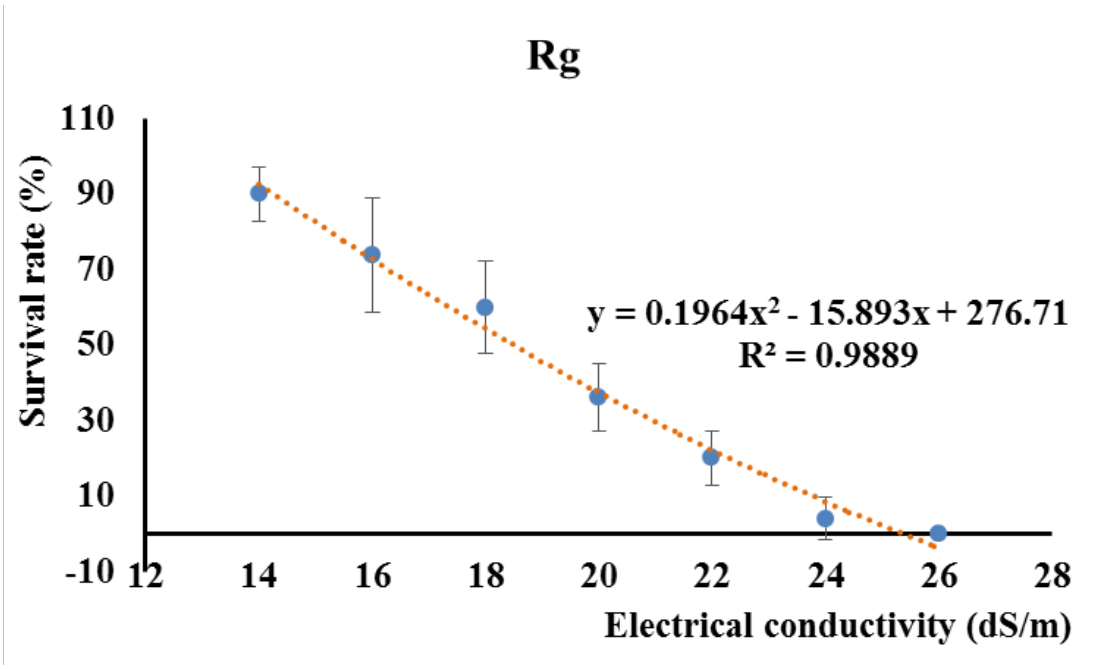


Figure 3.9 Data are the means (n = 5), representing the negative effects of salinity of the growing medium solution on the survival rate of the Rg seedlings

The symptom in rootstock and scion seedlings with high salinity levels, such as yellow leaf, stunted and wilted, in this study is shown in Figure 3.11.

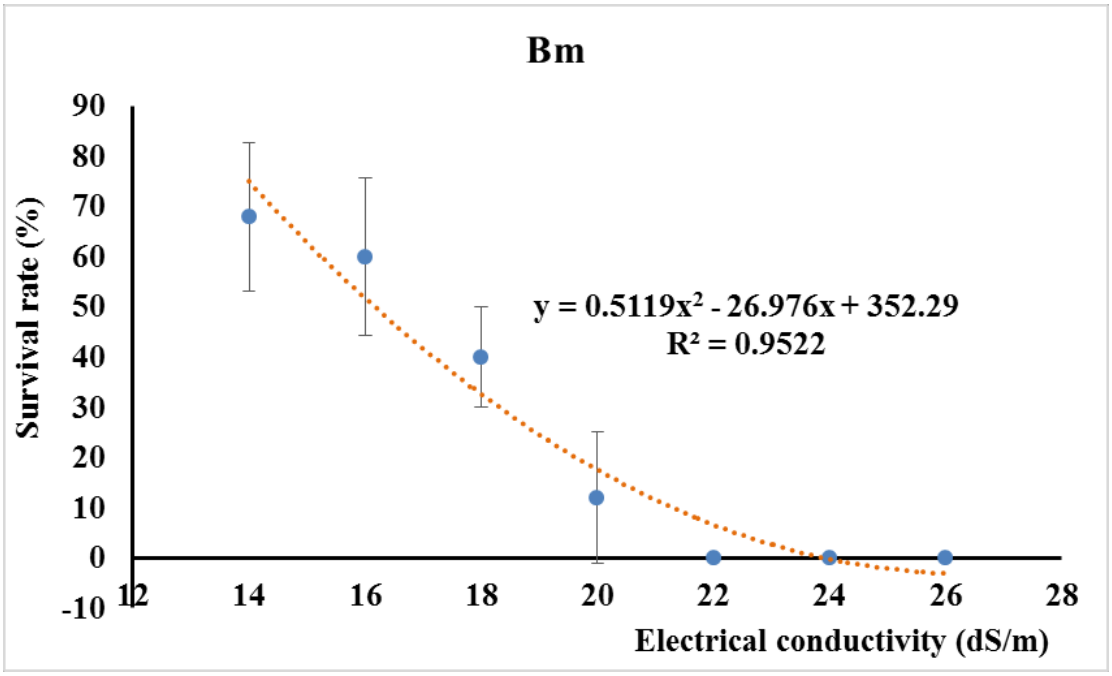


Figure 3.10 Data are the means (n = 5), presenting the negative effects of salinity of the growing medium solution on the survival rate of the Bm seedlings.

A two-way ANOVA was conducted that examined the effect of rootstock and salinity level on survival rate. There was a statistically significant interaction between the effects of rootstock and salinity level on survival rate of seedling, F (18, 112) = 13.430, p =0.001, (n=5).

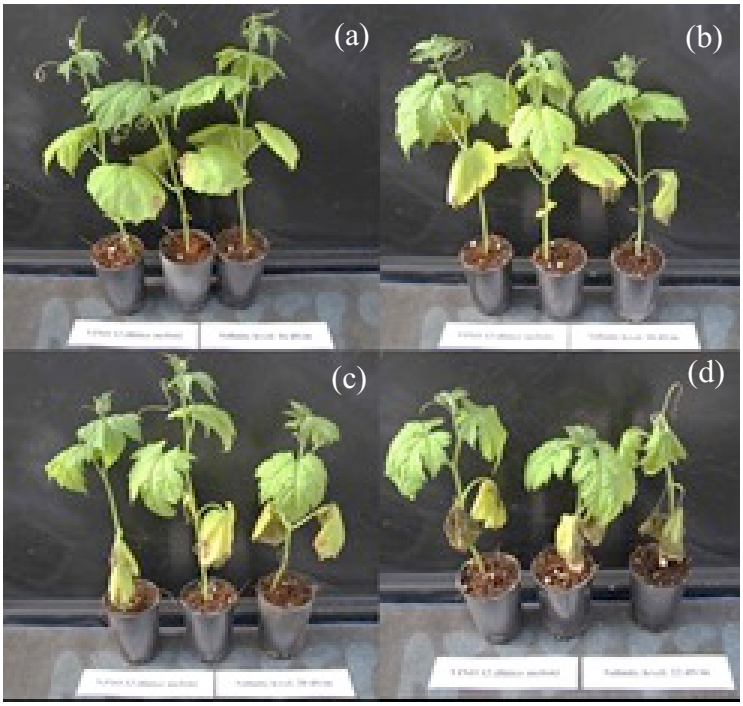


Figure 3.11 The symptoms of salinity effects on bitter melon seedlings at the different salinity levels: a (16.0 dSm^{-1}), b (18.0 dSm^{-1}), c (20.0 dSm^{-1}) and d (22.0 dSm^{-1}).

3.3.4 The resistance of Pythium on the rootstocks and scions

Similar to the salinity tests, the resistance of Pythium on the rootstocks is also an important test for both scion and rootstocks. A symptom of Pythium diseases are characterised by damping of young plants and a yellow wilt on the young seedlings. Root infection manifests as browning and rotting. These symptoms typically appeared on inoculated seedlings (Figure 3.5). However, there was a significant difference in the latent period among different rootstocks and scion. The results (Table 3.8) indicated that the seedlings of the Qb variety showed symptoms of yellow and wilt after inoculation from three to 11 days and the mortality rate was the highest compared to the other varieties, with 96.27%. The symptoms also appeared in the Bm, Sp and Rg at the fourth, fifth and sixth day and ended on the ninth day respectively. The rate of seedling death of these three varieties was 62.67%, 29.07% and 44.77% respectively. Both rootstocks Sampson and Ringer had lower mortality rate than Bitter melon. Therefore, the use of these varieties as rootstocks can reduce the effect of PA on the death of Bitter melon plants.

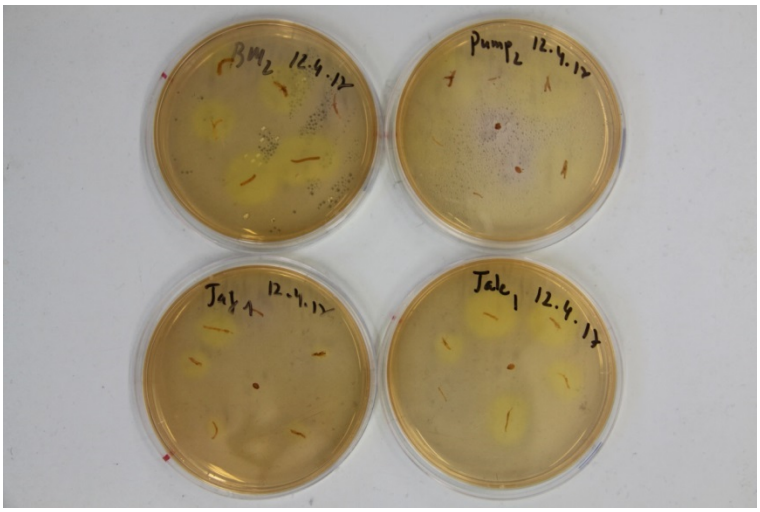


Figure 3.12 *Pythium aphanidermatum* recovered from the roots of the seedlings after 24 hours incubation at 25°C.

Table 3.7 The effects of Pythium on rootstock and scion seedlings

Replication	Rate of seedling death (%)			
	Queensland Blue	Sampson	Ringer	Bitter melon
1	100.00	30.67	49.33	65.33
2	100.00	32.00	46.67	64.00
3	96.00	30.67	43.84	62.67
4	93.33	26.67	44.00	60.00
5	92.00	25.33	40.00	61.33
Average (%)	**96.27 ± 3.70**	**29.07 ± 2.89**	**44.77 ± 3.49**	**62.67 ± 2.11**

Table 3.8 Salinity resistance of rootstock seedlings

Variety	Salinity level and Survival rate (%)						
	14 dSm^{-1}	**16 dSm^{-1}**	**18 dSm^{-1}**	**20 dSm^{-1}**	**22 dSm^{-1}**	**24 dSm^{-1}**	**26 dSm^{-1}**
Qb	100.00 ± 0.00^{aA}	100.00 ± 0.00^{aA}	96.00 ± 5.48^{aA}	92.00 ± 8.37^{abA}	86.00 ± 11.40^{abA}	78.00 ± 13.04^{bA}	52.00 ± 8.37^{cB}
Sp	100.00 ± 0.00^{aA}	100.00 ± 0.00^{aA}	100.00 ± 0.00^{aA}	98.00 ± 4.47^{aA}	92.00 ± 8.37^{abA}	84.00 ± 8.94^{bcA}	76.00 ± 11.40^{cA}
Rg	90.00 ± 7.07^{aA}	74.00 ± 15.17^{abB}	60.00 ± 12.25^{bB}	36.00 ± 8.94^{cB}	20.00 ± 7.07^{deB}	4.00 ± 5.48^{efB}	0.00 ± 0.00^{fC}
Bm	68.00 ± 14.83^{aB}	60.00 ± 15.81^{abB}	40.00 ± 10.00^{bC}	12.00 ± 13.04^{cC}	0.00 ± 0.00^{cC}	0.00 ± 0.00^{cB}	0.00 ± 0.00^{cC}

Data are the means ± standard deviations (n = 5). Data sharing different superscript letters (a, b, c) in the same row indicate significant difference in the germination rates of one variety at different dates ($P < 0.001$).

Data sharing different superscript letters (A, B, C) in the same column indicate significant difference in the germination rates of four varieties at the same date, in the same period ($P< 0.001$).

3.4 Discussion

The results of this study highlighted the importance of determining germination times of both scions and rootstocks, to optimise grafting times and ensure selection of the most appppropriate grafting method (Davis et al. 2008). This study showed that the best grafting method varies with stage of development. . In addition, rootstock seedlings were tested for *Pythium* root rot and salinity tolerance before grafting, This approrach resulted in rootstocks that were more likely to be successful under harsh environmental conditions.

In this study, the temperature of incubators was set at 25±1ºC for the three pumpkin varieties (rootstocks) and bitter melon (scion). Studies have reported that this temperature is the optimal temperature range for germinating pumpkin (Zehatab-Salmasi 2006) and bitter melon (Wang et al. 2003, Nerson 2007). In the off season, gemination periods were typicallly longer due to lower ambient air temperature (13.3±2.0ºC) compared to the main season (22.6±1.8ºC). In addition, the temperature of tap water supplied daily to maintain humidity at 80% during germination process was also similar to the ambient air temperature (13.3±2.0ºC), resulting in the temperature in the pot falling to below 25ºC. Lower temperatures increase germination time in many species that belong to the Cucurbitaceae family. For example, the germination time of *Cucurbita moschata* and *Cucurbita maxima* reduced from 13 days and 11 days (at 18º C) to 6 and 6.3 days (at 25ºC), respectively (Yetisir and Sari 2003). Bitter melon had longer germination times and the germination rate was lower than three rootstocks because this is also a sensitive species to sub-optimal temperature. This has been previously shown to be due to a marked reduction in enzymatic activities associated with carbohydrate and lipid degradation (Nerson 2007).

To improve germination rate, some technical measures can allow obtaining the large number of seedlings in short time. For example, seeds can be immersed in warm water (40ºC) overnight and treated with H_2SO_4 for 20 min, they also can soak in α-Naphthaleneacetic acid (NAA) or gibberellic acid (GA3) in a period of time (Soyler and Khawar, 2007). Poor seed germination is a major problem in wide-scale agricultural farming and breaking seed dormancy plays an important role in seedling production. Balaguera-López et al. (2008) reported that the use of gibberellic acid (GA3) is not only to enhance the germination percentage and reduce the sprouting time, but also equally achieve faster growth speed, less time for seedling preparation.

The absorption of sodium differed between the four varieties, resulting in diferrent seedling mortality rates at each salinity level. This was clearly evident as salinity of the nutrient solution

was increased over time (Table 3.2). At a salinity level of 16.0 dSm^{-1}, the Qb and Sp had the highest survival rate and showed no symptoms of salt stress making these rootstocks ideal for use in saline soil conditions. The Bm was the most susceptible species to salinity as it showed severe symptoms of salt stress and low survival rate (60%) confirming the need to use a more salt tolerant rootstock for production in these adverse conditions. These results suggest that grafted bitter melon plants should be planted at the salinity level 16.0 dSm^{-1} in later experiments. This salinity level was much higher than that previously used for *Cucurbita maxima* and *Lagenarai siceraria* tests (8.0 dSm^{-1}), and similar to that used for cucumbers when tested at 16.2 dSm^{-1} (Chartzoulakis 1992, Yetisir and Uygur 2010). In addition, 16.0 dSm^{-1} is the salt level limit of the most saline soils located in the two major production areas, the Red and the Cuu Long River Deltas, in Vietnam (Đức et al. 2009, Đức and Đạo 2011).

Grafting method is the decisive factor determing the success rate. It was previous demonstrated by Davis et al (2008) that the survival rate of common Cucurbit grafted plants, such as watermelon, pumpkin, cucumber and bitter melon, was inversely correlated with the difference in diameters of scion and rootstock. Results from this study are consistent with the above finding since the combination with the lowest hypocotyl diameter ratio (bitter melon on Sampson) had the most successful outcome (Table 3.6). The SLS grafting technique had a significantly higher survival rate than the TA technique for all three rootstocks. In fact, the TA grafting method is generally preferred when rootstocks and scions have thin stems or narrow hypocotyls, such as watermelon, cucumber and oriental melons (Marsic and Osvald 2004, Davis et al. 2008). The success rate obtained from this study was slightly higher than that of grafting watermelon onto *Cucurbita moschata* and *Cucurbita maxima* (85.0%) and other Cucurbit rootstocks (Yetisir and Sari 2003). In addition, the SLS grafting method increases the chance of contact between the vascular bundles at the cut surface of hypocotyls in both scion and rootstock (Masayuki et al. 1993).

The use of the Sp and Rg rootstocks can reduce the level of damage caused by *Pythium aphanidermatum* (PA) disease compared to the other rootstock (Qb) and scions (Table 3.8). This result indicates that on bitter melon farms with a root rot disease history, growers can use bitter melon scions grafted onto the Sp and Rg rootstocks in order to reduce the effects of Pythium disease. In addition, PA survival is lower under saline soils, therefore, disease caused by this pathogen can be naturally limited in saline soils (Al-Sadi et al. 2010). On the other hand, *Cucurbita maxima* varieties are resistant to fusarium wilt and have good tolerance to low and

high soil temperatures making them very suitable rootstocks in soils infested by pathogens and in all seasons (Davis et al. 2008).

3.5 Conclusion

The germination proportion of both rootstocks and scion achieved around 96.5–99.5% in the main seasons and 80.0–90.0% in the off seasons, incubated at 25^{o}C and a humidity of 80%. The germination time of bitter melon seed was longer than that of rootstocks (Qb, Sp and Rg), and took 6-9 days depending on season. Future studies should focus on trying to shorten germination time further by using NAA or GA3 in breaking seed dormancy.SLS grafting method provided the highest success rate compared to TA grafting method with 90.0%, 81.1% and 91.1% for the Qb, Sp and Rg, respectively. This is the first report on improving the success rate of grafting bitter melon by controlling seed germination time. Further enhancements using different grafting methods, such as Hole insertion grafting method, may result in higher success rates. However, this method requires greater operator skill and optimum enviromental conditions to ensure success. The Sp rootstock was more tolerant to PA, isolated from infected bitter melon plants and used to inoculate healthy seedlings. This rootstock had the lowest mortality at 29.1% compared to bitter melon and the Qb and Rg rootstocks.

In addition, the three rootstocks and bitter melon on its own roots had survival rates greater than 60% under saline conditions of 16.0 dSm^{-1}. Therefore, we can predict that the new grafted plants and control (self-grafted plant) can grow under saline conditions at 16.0 dSm^{-1}. It is also the first report on salinity tolerance limits of some pumpkin varieties and bitter melon.

CHAPTER 4

THE EVALUATION OF ROOTSTOCKS ON BITTER MELON PRODUCTION AS AFFECTED BY SALINITY

4.1 Introduction

In saline conditions, an appropriate rootstock may reduce the effects of salinity because of its ability to alter leaf physiology, including ion saline accumulation and Na+ (Yamaguchi and Blumwald 2005). The Na+ and Cl− accumulation induced by salinity in leaves has been shown to be controlled predominantly by the genotype of the rootstock (Santa-Cruz et al. 2002). The characteristics of the rootstock able to induce salt tolerance to the shoot depend on the salt tolerance mechanism of the shoot genotype through two phases: a rapid, osmotic phase that inhibits growth of young leaves, and a slower, ionic phase that accelerates senescence of mature leaves (Santa-Cruz et al. 2002, Munns and Tester 2008). Grafting may represent an effective tool to improve crop tolerance to salinity (Colla et al. 2006).

In grafted tomato plants, salinity has adverse effects not only on the biomass yield and relative growth rate, but also on other morphological parameters and the development of plants, such as plant height, number of leaves, number of flowers, stem diameter and height, root length and shoot/root weight ratio (Santa-Cruz et al. 2002). According to Gama et al. (Gama et al. 2007), photosynthesis, transpiration rate and stomatal conductance were adversely affected in common bean (*Phaseolus vulgaris* L.). As a result, these parameters impact vegetable yield and quality. However, salinity resistant rootstocks can improve the number of leaves, flowers, laterals, fruits, stem diameter, stem fresh height and fruit yield in comparison to non-grafted plants (Sivritepe et al. 2003). In Cucurbit species, the utilization of salt resistance rootstocks has not been investigated but the initial results of grafted tomato plants, cucumber and melon plants demonstrated the prospects for this new direction (Fernández-Garcia et al. 2004, Huang et al. 2010).

In non-saline conditions, rootstocks have both negative and positive influences on the development of grafted plants. Rootstocks can improve the development of grafted plants, including an increased number of leaves, wider stem diameter and taller height (Davis et al. 2008). For example, Watermelon cv. Sugar Baby and Crimson Sweet scion grafted onto pumpkin and bottle gourd were taller and had a larger leaf area and fresh weight than the self-

rooted plants in the first year. This advantage continued into the second year, with grafted plants outperforming self-rooted plants across these parameters (Petropoulos et al. 2012). Rootstocks can also impact fruit yield and quality through the elongating rate of the main laterals, the number of female flowers and the position of these flowers on plants and as well as the ratio of female: male flowers and basal stem diameters (Cansev and Ozgur 2010). In addition, rootstocks can affect the plant weight and fruit firmness (Yetisir and Sari 2003, El-Sayed et al. 2015). For example, watermelon scion grafted onto *Lagenaria* type rootstocks produced 27–106% higher yield, 42–180% higher dry stem weight, 58–100% more leaves and larger leaf area than their ungrafted plants. Total fruit yield in grafted plants between watermelon scion onto 2 rootstocks, including luffa (Macis) and pumpkin (Ercole) had a higher crop yield than in ungrafted plants by 81% (Colla et al. 2006). In contrast, graft incompatibility commonly causes physiological disorders, reduction in flower formation, fruit yield and fruit quality (Edelstein 2004). Grafting watermelon onto *Cucurbita* sp rootstocks had 127–240% less yield than the ungrafted plants. This result could be attributed to incompatibility of *Cucurbita* rootstocks because some of the plants died before harvesting (Yetisir and Sari 2003).

Rootstocks can impact fruit characteristics and lead to a change in fruit number and fruit productivity (Yetisir and Sari 2003, El-Sayed et al. 2015). However, the effects of rootstocks on fruit size and weight depended on the specific species as well as the rootstocks that were used for grafting (Giorgi et al. 2005). Grafting increased fruit size, resulting in higher yields than in the non-grafted control. It is considered that these differences in fruit characteristics do not constitute serious quality defects and therefore grafting of this crop is advantageous (Alexopoulos et al. 2007). Studies have shown that grafting applications did not significantly affect the diameter, length and volume of fruits, but improved yield and quality of several fruit-bearing vegetables such as watermelon, cucumber, and muskmelon (Lee 2006).

In South Korea, bitter melon grafted onto Luffa IL9 and IL16 as rootstocks improved the fruit quality more than other rootstocks (Zhen Dong et al. 2013). Other studies have reported that bitter melon grafted plants with different luffa rootstocks can improve fruit yield from 38% (Jiebao and Tianlun 1997) to 258.5% (Xingxue et al. 2012). Therefore, the grafting combination has a major effect in terms of both yield and quality of scion fruit. Therefore the selection of the right combination would be a useful means of improving fruit production and quality (Petropoulos et al. 2012).

The effects of growing seasons, including main seasons and off seasons, on the development of plants and crop yields are essentially the impacts of temperature and relative humidity, and

light intensity to these parameters. Temperature is a primary factor affecting the rate of plant development. In controlled environment studies, warm temperatures increased the rate of phenological development but there was no effect on leaf area or vegetative biomass compared to normal temperatures. However, temperature can affect pollination, one of the most sensitive phenological stages to temperature, in all species and would greatly affect production (Hatfield and Prueger 2015). Temperature also can affect the rates of fruit growth in volume. In addition, fruits are more sensitive to elevated temperature in their later stages of maturation (Adams et al. 2001). Specially, the increase of temperature relates to the increase of salinity levels (Hanson et al. 2006). At a salinity level of 16.0 dSm^{-1}, the summer in both Vietnam and Australia with an average temperature regularly exceeds 30^{o}C will directly affect bitter melon. In addition, 16.0 dSm^{-1} is also the limit of bitter melon's saline condition.

***Specific objective of this study were to:**

- Evaluate the effects of rootstocks on the development of bitter melon grafted plants, including the number of leaves, laterals, female flowers, stem diameter and height, when grown under saline and non-saline conditions both indoors and outdoors.
- Determine the effects of rootstocks on fruit production (fruit number, size and weight) and green yield (leaf and stem fresh weight) harvested from grafted plants grown under saline and non-saline conditions.

4.2 Materials and Methods

This section is described in Chapter 2. Based on the results in Chapter 3, grafted seedlings were created by single leaf splice grafting method (SLS). Salinity and other factors, such as type of rootstocks, volume of saline nutrition supply, were considered as homogenous. An experiment was treated as a repeat in evaluating the effects of temperature and relative humidity on bitter melon productivity through growing grafted plants in different seasons, mains and off seasons.

The effects of growing seasons and growing conditions on fruit yield are essentially the combined result of many impacting factors on the number of fruits, and fruit weight, such as temperature and relative humidity, light intensity and lighting time (day length). However, our study only mentioned of two factors of environment temperature and humidity. A combination of three experiments in three different environmental conditions corresponding to three replicates, including one in the 2016 off season (Expt 1), two in the 2017 main season, including indoors (Expt 2) and outdoors (Expt 3).

The self-grafted plant for the control used a bitter melon scion grafted onto a bitter melon rootstock.

4.2.1 The effects of rootstock on the development of bitter melon plants that were grown under saline conditions in off season and main season

4.2.1.1 Growing time and conditions

In the off season, experimental plants (grafted and self-grafted plants) were grown at the NSW Department of Central Coast Primary Industries Centre (DPI), Ourimbah, NSW, Australia (151° 22'E, 33° 21'S). In 2016, growing commenced in May and finished in June (outdoors) and October (indoors). In the main season, experimental plants were grown at the same location from October, 2016 to March, 2017 both indoors and outdoors.

In the climate-controlled greenhouse, the air temperature was maintained between 18 °C and 24°C with relative humidity between 78% and 92%. In winter, however, the average temperature on the Central Coast, Australia was 13ºC (max: 24.2ºC, min: 4.2ºC) with equivalent humidity of 61% (Table 2.2). Therefore, the experimental plants growing outdoors in winter performed poorly and did not survive beyond six weeks. All plants grown under these sub-optimal conditions grew slowly producing smaller stems, small leaves and were impacted by disease.

4.2.1.2 The effects of rootstocks on the grafted bitter melon salinity response growing inside greenhouse and outside

The aim of this experiment was to compare the development of grafted plants under saline conditions with grafted plants in non-saline conditions.

Depending on plant development, the volume of saline solution (ml) increased by 2.0 dSm^{-1} per week, from 4.0 to 16.0 dSm^{-1}, and was provided two days per week (Monday and Thursday). The saline treatment was achieved by supplying individual plant with a saline solution on a weekly basis. From week nine, the saline treatment for plants was stabilised at 16.0 dSm^{-1} and provided two days a week. The volume of salinity nutrient solution divided into two times a day with 1000ml per plant/time (Figure 4.1).

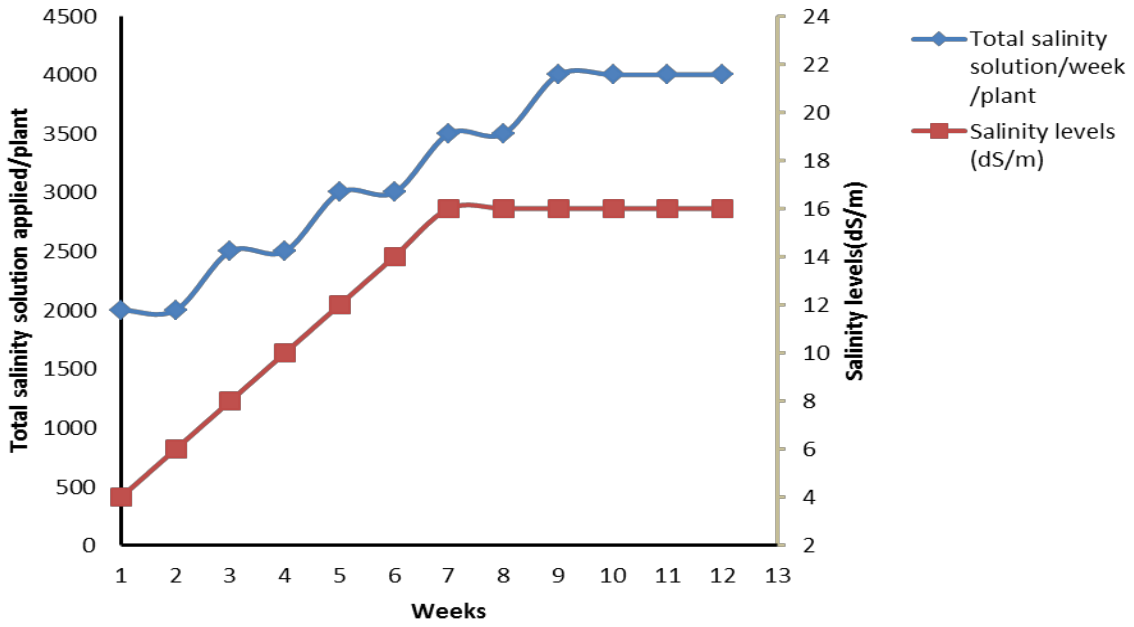


Figure 4.1 The increase of saline solutions applied per an experimental plant and maintained weekly from week 9.

The automatic nutrient supply was stopped two hours before the salinity nutrient was supplied by hand. The volume of salinity nutrient supply to the experimental plants increased from 1000ml/plant to 2000ml/plant after nine weeks. The control plants with non-salinity nutrient were also provided with an adequate volume. It was poured slowly onto the plant until water emerged from the base of the bag. The automatic nutrient supply was restarted after a further two hours.

Each treatment included three plants with three replicates for all treatments. The varieties were denoted as follows:

Bm/Qb was bitter melon scion grafted onto Queensland blue rootstock: Six plants (three planted under saline conditions).

Bm/Sp was bitter melon scion grafted onto Sampson: Six plants (three planted under saline conditions).

Bm/Rg was bitter melon scion grafted onto Ringer: Six plants (three planted under saline conditions).

Bm (control 1): Seven ungrafted bitter melon plants were used in the off season 2016 (three planted under saline conditions).

Bm/Bm (control 2, self-grafted plant): Seven plants. These plants were used in the main season 2017, (four planted under saline conditions indoor experiments and three planted outdoor experiments). The success of using grafted plants was analysed by the following measurements:

- Number of leaves
- Leaf area
- Number of male and female flowers
- Number of laterals
- Stem height
- Stem fresh weight

Measurements of some parameters, such as the number of leaves, laterals and main stem height, were stopped at Week 6 because the plants had reached the top of the trellis and had started to flower. Other parameter measurements, such as the number of laterals and stem fresh weight were stopped at the end of the experiment.

Leaf area (LA) of bitter melon was measured using the equation LA=0.88LW – 4.72, for all grafting and saline conditions: L: leaf length; W: leaf width (Blanco and Folegatti 2005).

The number of laterals was counted at week six with the laterals over 80cm in length.

Stem fresh weight was harvested five months after growing, which is equivalent to the growing time of bitter melon in the fields in Vietnam.

4.2.2 The effects of rootstock on fruit productivity in grafted plants growing under saline conditions in off seasons and main seasons

This experiment was conducted in conjunction with the experiment in part 4.2.1. All fruit, after harvesting, were evaluated by measuring parameters as follow:

- Fruit characteristics: diameter, length
- Fruit weight
- Number of fruits per plant
- Total fruit weight

In the off season, all marketable fruits were harvested at 29-30 days from pollination to harvest (days after pollinating: DAP) with the plants grown in greenhouse conditions.

In the main season, all marketable fruits were harvested at 14-15 DAP and 19–20 DAP, with the plants grown inside and outside the greenhouse respectively. The fruits obtained above 80 grams in weight were harvested and the yield of each individual plant was measured.

In the greenhouse, once flowering commenced, most of the female flowers were hand pollinated with pollen from male flowers of the same plant or other plants in the same group. For outside greenhouse crops, pollination was totally depended on nature (insect pollination).

Each female was labelled the date of the pollination (indoor) or bloom time (flower opening). All experiments stopped after 5 months although all the plants were growing normally. This means that the number of fruit and the yield of the plants were even higher than the figures reported in this research. (During this study period, only one greenhouse was required, therefore, some experiments were completed early to conduct other experiments).

* *The variation of fruit diameter, plant fresh weight and fruit yield are calculated by the following equation:*

$$\text{The variation } = \left(\frac{The\ value\ of\ treatment}{The\ value\ of\ control} - 1\right) x\ 100\ \%$$

+ The value of treatment: bitter melon scion grafted onto 3 rootstocks.

+ The value of control: non-grafted plants and bitter melon grafted onto bitter melon.

* *The fruit success (%) is calculated by the following equation:*

$$\text{The fruit success } = \left(\frac{\text{The number of mature fruit harvested}}{\text{The number of female flowers pollinated}}\right) x\ 100\ \%$$

4.2.3 Statistical analysis

Data were analysed using Two Way ANOVA. Statistical analyses were performed using SPSS 22.0 version software. The Tukey multiple comparison test ($P < 0.05$) was used to compare the stem fresh weight, the number of leaves, laterals and the fruit set. The Tukey multiple comparison test ($P < 0.05$) was also used to compare the fruit number, diameter, length, weight of fruit and yield in different rootstocks and growing conditions.

4.3 Results

In this study, grafted plants were planted once in the off-season of 2016 and once in the main season of 2017 under climate-controlled greenhouse conditions, with the temperature set from 18°C–24°C and humidity around 78-92% (Table 2.2). Therefore, environmental factors which may affect the experiment were excluded due to lack of replication of these environments. Rootstocks and saline conditions did not affect the development of plants but influenced bitter melon fruit number and yield.

4.3.1 Effects of rootstocks and saline conditions on the development of grafted plants grown in the off season 2016.

The analysis of variance indicated that saline treatment and three rootstocks did not affect the development of bitter melon grafted plants, including the main stem height, the number of

leaves and number of laterals (Table 4.1). There were no significant differences in the main stem height in three rootstocks grown under saline conditions and non-saline conditions at week 6, compared to the control plants (P>0.05). In a similar trend, rootstocks and saline condition did not affect the number of leaves and laterals in the grafted plants and control - ungrafted bitter melon (P>0.05). A two-way ANOVA analysis was conducted that examined the effect of rootstock and salinity condition on the stem height, the number of leaves and the number of laterals. There was not a statistically significant interaction between the effects of rootstock and salinity level on the stem height, the number of leaves and the number of laterals, of grafted plant (P>0.05, n=3).

Our results (Table 4.2) showed that rootstocks did not affect the size of the leaves, including length and width, and leaf area in the grafted bitter melon plants in both saline and non-saline conditions (P>0.05). A two-way ANOVA was conducted that examined the effect of rootstocks and saline conditions on the size of leaves. There was not a statistically significant interaction between the effects of rootstock and salinity condition on the length of leaves, the width of leaves or leaf area (p>0.05, n=3).

Table 4.1 Effects of rootstocks on the leaf size and number (in the 2016 off season)

Growing Conditions	**Experimental plants**	**Leaf size**		**Leaf area (cm^2)**
		Length (cm)	Width (cm)	
Saline ($16\ dSm^{-1}$)	Bm/Qb	16.67 ± 0.29^a	19.17 ± 2.08^a	276.95 ± 27.33^a
	Bm/Sp	19.00 ± 1.00^a	20.67 ± 0.67^a	341.33 ± 30.93^a
	Bm/Rg	19.67 ± 1.44^a	23.50 ± 1.50^a	402.51 ± 40.29^a
	Control	18.83 ± 1.89^a	20.00 ± 3.12^a	327.14 ± 80.86^a
Non-saline ($0.5\text{-}1.6\ dSm^{-1}$)	Bm/Qb	18.67 ± 1.89^a	20.00 ± 3.12^a	324.32 ± 81.98^a
	Bm/Sp	19.83 ± 1.04^a	21.83 ± 3.21^a	368.03 ± 76.64^a
	Bm/Rg	19.67 ± 0.29^a	23.17 ± 2.08^a	396.79 ± 32.18^a
	Control	19.13 ± 2.53^a	20.88 ± 2.38^a	347.23 ± 86.50^a

The data are means ± standard deviations (n=3) and those sharing the same superscript letter in the same column are not significantly different as determined by ANOVA and the means within columns separated using Turkey's multiple comparison test, (P>0.05).

Table 4.2 Effects of rootstocks on the growth of grafted plants (off season 2016)

Condition	**Salinity** (16 dSm^{-1})			**Non-salinity** (0.5-1.6 dSm^{-1})		
Variety	**Number of leaves**	**Number of laterals**	**Main stem height**	**Number of leaves**	**Number of laterals**	**Main stem height**
Bm/Qb	23.67 ± 2.08^{a}	14.33 ± 2.08^{a}	233.67 ± 27.79^{a}	24.67 ± 3.79^{a}	13.67 ± 1.15^{a}	261.00 ± 37.32^{a}
Bm/Sp	28.67 ± 2.52^{a}	13.67 ± 3.21^{a}	275.33 ± 29.96^{a}	28.33 ± 1.53^{a}	17.67 ± 2.52^{a}	280.00 ± 17.32^{a}
Bm/Rg	24.33 ± 2.08^{a}	15.33 ± 3.51^{a}	277.67 ± 6.80^{a}	26.00 ± 0.00^{a}	18.00 ± 2.00^{a}	274.00 ± 29.46^{a}
Bm (Control 1)	26.33 ± 2.31^{a}	15.33 ± 3.79^{a}	270.33 ± 17.62^{a}	25.00 ± 1.15^{a}	16.25 ± 1.71^{a}	257.75 ± 32.36^{a}

The data are means ± standard deviations (n=3) and those sharing the same superscript letter in the same column are not significantly different as determined by ANOVA and the Turkey's multiple comparison test, for saline condition and non – saline conditions ($P>0.05$).

Rootstocks and saline condition did not affect the development of grafted plants grown in the 2016 off season. Therefore, we decided to stop measuring these parameters in subsequent studies in the 2017 main season. The number of male flowers was not used to compare the differences between rootstocks grown in both conditions. There was a sufficient number of male flowers to use for hand pollination with the female flowers from the same rootstocks.

4.3.2 Effects of rootstocks on the number of female flowers

There were significant differences in the number of female flowers produced by different rootstocks in both saline and non–saline conditions with the plants grown indoors. All three rootstocks had an increased number of female flowers compared to the control when they were planted indoors. In the same rootstock, the number of female flowers in the plants grown under saline conditions was higher than that of plants grown under non-saline conditions (Table 4.3).

In the 2016 off season, the Rg rootstock produced the most flowers under non-saline conditions at 79 flowers per plant, while the Sp rootstock was the best performer under saline conditions producing 70 flowers. This compared to the control with 43 and 36 flowers per plant when produced under the same conditions respectively ($F=3.860$, $P=0.011$).

In the 2017 main season and indoor production, the number of female flowers in Rg rootstock was the highest with 124 (in saline conditions) and 156 (in non-saline conditions) flowers per plant, compared to the control producing only 97.7 and 98.0 flowers under the same growing conditions, respectively ($F=3.123$, $P=0.026$). However, there were no significant differences in the number of female flowers grown under saline and non-saline conditions, in the 2017 main season and outdoor production ($F=1.038$, $P=0.442$).

A two-way ANOVA was conducted to examine the effects of rootstocks and saline conditions and interaction between rootstocks and salinity on the number of female flowers. There was not a statistically significant interaction ($P>0.05$) between the effects of rootstock and salinity level on the number of female flower grown indoors and outdoors in both main seasons and off season ($n=3$).

Table 4.3 Effects of rootstocks and salinity on the number of female flowers

Growing conditions	Experimental plants	Number of female flowers		
		Indoors		Outdoors
		Off season 2016 (Expt 1)	Main season 2017 (Expt 2)	Main season 2017 (Expt 3)
Salinity (16 dSm^{-1})	Bm/Qb	42.00 ± 6.66^{ab}	108.00 ± 14.42^{ab}	85.33 ± 20.43^{a}
	Bm/Sp	70.00 ± 23.35^{ab}	119.33± 17.04^{ab}	89.33 ± 5.51^{a}
	Bm/Rg	62.33 ± 11.06^{ab}	124.33 ± 18.01^{ab}	99.00 ± 6.24^{a}
	Control	36.33 ± 7.51^{b}	84.25 ± 28.05^{b}	71.33 ± 19.04^{a}
Non-salinity (0.5-1.6 dSm^{-1})	Bm/Qb	46.33 ± 17.35^{ab}	112.67 ± 16.26^{ab}	81.33 ± 7.02^{a}
	Bm/Sp	62.33 ± 17.06^{ab}	108.33 ± 24.01^{ab}	90.33 ± 17.79^{a}
	Bm/Rg	79.33 ± 14.22^{a}	156.00± 21.66^{a}	83.67 ± 22.48^{a}
	Control	41.00 ± 8.68^{ab}	98.00 ± 25.36^{ab}	72.75 ± 18.15^{a}

The data are means ± standard deviations and those sharing the same superscript letter in the same column are not significantly different as determined by ANOVA and the means within columns separated using Turkey multiple comparison test, for salinity condition and non – salinity condition (P>0.05).

4.3.3 Effects of rootstocks on the stem diameter and fresh weight

Rootstocks improved the diameter of stem in the grafted plants in off season but had no impact in the main season. The increase of stem diameter in grafted plants was very clear and the analysis variance showed these values were significantly different in the 2016 off season and indoors. Correspondingly, they improved the stem fresh weight in this growing time, which were harvested at the end of the experiments.

In the 2016 off season (Expt 1), rootstocks did affect the increase in stem diameters (F=5.071, P=0.003). The stem diameter of the grafted plants was larger than that of control (ungrafted plants) when they were grown in the same conditions (Table 4.5). In the 2017 main season, rootstocks (Expt 2) had no affect the diameter of stems between all grafted plants grown indoors and under saline and non-saline conditions (F=0.845, p=0.566). In a similar trend, outdoors (Expt 3) rootstocks had no affect the stem diameters (F=0.894, p=0.533). Specially, the incremental values of stem diameters for grafted plants grown in the off season in both conditions were higher than that of main season and this value outdoors were smaller than indoors (Table 4.4).

The average fresh weight of plants in different rootstocks is presented in Table 4.4. The increase of stem diameter led to the increase of stem fresh weight in the off-season but not in the main season. In the 2016 off-season, the plant fresh weight increased from 2.73kg to 3.5kg with the plants grown under saline conditions and 2.83kg to 5.83kg with the plants grown under non-saline conditions, compared to the controls ($F=3.146$, $p=0.025$). However, there were no significant difference in the average of fresh weight of plants grown indoors and outdoors in the 2017 main seasons, $F=1.890$, $p=0.134$ and $F=0.952$, $p=0.494$, respectively. Although the values of plant fresh weight in the 2017 main season reduced from 6.27kg to 6.14kg (indoors and salinity) and from 5.75kg to 5.01kg (indoors and non-salinity), except for the Qb rootstock grown under saline conditions and the Rg rootstock grown in non-saline conditions (Table 4.5). Specifically, rootstocks reduced the plant fresh weight grown outdoors under saline and non-saline conditions from 2.49kg to 2.45kg (saline conditions) and from 2.56kg to 1.55kg (non-saline conditions). A two-way ANOVA was conducted to examine the effects of rootstocks and saline conditions and interaction between rootstocks and salinity on the diameter of stems and plant fresh weight. There was not a statistically significant interaction ($p>0.05$) between the effects of rootstock and salinity level on the diameter of stems and plant fresh weight grown indoors and outdoors in both main season and off season ($n=3$).

Table 4.4 Effects of grafting combination and saline condition on the diameter of scion and plant fresh weight grown in climate-controlled greenhouses and outdoor, in the off seasons and main seasons

Growing conditions	Experimental plants	Off season 2016		Main season 2017			
		Indoor (Expt 1)		Indoor (Expt 2)		Outdoor (Expt 3)	
		Diameter of stem (cm)	Plant fresh weight (Kg)	Diameter of stem (cm)	Plant fresh weight (Kg)	Diameter of stem (cm)	Plant fresh weight (Kg)
Salinity (16 dSm^{-1})	Bm/Qb	3.38 ± 0.34^{bc}	2.73 ± 1.17^{ab}	2.65 ± 0.76^{a}	8.71 ± 2.65^{a}	2.58 ± 0.37^{a}	2.48 ± 0.82^{a}
	Bm/Sp	3.56 ± 0.42^{abc}	3.17 ± 1.56^{ab}	2.55 ± 0.89^{a}	6.14 ± 1.91^{a}	2.90 ± 0.76^{a}	2.45 ± 0.34^{a}
	Bm/Rg	4.42 ± 0.95^{ab}	3.50 ± 1.99^{ab}	2.99 ± 0.65^{a}	6.27 ± 1.49^{a}	2.84 ± 0.52^{a}	2.49 ± 0.20^{a}
	Bm	2.96 ± 0.30^{c}	2.13 ± 0.47^{b}	2.28 ± 0.35^{a}	7.18 ± 0.80^{a}	2.49 ± 0.23^{a}	2.52 ± 1.56^{a}
Non-salinity (0.5-1.6 dSm^{-1})	Bm/Qb	4.10 ± 0.20^{abc}	2.83 ± 0.67^{ab}	2.30 ± 0.56^{a}	5.75 ± 1.04^{a}	2.41 ± 0.34^{a}	2.41 ± 0.60^{a}
	Bm/Sp	3.71 ± 0.56^{abc}	3.57 ± 1.10^{ab}	2.95 ± 0.20^{a}	5.01 ± 0.61^{a}	2.73 ± 0.43^{a}	1.55 ± 0.16^{a}
	Bm/Rg	4.82 ± 0.37^{a}	5.83 ± 1.10^{a}	2.70 ± 0.36^{a}	8.13 ± 1.49^{a}	2.97 ± 0.59^{a}	2.56 ± 1.19^{a}
	Bm	3.32 ± 0.37^{bc}	2.35 ± 0.40^{b}	2.34 ± 0.29^{a}	7.07 ± 1.73^{a}	2.33 ± 0.22^{a}	3.24 ± 0.89^{a}

The data are means ± standard deviations and those sharing the same superscript letter in the same column are not significantly different as determined by ANOVA and the means within columns separated using Turkey multiple comparison test, for salinity condition and non – salinity condition ($P>0.05$).

4.3.4 The comparison between the fruit set of grafted bitter melon grown inside greenhouse in the 2016 off-season and 2017 main season

The effects of rootstocks and saline conditions are presented in Table 4.5. There were no significant differences between the fruiting rates of the grafted plants grown under saline and non-saline conditions (P>0.05), except for grafted plants grown in the 2016 off-season (F=3.003, P=0.05).

Table 4.5 Fruit set (%) under both growing conditions in the 2016 off-season and the 2017 main season for plants grown in climate-controlled greenhouses and hand pollination

Conditions	Treatments	Indoors		Outdoors
		In off season 2016	In main season 2017	In main season 2017
Salinity (16 dSm^{-1})	Bm/Qb	74.02 ± 18.19^{ab}	76.08 ± 8.21^{a}	67.40 ±13.74^{a}
	Bm/Sp	42.38 ± 3.24^{b}	67.22 ± 5.10^{a}	73.65 ±5.56^{a}
	Bm/Rg	60.75 ± 18.60^{ab}	79.52 ± 9.46^{a}	58.60±8.60^{a}
	Bm/Bm	85.14 ± 12.57^{a}	76.04 ± 9.10^{a}	72.49 ±11.45^{a}
Non-salinity (0.5-1.6 dSm^{-1})	Bm/Qb	70.25 ± 13.28^{ab}	80.18 ± 8.98^{a}	64.59 ±13.14^{a}
	Bm/Sp	69.89 ± 27.23^{ab}	73.54 ± 4.27^{a}	57.57 ±12.07^{a}
	Bm/Rg	71.36 ± 5.77^{ab}	80.48 ± 8.17^{a}	69.32 ±6.40^{a}
	Bm/Bm	64.80 ± 7.39^{ab}	81.31 ± 4.33^{a}	72.29 ±31.50^{a}

The data are means ± standard deviations and those sharing the same superscript letter in the same column are not significantly different as determined by ANOVA and the means within columns separated using Turkey multiple comparison test, for salinity condition and non – salinity condition (P>0.05).

Whilst there was a trend showing a decline in % fruit set for grafted plants in both saline and non-saline conditions, these differences were largely not significant (p>0.05). For the main season, there was also no significance difference in fruit set between those plants grown indoors or outdoors. In non-saline conditions the analysis of variance indicated that rootstocks did not affect the fruit set in both offseason and main season (Table 4.5). This trend was similar to the plants grown in the main season, 2017 with F=1.161, p=0.374 (indoors) and F=0.452, p=0.855 (outdoors).

A two-way ANOVA was conducted that examined the effects of rootstocks and saline conditions on the fruit set (%) for the plants grown in three experiments. There was not a statistically significant interaction between rootstocks and saline conditions on the fruit set for

the grafted plants grown in the both growing times (main and off seasons), indoors and outdoors (P>0.05, n=3).

4.3.5 The effects of rootstocks on the number of fruit and yield

In general, all three rootstock treatments increased the number of fruits, which leads to significantly increased fruit yield in grafted plant compared with control. However, rootstocks did not affect the fruit characteristics, including diameter, length and weight of the fruit (P>0.05) in all experiments. The different rootstocks had differences in crop yield. The use of the Rg and Sp for rootstocks and growing in indoor conditions significantly increased fruit yield compared to the Qb rootstocks and control plants (non-grafted and self-grafted bitter melons). In addition, saline treatment reduced production compared with the plants grown in non-saline conditions.

4.3.5.1 The effects of rootstock on fruit characteristics and yield of plants grown in the 2016 off season

The effects of rootstocks and saline conditions on the number of fruits and total fruit weight (kg) are presented in Table 4.6. There were significant differences in the number of fruit from different rootstocks (F=3.689, P=0.013). The Rg rootstock had the highest number of fruit with 57 fruits per plant grown in non-saline conditions and 36 fruits per plant grown under saline conditions, and in normal conditions the Sp rootstock had 39 fruits per plant.

There were significant differences in the fruit yield in three rootstocks and control plants in both saline and non-saline conditions (F=3.347, P=0.020). The Rg rootstock also strongly increased productivity by 39.2% and 51.3% in non-saline and saline conditions, respectively. All rootstocks strongly improved fruit yield compared to the control plants when grown under saline conditions.

A two-way ANOVA was conducted to examine the effects of rootstocks and saline conditions on the size of fruit (diameter, length and weight), the number of fruits and fruit yield grown indoors and off seasons. There was not a statistically significant interaction between the effects of rootstocks and saline conditions on the size of fruit (P>0.05), the number of fruits, F(3,17)=1.768, P=0.191,(n=3) and the fruit yield F(3,17)=1.432, P=0.268,(n=3) grown indoors in off seasons.

4.3.5.2 The effects of rootstocks on the fruit characteristic and yield of plant grown indoors in the 2017 main season

Like grafted plants grown in the 2016 off-season, rootstocks affected the number of fruits and fruit yield in both indoors and outdoors, in the 2017 main seasons. There were significant differences in the number of fruit ($F=12.113$, $P<0.001$) and yield ($F=4.858$, $p=0.004$) from different rootstocks and control plants (self-grafted). Rootstocks strongly increased the number of fruits grown indoors compared to the control plants. The Rg rootstock produced a remarkable amount of fruit with 125.3 fruits per plant in non-saline conditions and 99.7 fruits per plant in saline conditions, which led to increasing productivity by 48.9% and 45.5%, respectively, compared to the control. The saline tolerance of both the Qb and Sp rootstocks are shown by the fruit yield index which higher than that of the control with 36.2% and 37.7%, respectively (Table 4.7). A two-way ANOVA was conducted that examined the effects of rootstocks and saline conditions on fruit size (diameter and length) and fruit weight, the number of fruits and fruit yield grown indoors and off seasons. There was not a statistically significant interaction between the effects of rootstocks and saline conditions on the size of fruit ($P>0.05$), the number of fruits, $F(3,17)=2.013$, $P=0.150$ ($n=3$) and the fruit yield $F(3,17)=1.331$; $P=0.297$,($n=3$) grown indoors in the 2017 main seasons.

4.3.5.3 The effects of rootstocks on fruit characteristics and yield with the grafted plant grown outdoors in the 2017 main season

The analysis of variance showed significant differences in fruit numbers ($F=10.474$, $P<0.0001$) and fruit yield ($F=4.241$, $P=0.007$) between different rootstocks and control plants (self-grafted). In saline conditions, the number of fruit in the Sp rootstock was the highest ranking, with an average of 66 fruits per plant compared to the control plants with an average 35 fruit per plant. In non-saline conditions, the Rg rootstock was the most productive with 79 fruits per plant compared to the control with 45 fruits per plant (Table 4.8). Specially, fruit productivity has increased significantly with 70.6% and 53.4% under saline conditions and 31.2% and 64.0% under non-saline conditions, respectively. In main season and outdoor productions, the combination between rootstocks and salinity gave a different results compared to two indoor experiments. A Two-way ANOVA was conducted that examined the effects of rootstocks and saline conditions on the number of fruits and fruit yield grown outdoors and main seasons. There was a statistically significant interaction between the effects of rootstocks and saline conditions on the number of fruit, $F(3,17)=3.783$, $P=0.03$, ($n=3$) but that was not a statistically significant interaction on the fruit yield, $F(3,17)=1.369$, $P=0.286$, ($n=3$).

Table 4.6 Effects of saline conditions and grafting combination on fruit number, diameter, length, weight and yield of the plants grown indoors in the off season 2016

Growing conditions	Grafting combination	Number of fruit	Individual fruit diameter (cm)	Individual fruit length (cm)	Individual fruit weight (gram)	Total fruit weight (kg)	Variation of yield (%)
Salinity (16 dSm^{-1})	Bm/Qb	30.33 ± 10.69^{b}	5.25 ± 0.47^{a}	18.20 ± 1.31^{a}	188.96 ± 16.72^{a}	**5.87 ± 1.02^{ab}**	**+31.03**
	Bm/Sp	31.00 ± 8.00^{b}	5.01 ± 0.29^{a}	17.93 ± 0.26^{a}	196.38± 26.51^{a}	**5.96 ± 1.53^{ab}**	**+33.04**
	Bm/Rg	36.33 ± 6.03^{ab}	5.01 ± 0.42^{a}	19.33 ± 0.94^{a}	178.12 ± 7.58^{a}	**6.78 ± 1.66^{ab}**	**+51.34**
	Bm	26.33 ± 13.32^{b}	5.02 ± 0.32^{a}	17.25 ± 1.21^{a}	175.19 ± 35.59^{a}	**4.48 ± 1.18^{b}**	**0.0**
Non – salinity (0.5-1.6 dSm^{-1})	Bm/Qb	32.00 ± 2.65^{b}	5.08 ± 0.07^{a}	17.17 ± 0.74^{a}	196.52 ± 4.04^{a}	**6.28 ± 0.42^{ab}**	**-13.85**
	Bm/Sp	39.33 ± 2.52^{ab}	5.27 ± 0.40^{a}	18.40 ± 1.58^{a}	208.44 ± 54.35^{a}	**8.13 ± 1.72^{ab}**	**+11.52**
	Bm/Rg	57.00 ± 3.61^{a}	5.47 ± 0.38^{a}	18.62 ± 0.28^{a}	204.96 ± 49.07^{a}	**10.15 ± 0.80^{a}**	**+39.23**
	Bm/ Bm	33.25 ± 11.59^{b}	5.37 ± 0.17^{a}	18.04 ± 0.52^{a}	217.57 ± 30.48^{a}	**7.29 ± 2.71^{ab}**	**0.0**

The data are means ± standard deviations (n=3) and those sharing the same superscript letter in the same column are not significantly different as determined by ANOVA and the means within columns separated using Turkey multiple comparison test, for salinity condition and non – salinity condition (P>0.05).

Table 4.7 Effects of saline conditions and grafting combination on fruit number, diameter, length, weight and yield of the plants grown indoors in the main season 2017

Growing conditions	Grafting combination	Number of fruit	Individual fruit diameter (cm)	Individual fruit length (cm)	Individual fruit weight (gram)	Total fruit weight (kg)	Variation of yield (%)
Salinity ($16\ dSm^{-1}$)	Bm/Qb	82.67 ± 17.62^{ab}	6.49 ± 1.03^{a}	26.25 ± 4.71^{a}	336.31 ± 106.46^{a}	**28.20 ± 1.70^{ab}**	**+36.23**
	Bm/Sp	80.67 ± 17.01^{ab}	6.76 ± 0.98^{a}	28.27 ± 6.10^{a}	356.99 ± 110.62^{a}	**28.51 ± 1.29^{ab}**	**+37.73**
	Bm/Rg	99.67 ± 24.95^{ab}	6.79 ± 0.97^{a}	26.34 ± 5.33^{a}	308.73 ± 95.84^{a}	**30.11 ± 2.04^{ab}**	**+45.46**
	Bm/Bm	65.50 ± 25.20^{b}	6.37 ± 0.81^{a}	26.59 ± 5.26^{a}	308.94 ± 131.82^{a}	**20.70 ± 1.49^{c}**	**0.0**
Non – salinity ($0.5\text{-}1.6\ dSm^{-1}$)	Bm/Qb	89.67 ± 9.87^{ab}	6.61 ± 0.82^{a}	27.18 ± 5.29^{a}	327.72 ± 107.62^{a}	**29.47 ± 1.57^{ab}**	**+12.18**
	Bm/Sp	79.00 ± 13.11^{ab}	6.43 ± 0.92^{a}	25.67 ± 4.75^{a}	366.17 ± 142.22^{a}	**29.00 ± 1.46^{bc}**	**+10.39**
	Bm/Rg	125.33 ± 20.50^{a}	7.16 ± 0.89^{a}	28.57 ± 4.17^{a}	335.82 ± 115.86^{a}	**39.12 ± 2.47^{a}**	**+48.92**
	Bm/Bm	79.33 ± 18.77^{ab}	6.69 ± 0.94^{a}	26.83 ± 5.38^{a}	356.16 ± 106.40^{a}	**26.27 ± 1.09^{b}**	**0.0**

The data are means ± standard deviations (n=3) and those sharing the same superscript letter in the same column are not significantly different as determined by ANOVA and the means within columns separated using Turkey multiple comparison test, for salinity condition and non – salinity condition ($P>0.05$).

Table 4.8 Effects of saline conditions and grafting combination on fruit number, diameter, length, weight and yield of the plants grown outdoors in the main season 2017

Growing conditions	Grafting combination	Number of fruits	Individual fruit diameter (cm)	Individual fruit length (cm)	Individual fruit weight (gram)	Total fruit weight per plant (kg)	Variation of yield (%)
	Bm/Qb	59.33 ± 8.50^{abc}	5.52 ± 0.34^{a}	20.33 ± 0.83^{a}	162.52 ± 106.22^{a}	**11.09 ± 2.53^{abc}**	**+37.08**
Salinity	Bm/Sp	66.00 ± 9.00^{abc}	5.69 ± 0.11^{a}	20.68 ± 1.85^{a}	209.47 ± 114.60^{a}	**13.80 ± 1.72^{ab}**	**+70.58**
(16 dSm^{-1})	Bm/Rg	58.33 ± 6.66^{bc}	5.73 ± 0.35^{a}	20.88 ± 1.20^{a}	214.90 ± 97.67^{a}	**12.41 ± 2.49^{abc}**	**+53.40**
	Bm/Bm	35.00 ± 2.00^{d}	5.77 ± 0.19^{a}	21.11 ± 0.62^{a}	212.78 ± 76.16^{a}	**8.09 ± 1.25^{c}**	**0.0**
Non – salinity	Bm/Qb	52.00 ± 5.35^{bcd}	5.59 ± 0.33^{a}	20.68 ± 1.08^{a}	209.35 ± 59.9^{a}	**10.79 ± 0.24^{abc}**	**+18.44**
	Bm/Sp	69.33 ± 4.04^{ab}	5.22 ± 0.35^{a}	18.64 ± 1.66^{b}	165.80 ± 77.29^{a}	**11.95 ± 1.79^{abc}**	**+31.17**
(0.5-1.6	Bm/Rg	79.33 ± 1.53^{a}	5.50 ± 0.28^{a}	20.23 ± 2.68^{a}	185.70 ± 60.25^{a}	**14.94 ± 1.24^{a}**	**+64.00%**
dSm^{-1})	Bm/Bm	45.50 ± 12.07^{cd}	5.60 ± 0.08^{a}	20.27 ± 1.19^{a}	208.98 ± 105.60^{a}	**9.11 ± 2.67^{bc}**	**0.0**

The data are means ± standard deviations (n=3) and those sharing the same superscript letter in the same column are not significantly different as determined by ANOVA and the means within columns separated using Turkey multiple comparison test, for salinity condition and non – salinity condition (P>0.05).

4.4 Effects of growing conditions and growing seasons on fruit yield of grafted bitter melon plants

The differences in the number of fruit, fruit yield harvested indoors and outdoors at two different growing seasons is very clear as shown in Table 4.9 and Table 4.10. In the 2017 main season, fruits maturity were started to harvest at week 8 since the plants were planted for both experiments growing indoors and outdoors, while in the 2016 off season and indoor conditions the fruits maturity were started pick at week 10.

4.4.1 Effects of growing conditions and growing seasons on the number of fruit and fruit weight harvested weekly in grafted bitter melon plants

The effects of growing seasons and growing conditions on fruit yield harvested weekly indicated that there were significant differences ($P<0.0001$) in the number of fruit and fruit weight between plants grown in main seasons (indoors, outdoors) and plants grown in off seasons (indoors). Data were recorded continuously for 10 weeks, from week 9 to week 19 in main seasons and from week 10 to week 22 in off seasons, when all the treatment plants were harvested. Our research has shown that, as the temperature rises, the number of fruits harvested increases. The numbers of bitter melon fruits increased in direct proportion to the increase in environmental temperature within the suitable temperature ranges of bitter melon plants (Table 4.9).

Table 4.9 Effects of growing conditions and growing seasons on the number of fruit and average of fruit weight harvested weekly

Growing times	Off season 2016	Main season 2017	
Average/week	Indoors	Indoors	Outdoors
Number of fruit	21.38 ± 11.65^{b}	59.76 ± 32.89^{a}	40.57 ± 21.16^{ab}
Weight of fruit (gram)	241.96 ± 62.30^{b}	364.98 ± 85.86^{a}	243.33 ± 50.19^{b}

The data are means ± standard deviations (n=3) and those sharing the same superscript letter in the same row are not significantly different as determined by ANOVA and the means within columns separated using Turkey multiple comparison test, for salinity condition and non – salinity condition ($P>0.05$).

In fact, temperature and relative humidity may not be two main factors affecting these results. They have little effect on the number of fruits harvested indoors and outdoors per week, in both main seasons and off seasons. A liner regression was developed to predict fruit production with rises in temperature. However, the value of R-square is small ($R^2 = 0.2115$), so it is not

sufficient to conclude that temperature and humidity affected fruit yield (Figure 4.2). This value only can be indicated that the number of fruits had less correlation with temperature.

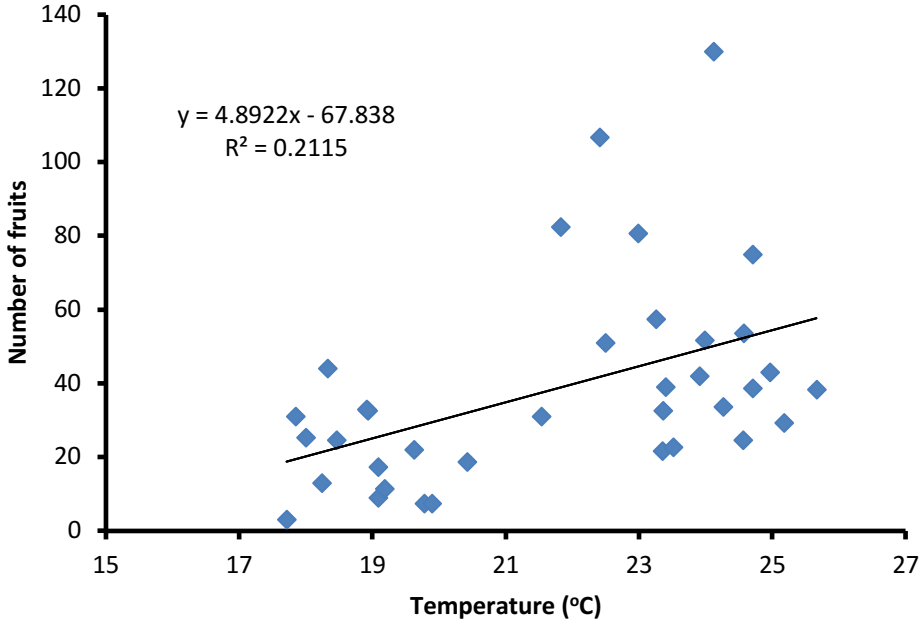


Figure 4.2 The correlation between ambient temperature and number of fruits. Data were collected indoors (2 crops) and outdoors (1 crop)

4.4.2 Effects of growing conditions and growing seasons on the numbers of fruits, fruit weight and total yield

There were significant differences in the numbers of fruits, weight of fruits and yield ($p<0.0001$) from different growing times and growing conditions (Table 4.10). Going back to the Table 2.2, the temperature varied within 18.5±0.9°C and the relative humidity varied within 78.1±5.4% in the 2016 off season. In the 2017 main season, these factors varied higher with 22.6±1.8°C and 77.1±3.6% outdoors, 23.2±0.9°C and 92.2±4.2% indoors, respectively.

Similar to the weekly harvested fruit output in part 4.4.1, the value of R-square is too small ($R^2 = 0.1388$). The increase of temperature has related to an increase in the average weight of fruit. A liner regression was established to predict the fruit yield with the increase in temperature. Although this variable statistically predicted the fruit yield but the R-square value indicated that the fruit weight had less correlation with temperature (Figure 4.3).

Table 4.10 Effects of temperature and relative humidity on the number of fruit per bitter melon plant, the weight of fruit and total yield in differences growing seasons and conditions

Growing times	Off season 2016	Main season 2017	
Fruit production	**Indoors**	**Indoors**	**Outdoors**
Number of fruit (per plant)	35.70±9.45^{c}	87.73±16.87^{a}	58.10±13.99^{b}
Weight of fruit (gram)	195.77±14.65^{b}	337.11±20.29^{a}	196.19±21.73^{b}
Yield (kg)	6.87±1.71^{c}	28.92±4.76^{a}	11.52±2.27^{b}

The data are means ± standard deviations (n=3) and those sharing the same superscript letter in the same row are not significantly different as determined by ANOVA and the means within columns separated using Turkey multiple comparison test, for salinity condition and non – salinity condition (P>0.05).

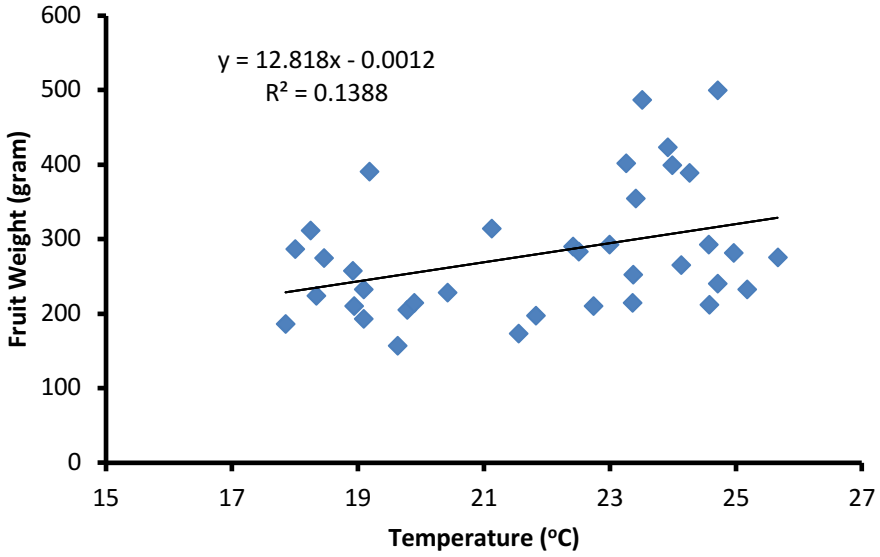


Figure 4.3 The correlation between ambient temperature and fruit weight. Data were collected indoors (2crops) and outdoors (1 crop)

4.5 Discussion

This study clearly demonstrated that rootstocks improved bitter melon yield performance irrespective of season, growing system (greenhouse or outdoor) or salinity conditions. Under both non-saline and saline conditions, the combination of bitter melon scions and these rootstocks did not affect the vegetative development of grafted plants, including the number of leaves and laterals; main stem diameter and height.

Factors affecting the growth and development of grafted bitter melon plants under saline and non-saline conditions.

Three rootstocks, Queensland Blue (Qb), Sampson (Sp) and Ringer (Rg) resulted ingrafted plants with normal morphological development in both favorable and adverse environments. Generally, an incompatibility between scion and rootstock can be seen where plant morphological development is altered (Yetisir and Sari 2003, Akihisa et al. 2007). However, our study showed that, the use of diffirent rootstocks did not affect grafted plant growth and morphology, including the number of leaves, laterals, main stem height and diameter and plant fresh weigh. In contrast, watermelon scions grafted onto *Cucurbita* and *Lagenaria* type rootstocks produced fewer leaves (number of leaves and leaf area), and showed a reduction in stem fresh and dry weights (Yetisir and Sari 2003). Watermelon scions grafted onto commercial hybrids of rootstocks improved the main stem and root length, as well as the number of lateral vines (Akihisa et al. 2007).

Rootstocks had no effect on fruit characteristics, including fruit diameter, length and weight. This was irrespective of season or whether the plants were grown indoor or outdoors.. Davis et al.(2005) found similar results for watermelon scions grafted ontosquash and gourd rootstocks. These results confirmed that the Sp and Rg rootstocks are completely compatible with VINO 12 bitter melon variety.

In the field, the pollination of bitter melon flowers depends on natural factors, such as weather (temperature, humidity and wind) and pollinator species (Oronje et al. 2012). For the indoor experiments, bitter melon flowers are pollinated by hand, therefore, the rate of fruit set depended on the time the flowers are pollinated. The time suitable for pollinating bitter melon is 9:00-11:00am in winter and 7:00-9:00am in summer (Hoi et al. 2013). In fact, both male and female flowers fully open during these periods. The pollens are dry enough to fall off and the pistil has enough stickiness to hold the pollen when performing pollination by hands.

Effects of rootstocks and saline conditions on grafted bitter melon productions

The use of rootstocks resulted in an increase in the number of female flowers, which led to an increase in fruit numbers and yield, compared to ungrafted plants (Petropoulos et al. 2012). In this study, different rootstocks have changed a number of particularly important indicators, such as the number of female flowers (Table 4.3), plant fresh weight (Table 4.4) and fruit set (Table 4.5). Especially, the Sp and Rg rootstocks significantly increased three critical criterias, including the numbers of female flowers (i), fruit numbers (ii) and fruit yield (iii), compared to the Qb

rootstocks and control plants (ungrafted and self-grafted). Thus, these results shown that fruit set depended on growing time (off season) but not on inside or outside conditions, while fruit number and fruit yield influenced by rootstocks. Both Sp and Rg rootstocks improved fruit numbers and yields and also highlighted that they have more compatibility with bitter melon scion than Qb rootstocks (Table 4.6, Table 4.7 and Table 4.8). Rootstock plays an important role in fruit yield and quality. For example, bitter melon scion grafted onto some Luffa varieties increased fruit yield from 38.0% (Jiebao and Tianlun 1997) to 258.5% (Xingxue et al. 2012).

Saline conditions at 16.0 dSm^{-1} slightly reduced the fruit production of grafted plants from three rootstocks but strongly reduced the fruit yield of controls within the indoor crops. Salinity is harmful to plant growth and often reduces fruit numbers and yield (Santa-Cruz et al. 2002, Yamaguchi and Blumwald 2005). At the salinity level 16.0 dSm^{-1}, the fruit yield of grafted plants was higher than that of ungrafted and self-grafted plants. However, these values may be further differences if all experiments are maintained for longer periods of time, instead of being ended after five months.

The number of fruit and fruit yield in all treatment plants could be higher than these values harvested in five months. When the plants were harvested, yet despite the short experiment time the yield of Vietnamese bitter melon was high compared to previous cultivations in Australia of other varieties. In climate-controlled greenhouse, the Sp and Rg rootstocks achieved 5.96 – 10.15 kg/plant in the off seasons and 28.51-39.12kg/ plant in the main season, compared to other high yield bitter melon varieties that were previously cultivated in Australia during one season and the same growing conditions, such as Jade, Hanuman, Niddhi and Indra (Tan et al. 2014). In fact, greenhouse productions have more the advantage of prolonging the season in a temperate region than outdoor productions (Gruda 2005, Tan et al. 2014). The fruit yield of Sp rootstock is lowers than that of the Rg rootstock except for growing outdoors in main season. This is only explained by the good adaptation of grafting combination to environmental conditions, the Rg with indoors and the Sp with outdoors.

The influence of temperature and relative humidity on fruit yield of grafted bitter melon plant

Temperature is the primary factor affecting the rate of plant development, the number of female flowers, fruit numbers and yields (Adams et al. 2001). In saline conditions, the increase of temperature leads to the increase of salinity levels. For example, if the EC is 5.0 dSm^{-1} at 25^{o}C, it will be 5.5 dSm^{-1} at 30^{o}C (Hanson et al. 2006). In 2016, experiments were conducted in a

climate-controlled greenhouse, with the average number of fruit per plant and yield lower than that of the main season in 2017.

Our results indicated that temperature and relative humidity were not sufficiently convincing that they could not become the most important factors affecting fruit yields. However, we still want to emphasise that bitter melon grafted plants growing under greenhouse conditions achieved higher fruit yields from 2 to 3 times than that of outdoor productions. In fact, both the highest and the lowest temperatures outside (Table 2.2) are not suitable for bitter melon growth (Davis et al., 2008). As a result, the fruit yield indoors is much higher than that of outdoors in main seasons. In addition, there are significant differences in fruit yield of greenhouse productions in two different seasons, main seasons and off seasons (Table 4.10). It is clear that cropping time and temperature influenced rootstock, therefore, affected the crop yield, similar to the results of studies on grafted watermelon plants (Yetisir and Sari 2003, Petropoulos et al. 2012).

In the greenhouse, Davis et al. (2008) identified that grafting influences absorption and translocation of phosphorus, nitrogen, magnesium, and calcium. Moreover, other studies suggested that improved nutrient uptake in grafted seedlings increases photosynthesis, which is particularly noticeable under less than optimal growing conditions, such as weak sunlight and low CO_2 content in solar greenhouses during winter months (Hu et al. 2006, Jang et al. 2013). Therefore, these conditions can allow grafted plants to improve fruit yields, sometimes with higher fruit quality.

4.6 Conclusion

Bitter melon scion grafted onto appropriate rootstocks could reduce the effects of salinity. Rootstocks did not affect the development of grafted bitter melon plants but affected the number of fruits and consequently fruit yield. The results would be more significant with grafted bitter melon in greenhouse production if the plants were maintained and harvested after the 19^{th} week in main seasons. For future studies, it is worth evaluating the total number of fruits and yield for the whole grafted bitter melon life cycle.

The Rg and Sp varieties provided excellent rootstocks for the Vietnamese bitter melon variety. The Rg and Sp grafting combination resulted in a better yield than the Qb rootstock or the control plants. The yield of the Rg and Sp grafting combination improved by 39.23-64.0%, 10.39-31.17% in non-saline conditions and 45.46%-53.40%, 33.04-70.58% under saline

conditions at level of 16.0 dSm^{-1}, respectively, compared to the controls (ungrafted and self-grafted) plants.

The use of rootstocks resulted in clear improvements in grafted bitter melon fruit yield under saline growing conditions.This study is limited at the salinity level of 16.0 dSm^{-1} but in facts, the grafted bitter melon plants may grow well at higher salinity levels. Thus, it is neccessary to conduct a further experiment with higher salinity levels approaching the salinity range of 29.25 dSm^{-1} to 33.44 dSm^{-1}, typical of saline levels in the Mekong Delta region. In addition, the number of fruits, individual fruit weight and fruit yield are not completely affected by temperature and relative humidity. They may be affected by other environmental factors, such as light intensity and lighting time (day length).

CHAPTER 5

THE EFFECT OF ROOTSTOCKS ON BITTER MELON FRUIT QUALITY UNDER DIFFERENT GROWING CONDITIONS

5.1 Introduction

Rootstocks and saline conditions impact the quality of fruits. These depend upon the species that are used for rootstocks and the levels of salinity. Rootstocks and saline conditions have been found to have positive and negative influences on the quality of fruit harvested from grafted plants.

Salt tolerant rootstocks can provide a useful tool to improve fruit yield and quality of Cucurbits under NaCl stress. Grafting *Cucumis sativus* scions onto the commercial salt tolerant rootstock *Cucurbita ficifolia* and *Lagenaria siceraria* resulted in increasing the content of dry matter, soluble sugars and titratable acidity in the fruits of all the plants, but had no significant effect on vitamin C content (Huang et al. 2009). Increasing water salinity from 0.5 dSm^{-1} (non-saline as control) to 15.7dSm^{-1} led to a decrease in both fruit size and water content in ungrafted tomatoes (*Lycopersicon esculentum* Mill.). In contrast, irrigation with saline water containing NaCl up to 3.9 dSm^{-1} improved the carotenoid content and the antioxidant activity of these fruits (Stefania De Pascale 2001). At some salt levels, the content of soluble solids, glucose and fructose was increased but the content of carotene and lycopene was not affected. Grafting may be a useful tool to increase tomato quality because the sodium-chloride and nitrate ion concentrations in the ungrafted plants are higher than those in the grafted plants (Fernández-Garcia et al. 2004).

Rootstocks and saline conditions can impact the moisture content in fruits, which directly relates to the drying time it takes to reduce the fruit moisture to a suitable level before extraction of bioactive compounds. Thus, it is valuable to understand the impact of these factors (rootstocks and saline conditions) on the moisture content of the fruits. In ripe tomatoes, water accounted for more than 90% of the total weight, and dry matter was only 5-8% of the fruit weight (Davies & Hobson, 1981, Ho, 1980). Fruits from the plants grown at high salinity accumulated less water but not less dry matter than the fruits from the plants grown at low salinity (Ehret & Ho, 1986). However, salinity slightly increased the amount of water in the ungrafted tomato plants of the "Fanny" variety and in the grafted plants of the "Goldmar" variety (Nieves Fena'ndez – Garcia, 2004). In some orange (*Citrus)* varieties, fruit rind

thickness varied depending on rootstock and sampling time and salinity reduced the water content of the fruits (Treeby et al. 2007).

The rootstock and the salinity of the growing medium can affect the fruit colour and firmness in grafted plants. However, studies on crops in saline conditions have yielded conflicting results in that saline conditions can increase or decrease the colour and firmness of fruits (Colla et al. 2006). The impacts of saline conditions depend on the species (ungrafted) and rootstocks (grafted plants). Rootstocks can affect the fruit quality attributes, including colour and firmness (Alan et al. 2007, Borghesi et al. 2011). Generally, fruits from grafted watermelon plants had a thicker rind and slightly lower total soluble solids content than the fruits from the non-grafted plants (Alexopoulos et al. 2007). This is evidenced by other studies on watermelon as scion and four pumpkin rootstocks. As a result, these rootstocks had little influence on the fruit shape, fruit index, rind and peel thickness (Alan et al. 2007). Therefore, these differences in fruit characteristics were not considered to be serious quality defects and therefore grafting of this crop was seen as advantageous (Alexopoulos et al. 2007). However, rootstocks can affect fruit weight, total yield and marketable yield were significantly influenced by grafting (Turhan et al. 2012).

Fruit size, quality and taste have significant influences on the value of a crop, with sugar content being one of the most significant aspects, which can be influenced by salinity (Gao and Liao 2006). Salinity has been observed to increase melon fruit quality by increasing firmness, total sugars, soluble solids, sucrose, fructose and glucose. However, this effect was greater under low saline conditions than under high saline conditions (Navarro 2015). Muskmelons and some other melons grown on saline soils were also found to be markedly softer and juicier at the normal harvest stage than fruit from non-saline soils. Therefore, saline conditions can influence the size and organoleptic characteristics of fruits (Franco et al. 2015).

The fruit bioactive compounds may also be influenced by the chosen rootstocks. In some species, rootstocks have a positive effect on the fruit quality. Peach fruit harvested from grafted plants have increased antioxidant activity and total phenolic content (Giorgi et al. 2005). Grafting watermelon increased total carotenoids and amino acids by 20 and 35% in fruits, respectively (Davis et al. 2008). However, there were no significant differences in the ratio of sugar to acid content in tomato fruits between grafted and ungrafted plants (Pogonyi et al. 2005). In another study, grafting tomato scions on suitable rootstocks had positive effects on the cultivation performance but it decreased the nutritional quality of the fruits (Vrcek et al. 2011).

Different rootstocks can have different effects with the same scion. For example, watermelon scions grafted onto *Lagenaria* rootstocks only had minor differences in fruit quality compared to the control plants but the quality parameters in the fruits for the scions grafted onto Cucurbita rootstocks were much lower than in the control (Yetışır et al. 2003).

To date, no studies have been published on the effect of rootstocks and saline conditions on the quality of bitter melon fruit. In this study, three rootstocks did not affect the growth and development of the grafted plants (Chapter 3) but they greatly affected fruit yield (Chapter 4) depending on growing conditions: salinity and non-salinity; indoors and outdoors; main seasons and off seasons. This chapter investigates whether rootstocks affect the fruit quality or not and how they affect to the quality of fruits.

The specific objective of this study was to:

Examine the effects of rootstocks, salinity level of the growing medium, growing season, indoor and outdoor conditions on the physiochemical characteristics of the bitter melon fruit, including moisture content, firmness, colour, total saponin content, total phenolic compound content and antioxidant capacity.

5.2 Materials and Methods

5.2.1 Materials

The fruits of the bitter melon grafted on different rootstocks and grown under various saline conditions (as described in Chapter 2) were collected in the off season 2016 (from 15th July to 15th October, 2016), and the main season 2017 (from 10th December, 2016 to 10th March, 2017) at the DPI, Ourimbah, NSW, Australia. They were harvested at 30 days in the off season and 15 days in the main season. Three fruits were randomly taken for each treatment. After harvesting, each individual fruit was labelled and measured for physical properties. The fruits were then stored in a freezer at –18^{o}C for later analysis of chemical and antioxidant properties.

Analytical chemicals

All chemicals used in this study were of analytical grade. Methanol was obtained from *Merck Pty Ltd.* Folin-Ciocalteu reagent, 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2,2-diphenyl1-picryl-hydrazil (DPPH), ferric-reducing antioxidant power (FRAP), gallic acid, escin, 2,4,6-tripyridyl-s-triazine (TPTZ), and iron(III) chloride and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were obtained from Sigma–Aldrich Pty Ltd. (Castle Hill, Sydney, NSW Australia). H_2SO_4 72% and HCl were purchased from *Ajax*

Finechemicals (Thermo Fisher Scientific, North Ryde NSW, Australia). AcOH was obtained from *BDH Laboratory Supplies* (Bio-Strategy, Tingalpa, QLD, Australia). Vanillin 8% (w/v in Ethanol), $K_2S_2O_8$, and MeOH were purchased from *Merck* (Bayswater, VIC, Australia). Na_2CO_3 was obtained from *Chem-supply* (Gillman, SA, Australia). Deionised water was prepared on the day of use with a Milli-Q Direct 16 water purification system (Millipore Australia Pty Ltd, North Ryde, NSW, Australia).

5.2.2 Methods for measurement of physical properties

Moisture content: The fresh fruits were sliced lengthwise to remove the seeds, the meat was then cut into small cubes. The fresh samples (2-3 grams) were then transferred into the pre-weight trays and then dried in a vacuum oven with reduced pressure for 24 hours at 80^o C. Moisture content was worked out based on weight difference as per the following equation.

$$\text{Moisture content (\%)} = \left(\frac{(M1-Mo)-(M2-M0)}{(M1-M0)}\right) x\ 100\ \%$$

M_0: weight of tray; M_1: weight of fresh sample; M_2: weight of dried sample

Fruit skin colour: The colour (L*, a* and b*) of fruit skin was measured using a Minolta Chroma Meter CR-400/410 (Minolta Corp, Osaka, Japan). The equatorial axis of the fruits was selected to take the measurements, six per fruit with symmetrical arrangement in six positions: three on each side of the fruit (top, middle and bottom). The colour of the fruit was expressed by the two colour parameters Chroma and Hue angle. Chroma [C* = (a*2 + b*2)0.5], and hue angle [Ho = arctan (b*/a*)] (Leo Sabatino 2017).

Fruit firmness: The firmness of individual bitter melon fruit was determined using a Penetrometer (Facchini, Alfonsine, Italy) with an 8 mm flat plunger under constant force to penetrate into the fruit. Means of three values per fruit were calculated and expressed as kilograms force (kgf) in six positions: top, middle and bottom of the fruit.

5.2.3 Methods for determination of chemical properties

***Extraction for analysis*:** Before extraction, the fruits were removed from the freezer and left in a cool room at a temperature of 4–5^{o}C for 24 hours. After thawing, fresh fruits were randomly selected, and the seeds were removed. The collected fruits were then extracted with ethanol as described in a previous study (Fang and Ng 2013). Briefly, the fresh sample (FS)

was ground with 75% (v/v) ethanol at a ratio of 1:2 g of FS/mL. The mixture was put in an ultrasonic bath (SONICLEAN 1000HD, Soniclean Pty Ltd, Thebarton SA, Australia) set at 40ºC and 60% power for 40 minutes to assist the extraction of bioactive compounds. The mixture was then centrifuged at 3500 *x g* using a Beckman J2-MC centrifuge (BECKMAN Coulter Ltd, Sydney, Australia) for 10 minutes to remove the un-extractable solids. Finally, the supernatant was filtered using a filter paper 90 mm (Toyo Roshi Kaisha, Ltd. Japan). The extract was collected and stored at -18ºC for further analysis. The sample (SP) was further diluted to determine total saponin content (TSC), total phenolic content (TPC) and antioxidant capacity using ABTS, DPPH and FRAP assays.

***Determination of total saponin content*:** Total saponin content (TSC) was determined according to the previously reported method by Hiai et al (1976) with some modifications. Briefly, 0.5mL of the extract was mixed with 0.5mL of 8% vanillin solution and 5mL of 72% sulfuric acid (H_2SO_4) was then added to the mixture. The mixture was kept in an ice water bath for 5 minutes, incubated at 60ºC for a further 15 minutes and rapidly cooled on ice to room temperature. The absorption of the mixture was measured at 560nm using a spectrophotometer. Escin was used to build up a standard curve and the levels of TSC were expressed as mg of escin equivalents per gram of dried mass (mg EE)/g DM).

The standard curve of total saponin:

Y = 0.9245X + 0.0069, where X: saponin content (mg escin/mL);

Y: Absorbance value (Abs)

$$\text{TSA} = \frac{(Abs - 0.0069) * f * V * 100}{0.9245 * W * (100 - M)} \ (mg\ ESE/g\ DM)$$

ESE: escin equivalents

f: dilution factor

V: volume of mixture (mL)

W: weight of fresh sample (g)

M: moisture content (%)

***Determination of total phenolic content in fruits*:** Total phenolic content (TPC) was determined based on the prior established method by Cicco et al (2009) with some modifications. Briefly, 0.5 mL of the extract was mixed with 2.5 mL of 10% Folin reagent and

kept for 6 minutes at ambient temperature before 2.0 mL of 7.5% Na_2CO_3 was added to the mixture. The mixture was incubated for 60 minutes at ambient temperature before measuring the absorption at 765 nm using a spectrophotometer. Gallic acid was used to build up a standard curve and the levels of TPC were expressed as mg gallic acid equivalents per gram of dried mass (mgGAE/g DM).

The standard curve of phenolic compounds:

Y = 11.068x + 0.0169, where X: total phenolic content (mg gallic acid/mL);

Y: Absorbance value (Abs)

$$\text{TPC} = \frac{(Abs - 0.0169) * f * V * 100}{11.068 * W * (100 - M)} \ (mg\ GAE/g\ DM)$$

TPC: total phenolic content

GAE: gallic acid equivalents

f: dilution factor

V: volume of mixture (mL)

W: weight of fresh sample (g)

M: moisture content (%)

5.2.4 Methods for determination of antioxidant capacity

Antioxidant capacity of the extracts of the grafted bitter melon fruits grown under various saline conditions was measured using three antioxidant assays, including ABTS radical scavenging capacity, DPPH radical scavenging capacity, and Ferric reducing antioxidant power assays

ABTS radical scavenging capacity: ABTS radical scavenging capacity was evaluated according to the method described by Thaipong et al. (2006) with some modifications. Briefly, 0.15 mL of the SP was thoroughly mixed with 2.85 mL ABTS working solution (absorbance value at 734 nm was 1.1). The mixture was incubated for 120 minutes in the dark. The absorption of the mixture was measured at 734 nm using a spectrophotometer. Trolox was used to build up a standard curve and the results were expressed as mg Trolox equivalents per gram of dried mass (mgTE/g DM).

The standard curve of the ABTS:

Y = 1.4805X - 0.0248, where X: concentration of trolox (mM);

Y: Absorbance value (Abs)

$$\text{ABTS} = \frac{(A + 0.0248) * f * MW * 100}{1.4805 * W * (100 - M) * 10} \ (mg\ TE/g\ DM)$$

ABTS: ABTS radical scavenging activity

A: Abs control – Abs sample

MW: Molecular weight of Trolox (= 250.29)

f: dilution factor

V: volume of mixture (mL)

W: weight of fresh sample (g)

M: moisture content (%)

TE: trolox equivalents

DPPH radical scavenging capacity: DPPH radical scavenging capacity was examined based on the established method by Nguyen et al (2017) with some modifications. Briefly, 0.15 mL of the SP was mixed with 2.85 mL DPPH stock solution (mixture of methanol and stock solution at a ratio of 1:4.5 by volume). The mixture was kept for 180 minutes in the dark. The absorption of the mixture was measured at 515 nm using a spectrophotometer. Trolox was used to build up a standard curve and the results were expressed as mg Trolox equivalents per gram of dried mass (mgTE/g DM).

The standard curve of the DPPH:

Y=1.1897x - 0.0191, where X: concentration of trolox (mM);

Y: Absorbance value (Abs)

$$\text{DPPH} = \frac{(A + 0.0191) * f * MW * 100}{1.1879 * W * (100 - M) * 10} \ (mg\ TE/g\ DM)$$

DPPH: DPPH radical scavenging activity

A: Abs control – Abs sample

MW: Molecular weight of trolox (= 250.29)

f: dilution factor

V: volume of mixture (mL)

W: weight of fresh sample (g)

M: moisture content (%)

TE: trolox equivalents

Ferric reducing antioxidant power: Ferric reducing antioxidant power (FRAP) was determined according to the described method by Nguyen et al. (2017) with some modifications. Briefly, 0.15 mL of the SP was thoroughly mixed with the 2.85 mL of working solution. The mixture was incubated for 30 minutes in the dark at ambient temperature before measuring the absorption at 593 nm using a spectrophotometer. Trolox was used to build up a standard curve and the results were expressed as mg Trolox equivalents per gram of dried mass (mgTE/g DM).

The standard curve of the FRAP:

Y=2.0953X - 0.0088, where X: Concentration of Trolox (mM);

Y: Absorbance value (Abs)

$$\text{FRAP} = \frac{(Abs + 0.0088) * f * MW * V}{2.0953 * W * (100 - M) * 10} \ (mg\ TE/g\ DM)$$

FRAP: ferric reducing antioxidant power

MW: Molecular weight of trolox is 250.29 g/mol

f: dilution factor

V: volume of mixture (mL)

W: weight of fresh sample (g)

M: moisture content (%)

TE: Trolox equivalents

5.2.5 Statistical analysis

Results were presented as means ± standard deviations. Data were assessed by analysis of variance (ANOVA) and the Tukey post-hoc test (95% confidence interval, $P< 0.05$) to compare means using the SPSS-PASW GradPack 22.0 for Mac (IBM Corp., Armonk, NY, USA). Correlation coefficients (r) were determined by Pearson's correlation matrix method using SPSS software.

5.3 Results

5.3.1 Effects of rootstocks and salinity of growing medium on physical properties

Moisture content: The one-way ANOVA performed to ascertain the effects of the rootstocks and the growing conditions on the moisture content of the bitter melon fruits showed that there were statistically significant differences in the moisture content of the fruits, with the values of $F(23.49) = 27.620$ and $P < 0.0001$ ($n=5$). However, the post-hoc test revealed that there were no significant differences in the moisture content between the fruits from the bitter melon plants whether or not they were grafted to the three rootstocks tested when they were grown under the same growing conditions (Table 5.1).

The differences were between the different growing conditions. The plants grown inside the greenhouses (indoors) during the main growing season under both saline and non-saline conditions had the highest moisture content irrespective of whether they were grafted to rootstock or not (Table 5.1). Being grown indoors compared to outdoors increased the moisture content of the bitter melon plants grown in the main season 2017; the moisture content in the fruits ranged from 94.15 to 94.31% for the plants grown indoors, which was higher than for the plants grown outdoors (from 92.48% in to 93.19%).

Fruit firmness: The one-way ANOVA performed to ascertain the effects of the rootstocks and the growing conditions on the firmness of the fruits indicated that there were no statistically significant differences in the firmness of the fruits, with the values of $F(23.48) = 1.033$ and $P > 0.05$ ($n=5$). Therefore, there was no effect found for the rootstocks on the fruit firmness although there were some effects of the growing conditions on the moisture content of the fruit, no effects were found for the growing conditions, including salinity, on firmness (Table 5.1).

Fruit skin colour: The one-way ANOVA was run to compare the colour of the fruits (L*, C* and h values) from different rootstocks and growing conditions (salinity and non-salinity) and it showed that the L* (L_x) values were not significantly different ($P > 0.05$), while there was a statistically significant effect on the C* (C_x) and h values, with $F(23.48) = 13.772$ and $P < 0.0001$ ($n=5$). However, the post-hoc test revealed that there were no significant differences in the C* and h values between the different rootstocks (Table 5.2).

The C* values were higher for the plants grown in the off season 2016 both under saline and non-saline conditions compared to all other growth conditions (Table 5.2). Most of the plants

grown in the off season 2016 both under saline and non-saline conditions also had lower h values compared to all other growth conditions (Table 5.2).

Table 5.1 Effects of rootstocks and salinity of growing medium on the moisture content and firmness of bitter melon fruits

Treatments	Time	Growing condition	Rootstock variety	Moisture content (%)	Firmness (kgf)
Salinity (16.0 dSm^{-1})	**Off season 2016**	**Indoor**	Bm/Qb	91.82 ± 0.16^{gh}	6.55 ± 0.52^{a}
			Bm/Sp	90.99 ± 0.36^{h}	6.35 ± 0.54^{a}
			Bm/Rg	91.90 ± 0.62^{fgh}	5.84 ± 0.32^{a}
			Bm	91.43 ± 0.83^{h}	5.90 ± 0.14^{a}
	Main season 2017	**Indoor**	Bm/Qb	94.22 ± 0.11^{ab}	6.63 ± 0.71^{a}
			Bm/Sp	94.19 ± 0.22^{ab}	6.24 ± 0.39^{a}
			Bm/Rg	94.15 ± 0.16^{abc}	6.47 ± 0.22^{a}
			Bm/Bm	94.19 ± 0.31^{ab}	6.33 ± 0.60^{a}
		Outdoor	Bm/Qb	92.48 ± 0.31^{defg}	7.20 ± 0.72^{a}
			Bm/Sp	92.98 ± 0.43^{def}	6.38 ± 0.41^{a}
			Bm/Rg	92.54 ± 0.24^{defg}	6.76 ± 0.21^{a}
			Bm/Bm	92.87 ± 0.36^{defg}	6.76 ± 0.21^{a}
Non – salinity (0.5 – 1.6 dSm^{-1})	**Off season 2016**	**Indoor**	Bm/Qb	92.28 ± 0.38^{defg}	6.16 ± 0.64^{a}
			Bm/Sp	91.79 ± 0.17^{gh}	6.45 ± 0.87^{a}
			Bm/Rg	92.04 ± 0.41^{efgh}	6.20 ± 0.64^{a}
			Bm	92.59 ± 0.69^{defg}	6.36 ± 0.15^{a}
	Main season 2017	**Indoor**	Bm/Qb	94.26 ± 0.13^{ab}	6.28 ± 0.24^{a}
			Bm/Sp	94.24 ± 0.12^{ab}	6.54 ± 0.53^{a}
			Bm/Rg	94.31 ± 0.33^{a}	6.21 ± 0.27^{a}
			Bm/Bm	94.21 ± 0.43^{ab}	6.04 ± 0.41^{a}
		Outdoor	Bm/Qb	93.19 ± 0.16^{bcd}	6.31 ± 0.55^{a}
			Bm/Sp	93.06 ± 0.13^{cde}	6.58 ± 0.45^{a}
			Bm/Rg	93.05 ± 0.31^{de}	6.65 ± 0.30^{a}
			Bm/Bm	93.07 ± 0.19^{cde}	6.52 ± 0.47^{a}

Data are the mean values ± standard deviations (n=5). Data in the same column sharing similar superscript letters are not significantly different ($P<0.05$).

Table 5.2 Effect of rootstock and salinity on the fruit colour

Seasons	Positions	Conditions	Varieties	Skin fruit colour		
				L*	C*	h
Off season 2016	**Indoor**	**Salinity (16 dSm^{-1})**	Bm/Qb	52.73 ± 1.30^{a}	32.81 ± 0.86^{a}	115.21 ± 0.51^{cdef}
			Bm/Sp	51.07 ± 1.77^{a}	34.73 ± 1.19^{a}	114.64 ± 1.05^{defg}
			Bm/Rg	49.92 ± 1.19^{a}	35.45 ± 1.96^{a}	111.73 ± 2.74^{fg}
			Bm	51.24 ± 2.02^{a}	34.99 ± 1.97^{a}	112.43 ± 2.86^{fg}
		Non-salinity (0.5-1.6 dSm^{-1})	Bm/Qb	50.24 ± 0.35^{a}	33.72 ± 0.49^{a}	114.64 ± 0.30^{defg}
			Bm/Sp	51.83 ± 0.55^{a}	33.66 ± 1.32^{a}	114.51 ± 0.23^{efg}
			Bm/Rg	51.08 ± 2.72^{a}	34.08 ± 0.96^{a}	115.37 ± 0.84^{cdef}
			Bm	48.86 ± 1.29^{a}	36.72 ± 1.38^{a}	110.44 ± 2.07^{g}
Main season 2017	**Indoor**	**Salinity (16 dSm^{-1})**	Bm/Qb	38.15 ± 2.88^{b}	25.33 ± 1.18^{b}	120.40 ± 0.33^{ab}
			Bm/Sp	39.17 ± 2.01^{b}	27.36 ± 2.28^{b}	116.06 ± 1.65^{bcdef}
			Bm/Rg	34.90 ± 1.60^{ab}	26.52 ± 0.58^{b}	120.05 ± 1.00^{ab}
			Bm/Bm	34.11 ± 2.29^{ab}	24.95 ± 1.14^{b}	118.11 ± 1.71^{abcde}
		Non-salinity (0.5-1.6 dSm^{-1})	Bm/Qb	37.17 ± 1.73^{ab}	27.10 ± 1.79^{b}	120.17 ± 1.04^{ab}
			Bm/Sp	34.69 ± 1.72^{ab}	25.62 ± 2.21^{b}	117.94 ± 0.81^{abcde}
			Bm/Rg	35.53 ± 2.93^{ab}	27.24 ± 0.75^{b}	118.65 ± 1.31^{abcde}
			Bm/Bm	32.14 ± 2.24^{c}	25.46 ± 0.34^{b}	115.92 ± 2.28^{bcdef}
	Outdoor	**Salinity**	Bm/Qb	35.82 ± 1.00^{ab}	26.36 ± 0.60^{b}	120.06 ± 0.45^{ab}

Seasons	Positions	Conditions	Varieties	Skin fruit colour		
				L*	C*	h
		(16 dSm^{-1})	Bm/Sp	35.77 ± 0.92^{ab}	25.41 ± 1.53^{b}	120.46 ± 0.79^{ab}
			Bm/Rg	34.14 ± 2.14^{ab}	25.82 ± 2.09^{b}	120.38 ± 0.54^{ab}
			Bm/Bm	33.99 ± 1.35^{ab}	26.97 ± 1.67^{b}	120.71 ± 0.61^{a}
			Bm/Qb	36.91 ± 1.63^{ab}	25.69 ± 1.27^{b}	119.21 ± 1.71^{abcd}
		Non-salinity	Bm/Sp	36.22 ± 2.73^{ab}	26.79 ± 2.30^{b}	119.39 ± 2.02^{abc}
		(0.5-1.6 dSm^{-1})	Bm/Rg	37.32 ± 1.31^{ab}	26.16 ± 2.40^{b}	119.63 ± 2.06^{abc}
			Bm/Bm	35.85 ± 1.10^{ab}	26.86 ± 1.97^{b}	120.12 ± 0.67^{ab}

Data are the mean values ± standard deviations (n=5). Data in the same column sharing similar superscript letters are not significantly different ($P<0.05$).

5.3.2 Effects of rootstocks and the salinity of the growing medium on the saponin and phenolic compound content

The one-way ANOVA was performed to consider the effects of rootstocks and growing conditions on the TSC and found that there were statistically significant differences in the TSC of the fruits, with the values of $F(23.49) = 2011.722$ and $P < 0.0001$ (n=5). The post-hoc test revealed that there were significant effects of the rootstock on the fruit TSC for all the growing conditions but no one rootstock gave the highest TSC under all the growing conditions (Table 5.3). For example, the Sampson rootstock (Bm/Sp) gave the overall highest TSC values (205.65 mg EE/g dried mass) when the plants (Bm/Hp) were grown outdoor under low salinity conditions during the main season 2017. However, this rootstock effect was not found for any of the other growing conditions. Similarly, there were no consistent patterns for the other rootstocks across all the growing conditions.

Most of the fruits from bitter melon plants grown under non-saline conditions during the main season had significantly higher TSC than that of the plants grown under saline conditions (Table 5.3). Most of the fruits from plants grown outdoors under both saline and non-saline conditions during the main season also had significantly higher TSC than that of the plants grown indoors (Table 5.3). The fruits from the main season also had significantly higher levels of TSC in comparison with those grown in the off season.

Similarly, the one-way ANOVA showed a significant difference in the TPC from plants grafted onto different rootstocks and grown under different growing conditions with the values of $F(23.49) = 17860.413$ and $P < 0.0001$ (n=5). The post-hoc test revealed that there were significant effects of the rootstock on the fruit TSC for all the growing conditions but no one rootstock gave the highest TSC under all the growing conditions.

The TPC in fruits grown outdoors was significantly higher than that of the plants grown indoors for all the rootstocks under both saline and non-saline conditions ($p<0.05$) (Table 5.3). For example, under saline conditions, the TPC in fruits harvested from outdoor was 7 to 9 times higher than for fruits harvested from indoor from the same rootstocks. Of note, plants grafted to all three rootstocks and grown indoors in the 2016 off season, under both saline and non-saline conditions, had the lowest levels of TPC ($P>0.05$).

Table 5.3 Effects of rootstocks and salinity of growing medium on total saponins and total phenolic compounds in bitter melon fruits

Treatments	Time	Growing condition	Rootstock variety	TSC (mg EE/g DM)	TPC (mg GAE/g DM)
Salinity (16 dSm^{-1})	**Off season 2016**	**Indoor**	Bm/Qb	82.22 ± 2.73^{l}	9.49 ± 2.77^{k}
			Bm/Sp	67.04 ± 2.64^{m}	11.00 ± 2.05^{k}
			Bm/Rg	90.23 ± 1.05^{k}	11.54 ± 2.65^{k}
			Bm	79.41 ± 1.01^{l}	11.15 ± 1.07^{k}
	Main season 2017	**Indoor**	Bm/Qb	136.74 ± 1.78^{h}	135.32 ± 3.92^{f}
			Bm/Sp	138.63 ± 1.68^{h}	96.15 ± 3.01^{gh}
			Bm/Rg	116.65 ± 1.74^{j}	85.80 ± 4.02^{i}
			Bm/Bm	126.17 ± 2.52^{i}	95.71 ± 3.86^{gh}
		Outdoor	Bm/Qb	170.69 ± 1.01^{f}	780.28 ± 9.47^{d}
			Bm/Sp	186.72 ± 1.60^{de}	851.68 ± 4.85^{ab}
			Bm/Rg	183.85 ± 5.24^{e}	768.96 ± 6.54^{d}
			Bm/Bm	183.05 ± 1.63^{e}	843.61 ± 8.27^{abc}
Non – salinity (0.5-1.6 dSm^{-1})	**Off season 2016**	**Indoor**	Bm/Qb	92.14 ± 1.07^{k}	9.83 ± 1.08^{k}
			Bm/Sp	71.20 ± 0.81^{m}	10.08 ± 0.92^{k}
			Bm/Rg	95.07 ± 1.52^{k}	12.69 ± 2.26^{k}
			Bm	95.79 ± 1.53^{k}	11.59 ± 1.07^{k}
	Main season 2017	**Indoor**	Bm/Qb	195.37 ± 2.38^{b}	101.55 ± 3.63^{g}
			Bm/Sp	191.62 ± 2.58^{bc}	70.70 ± 3.13^{j}
			Bm/Rg	153.25 ± 1.32^{g}	95.03 ± 3.65^{gh}
			Bm/Bm	197.28 ± 1.13^{b}	88.58 ± 3.50^{gh}
		Outdoor	Bm/Qb	175.39 ± 7.21^{d}	750.93 ± 9.17^{e}
			Bm/Sp	205.65 ± 2.49^{a}	855.58 ± 4.45^{ab}
			Bm/Rg	189.16 ± 1.56^{cd}	834.74 ± 7.31^{bc}
			Bm/Bm	188.29 ± 2.47^{cde}	831.56 ± 6.68^{c}

Data are the mean values ± standard deviations (n=5). Data in the same column sharing similar superscript letters are not significantly different ($P<0.05$). TSC: total saponins content, TPC: total phenolic content, EE: escin equivalents, GAE: gallic acid equivalents, DM: dried mass.

5.3.3 Effects of rootstocks and the salinity of growing medium on antioxidant activity

The one-way ANOVA performed to predict the effects of rootstocks and the growing conditions on the antioxidant activity as measured using the ABTS, DPPH and FRAP assays found that the differences in the ABTS values were statistically significant, with the values of $F(23.49) = 1340.217$ and $P < 0.0001$ for the ABTS assay, $F(23.49) = 6522.696$ and $P < 0.0001$ for the DPPH assay and $F(23.49) = 1248.415$ and $P < 0.0001$ for the FRAP assay. The post-hoc test revealed that there were no consistent significant effects of the rootstock on the fruit ABTS, DPPH and FRAP antioxidant activities for all the growing conditions (Table 5.4).

Similar to the TPC in fruits grown outdoors, the antioxidant activity as measured using the three assays was significantly higher than that of the plants grown indoors for all the rootstocks under both saline and non-saline conditions ($p<0.05$) (Table 5.4). For example, the fruits grown outdoor under non-saline conditions had ~20 times more DPPH activity than the fruits harvested from plants grown indoor during the off season 2016.

Antioxidant activity based on ABTS assay

The results from the ABTS assay (Table 5.4) showed that the antioxidant capacity was not significantly different between the three rootstocks and the control plants in all treatments except for the plants grown outdoor. For the plants grown outdoor in the main season 2017, the fruit of the plants grown with the three rootstocks had significantly higher levels of ABTS antioxidant activity compared to that of the fruits from the self-grafted bitter melon plants ($p<0.05$), whether they were grown under saline or non-saline conditions.

Antioxidant activity based on DPPH assay

The results from the DPPH assay (Table 5.4) showed that the antioxidant capacity was not significantly different between the three rootstocks and the control plants in all treatments except one. For the plants grown indoor in the off season 2016 under non-saline conditions, the fruit of the plants grown with the three rootstocks had significantly higher levels of DPPH antioxidant activity compared to that of the fruits from the self-grafted bitter melon plants ($p<0.05$). However, the main effect on the DPPH antioxidant activity was that the antioxidant capacity of the fruits grown outdoor was significantly higher than that of the fruits grown under all other conditions.

Table 5.4 Effects of rootstocks and saline conditions on the antioxidant property of the fruits (ABTS, DPPH and FRAP assays)

Treatments	Time	Growing condition	Rootstock variety	ABTS (mg TE/g DM)	DPPH (mg TE/g DM)	FRAP (mg TE/g DM)
Salinity (16 dSm^{-1})	**Off season 2016**	**Indoor**	Bm/Qb	140.84 ± 8.54^{g}	8.10 ± 0.06^{gh}	3.91 ± 0.18^{fg}
			Bm/Sp	149.54 ± 9.67^{fg}	8.69 ± 1.46^{gh}	4.10 ± 0.88^{efg}
			Bm/Rg	125.52 ± 8.00^{fg}	6.86 ± 0.42^{h}	4.15 ± 0.18^{efg}
			Bm	134.89 ± 7.71^{g}	7.83 ± 1.02^{gh}	4.21 ± 0.83^{efg}
	Main season 2017	**Indoor**	Bm/Qb	237.41 ± 5.55^{ef}	8.52 ± 1.57^{gh}	3.54 ± 0.47^{g}
			Bm/Sp	182.18 ± 10.20^{fg}	7.75 ± 1.75^{gh}	3.28 ± 0.25^{g}
			Bm/Rg	170.16 ± 4.54^{fg}	6.64 ± 0.41^{h}	4.84 ± 0.64^{ef}
			Bm/Bm	203.43 ± 2.63^{efg}	8.98 ± 2.49^{gh}	3.91 ± 0.06^{fg}
		Outdoor	Bm/Qb	1454.68 ± 55.01^{bc}	149.62 ± 1.35^{d}	21.15 ± 0.33^{ab}
			Bm/Sp	1505.50 ± 21.36^{ab}	161.02 ± 1.11^{b}	20.11 ± 0.21^{bc}
			Bm/Rg	1410.84 ± 66.21^{c}	154.74 ± 2.24^{c}	19.77 ± 0.41^{c}
			Bm/Bm	683.00 ± 41.26^{e}	158.78 ± 1.73^{bc}	22.37 ± 0.40^{a}
Non – salinity (0.5-1.6 dSm^{-1})	**Off season 2016**	**Indoor**	Bm/Qb	153.43 ± 2.39^{fg}	19.33 ± 1.27^{e}	5.22 ± 0.25^{e}
			Bm/Sp	150.29 ± 7.37^{fg}	16.05 ± 2.07^{ef}	3.41 ± 0.28^{g}
			Bm/Rg	154.83 ± 13.33^{fg}	19.51 ± 0.98^{e}	4.07 ± 0.79^{efg}
			Bm	150.04 ± 6.72^{fg}	9.94 ± 2.22^{gh}	3.91 ± 0.29^{fg}
		Indoor	Bm/Qb	292.92 ± 8.48^{e}	101.55 ± 3.63^{d}	6.81 ± 0.29^{d}
			Bm/Sp	215.01 ± 8.77^{efg}	70.70 ± 3.13^{defg}	3.92 ± 0.16^{fg}

Treatments	Time	Growing condition	Rootstock variety	ABTS (mg TE/g DM)	DPPH (mg TE/g DM)	FRAP (mg TE/g DM)
	Main season 2017		Bm/Rg	206.79 ± 7.34^{efg}	95.03 ± 3.65^{d}	3.73 ± 0.18^{fg}
			Bm/Bm	272.17 ± 15.65^{e}	88.58 ± 3.50^{de}	3.02 ± 0.13^{g}
		Outdoor	Bm/Qb	1557.95 ± 51.84^{a}	167.84 ± 2.62^{a}	21.63 ± 0.27^{a}
			Bm/Sp	1560.27 ± 9.48^{a}	163.35 ± 2.46^{ab}	21.19 ± 0.44^{ab}
			Bm/Rg	1486.61 ± 41.16^{abc}	163.64 ± 0.80^{ab}	22.06 ± 0.25^{a}
			Bm/Bm	683.00 ± 41.26^{d}	167.36 ± 2.34^{a}	22.32 ± 0.19^{a}

Data are the mean values ± standard deviations (n=5). Data in the same column sharing similar superscript letters are not significantly different (P>0.05). TE: trolox equivalents, DM: dried mass.

Antioxidant activity based on FRAP assay

The results from the FRAP assay (Table 5.4) showed that the antioxidant capacity was not significantly different between the three rootstocks and the control plants in all treatments except one. For the plants grown outdoor in the main season 2017 under saline conditions, the fruit of the plants grown with the two of the rootstocks (Sp and Rg) had significantly lower levels of FRAP antioxidant activity compared to that of the fruits from the self-grafted bitter melon plants ($p<0.05$). The fruits from the Rg rootstock also had a lower FRAP antioxidant activity compared to that of fruits from the Qb rootstock. However, the main effect on the FRAP antioxidant activity was that the antioxidant capacity of the fruits grown outdoor was significantly higher than that of the fruits grown under all other conditions.

5.4 Discussion

Our results showed that the rootstocks used for grafting bitter melon had no consistent effect on any of the measured fruit parameters. The salinity level of the growing medium also had minimal effects; the only consistent effect observed was that salinity decreased the TSC of the fruits grown indoors during the main season. The bitter melon fruit grown during the main season 2017 had higher moisture content, TSC, TPC and antioxidant capacity than the fruits grown during the off season 2016. Although the fruits grown outdoor during the main season 2017 had a lower moisture content than the fruits grown indoors during the main season, they had the highest TPC and antioxidant capacity irrespective of the rootstock or salinity level.

Effects on fruit physical properties

Our results (Table 5.1) revealed that the grafted fruits grown indoors had a higher moisture content than that of the fruits grown outdoors. In fact, Table 2.2 (Chapter 2) decribed that humidity indoors were higher than outdoors, these affected and contributed to the amount of water in the fruit. These findings were supported by previous studies on some crops, such as ungrafted strawberries (Yuan et al. 2004) and eggplants (Gruda 2005), under non-saline conditions. However, our results differed to those of Voutsela et al. (2012) study on grafted tomato plants cultivated outdoors and indoors under salinity stress.

Based on color standards (Weatheralla et al. 1992 and McLellan et al 1995), we resulted that the bitter melon fruits grown during the off season had a lighter green colour. These results were similar to those observed by Davis et al. (2005) in the grafted watermelon plants, and Singh and

Rao (2014), in grafted Cucurbitaceous crops, including bitter melon, cucumber, watermelon and pumpkin.

The impact of growing conditions: indoors and outdoors on the fruit moisture content can be caused by differences in temperature, humidity and irrigation. There is no previous study conducted on these issues in grafted bitter melon and also not much research on the relationship between the humidity of the environment and fruits. However, research results on peach fruits (Berman and Dejong 1996) are similar to those on bitter melon that we have presented. In the current study, the indoor temperature was lower than that of outdoor while the equivalent humidity was higher than that of outdoor (Table 2.2). This may have led to the observed difference in moisture content between the fruits grown indoors and outdoors. Outdoor plants are affected by other adverse enviromental factors relate to the amount of water evaporates, such as high temperature. Further, under field conditions, wind and precipitation are important natural sources relate to water evaporate, reducing salinity level, even mechanical stress (Joyce Griffin Latimer 1991).

It is interesting to note that the rootstocks and the saline and non-saline conditions did not significantly affect the moisture content of the fruits. These findings were different to those reported in a previous study, which found that irrigation with saline water had slightly reduced fruit water content (Mitchell et al. 1991). Another study also reported that grafting could increase the solid content in fruits grown under saline conditions (Flores et al. 2010). The differences may be explained by the different varieties, rootstocks and other growing conditions used in the different studies.

Firmness (crispness) is an important characteristic of fruits since it relates directly to the commercial value of the product (Batu 2004, Yoshioka et al. 2009). The rootstocks, the saline conditions and the other growing conditions did not affect the fruit firmness harvested from the grafted plants grown indoors, outdoors and under saline or non-saline conditions (Table 5.1). These are in agreement with the results obtained by Giorgi et al. (2005), who were working with grafted peach plants.

Fruit skin colour is directly related to the acceptance of fruit by customers; colour has a great impact on a consumer's decision to buy fruits. Acceptance by customers of colour was found to have a close relationship with colorimetric data (a, b, L) (Manera et al. 2013) usually expressed as lightness/darkness (L*), vividness/chroma (C*) and the tint of the colour/hue

angle (h). The rootstocks and saline conditions did not influence the lightness or darkness (L*), vividness (C*) and huge angle (h) of the fruit skins (Table 5.2).

The C* values were higher for the plants grown in the off season 2016 both under saline and non-saline conditions compared to all other growth conditions (Table 5.2). Most of the plants grown in the off season 2016 both under saline and non-saline conditions also had lower "h" values compared to all other growth conditions (Table 5.2). Based on color standards (Weatheralla et al. 1992 and McLellan et al 1995), we resulted that the bitter melon fruits grown during the off season had a lighter green colour. These results were similar to those observed by Davis et al. (2005) in the grafted watermelon plants, and Singh and Rao (2014), in grafted Cucurbitaceous crops, including bitter melon, cucumber, watermelon and pumpkin.

Effects on fruit saponins, phenolic compounds and antioxidant activity

Saponins (TSC) and phenolic compounds (TPC) are two major bioactive groups found in bitter melon fruits. These two major bioactive compounds have been linked with antioxidant activity and various health benefits (Tan et al. 2016). Antioxidant properties have been involved in defence mechanisms against pathogen associated with the attack of free radicals and thus linked with health benefits. However, more than one antioxidant assay is needed to measure the antioxidant properties due to each assay having advantages and also limitations (Vuong et al. 2015). Therefore, in the present study, the antioxidant capacity of the fruits from bitter melon plants grown with different rootstocks and under different conditions was measured using three different assays, the ABTS, DPPH and FRAP assays.

The rootstocks and salinity did affect the levels of TSC, TPC and antioxidant capacity in the grafted fruits but there was no consistent pattern across the growing conditions. These findings were similar to those of Moncada et al. (2013), who reported that rootstocks had little or no effect on phenolic content in fruits of the grafted eggplants (*Solanum melongena* L.). However, there were some effects of the rootstocks under specific conditions: the fruit of the plants grown with the three rootstocks had higher ABTS antioxidant activity when grown outdoor in the main season 2017, whether they were grown under saline or non-saline conditions, compared to that of the fruits from the self-grafted bitter melon plants. The fruit of the plants grown with the three rootstocks had no higher DPPH antioxidant activity when grown indoor in the main season 2017 under saline and non-saline conditions, compared to that of the fruits from the self-grafted bitter melon plants. In contrast, for the plants grown outdoor in the main season

2017 under saline conditions, the fruit of the plants grown with the rootstocks Qb had a higher FRAP antioxidant activity compared to that of fruits from the self-grafted bitter melon plants while the other rootstocks (Sp and Rg) had no effect on the FRAP activity. Therefore, under these specific conditions, the Qb rootstock gave the best results across the three antioxidant assays. However, even under these specific conditions, the rootstock had no consistent effects on the TSC and TPC.

Our main finding was that the fruit grown outside during the main growing season 2017 had much higher TPC and ABTS, DPPH and FRAP antioxidant activity compared to all other growing conditions. These results were somewhat similar to those observed by Vrcek et al. (2011) on tomato (*Solanum lycopersicum* L.), and Gómez-Caravaca et al. (2012) on Quinoa (*Chenopodium quinoa* Willd.). In the present study, the growing season significantly affected the TSC and TPC of bitter melon fruits in that the fruits grown during the main season were significantly higher in TSC and TPC than those grown in the off season, whether they were grown indoor or outdoor. However, this effect of the growing season was not seen for the antioxidant capacity when the fruits were grown indoor.

Antioxidant capacity is linked with potential health benefits and is known to be closely linked with TPC and TSC (Vuong et al. 2015). In the present study, the impact of growing the bitter melons outdoor had the biggest impact on the antioxidant capacity, which was closely mirrored by the TPC but not the TSC. These results were similar to those observed by Moncada et al. (2013) on the grafted eggplants (*Solanum melongena* L.). Tan et al. (2014), who found that bitter melon varieties grown indoor, including Hanuman, White, Jade and Indra, had lower TPC and antioxidant activities than a bitter melon variety (Moonlight) grown outdoors. This effect is most likely due to the fruits grown outdoor being directly exposed to sunlight and having to produce phenolic compounds as protection against ultraviolet radiation (Luthria et al. 2006, Erkan et al. 2008).

5.5 Conclusions

The rootstocks and salinity at 16.0 dSm^{-1} did not consistently affect the moisture content, firmness, colour, saponins, phenolic compounds and antioxidant activity in the bitter melon fruits. However, the bitter melon fruit grown during the main season 2017 had higher TSC, TPC and antioxidant capacity than the fruits grown during the off season 2016. Of these, the fruits grown outdoor during the main season 2017 also had the highest TSC, TPC and

antioxidant capacity. Also, under some specific conditions, the Qb rootstock gave the best results across the three antioxidant assays.

Total saponin in bitter melon fruits is the main material in the pharmaceutical industry to produce medicine for diabetics. Future study is necessary to explore types of saponin and the concentration of the saponin components. In addition, the vitamin C content in fruits were harvested from difference rootstocks should also be considered in case of using grafted bitter melon fruits for food.

Based on our research results, further studies on the salinity tolerance of grafted bitter melon plants under saline field conditions should be evaluated. In particular, high saline effects on bitter melon yield and fruit quality warrant further attention.

CHAPTER 6

GENERAL DISCUSSION

This study was conducted to examine the effect of grafted bitter melon on different rootstocks on fruit yield and quality. Vietnamese bitter melon (VINO 12) scion was grafted onto three pumpkin cultivars, 'Queensland Blue', 'Sampson', and 'Ringer' that are known to have salinity tolerance. These 3 rootstocks are also tolerant to *Fusarium* and *Pythium* diseases.

The hypothesis for this study was that grafting onto an appropriate rootstock would significantly improve Vietnamese bitter melon production in that rootstocks with resistance to soil-borne diseases and saline conditions could mitigate these poor conditions in terms of plant growth, fruit yield and fruit quality. Therefore, the main aim was to improve a Vietnamese bitter melon variety (VINO 12) by grafting it with three different rootstocks to improve its growth and resistance to soil-borne disease, its fruit productivity and its fruit quality, especially under saline conditions.

Specifically this study has looked at the effect of rootstock on growth, disease resistance and fruit yield and quality (including bioactives) of bitter melon produced under saline and non-saline conditions during main or off seasons. Several pumpkin varieties have demonstrated resistance to Fusarium (Davis et al. 2008) however, their performance as a rootstock for bitter melon is unknown. Three pumpkin rootstocks, Queensland blue (Qb), Sampson (Sp) and Ringer (Rg), were evaluated for their potential as rootstocks for bitter melon produced under both optimal and sub-optimal conditions and the most suitable of two grafting methods, single leaf splice (SLS) and tongue approach (TA), for bitter melon scion with rootstocks was also determined.

The rootstocks were evaluated for their resistance to sodium chloride (NaCl) and the root rot disease caused by *Pythium aphanidermatum* before being grafted onto bitter melon. The grafted plants were evaluated under different growing conditions (indoor – greenhouse and outdoor) during both the main and off-seasons. They were compared to ungrafted and self-grafted control plants. Fruit yield was evaluated as the total fruit weight obtained during a five month season, while fruit quality was assessed including total saponins (TSC), phenolic compounds (TPC) and antioxidant properties (ABTS, DPPH and FRAP activity).

In Chapter 3, it was found that the SLS grafting method provided a higher success rate than the TA grafting method: 90.0%, 81.1% and 91.1% for the Qb, Sp and Rg rootstocks, respectively. The Sp rootstock was more tolerant to *Pythium aphanidermatum* isolated from root rot bitter melon plants and used to inoculate healthy seedlings. This rootstock had a lower mortality rate (29.1%) than the bitter melon scion seedlings and the Qb and Rg rootstock seedlings. In addition, the three rootstocks and scion seedlings were all able to survive under saline conditions at 16.0 dSm^{-1}, with survival rates of over 60.0%. However, the Sp rootstock showed the highest resistance (100%) against this level of salinity. Therefore, it was predicted that the new grafted plants and control (self-grafted plants) would grow under these saline conditions.

In Chapter 4, the Rg and Sp varieties were found to be excellent rootstocks for the Vietnamese bitter melon VINO 12 variety. They both resulted in a better fruit yield than the Qb rootstocks or the control plants. Compared to the control bitter melon plants (ungrafted and self-grafted), the fruit yield for the Rg and Sp grafted bitter melon plants was improved by 39.23-64.0%, 10.39-31.17% in non-saline conditions and 45.46%-53.40%, 33.04-70.58% under saline conditions (16.0 dSm^{-1}), respectively.

In Chapter 5, the rootstocks and salinity at 16.0 dSm^{-1} did not consistently affect the moisture content, firmness, colour, saponins, phenolic compounds and antioxidant activity in the bitter melon fruits. However, the fruit grown during the main season 2017 had higher TSC, TPC and antioxidant capacity than the fruits grown during the off season 2016. Of these, the fruits grown outdoor during the main season 2017 also had the highest TSC, TPC and antioxidant capacity. Also, under some specific conditions, the Qb rootstock gave the best results across the three antioxidant assays.

6.1 Seed germination time, grafting methods and seedling resistance to *Pythium* and salinity

Developing resilient cropping systems that can cope with conditions like disease and salinity is a major challenge facing Vietnam (Dau et al. 2009, Tien 2010). Our results suggest that grafting of bitter melon onto a healthy rootstock using the SLS method is viable and may provide the bitter melon industry with an appropriate alternative for areaseither heavily infested with soil borne disease or where saline intrusion is becoming an urgent problem. Specifically, our results showed that the Sp rootstock has the greatest potential because it showed a high resistance against *Pythium aphanidermatum* and salinity. In addition, all three tested rootstocks

have potential for producing grafted bitter melon grown on soils with salinity levels at 16.0 dSm^{-1} or higher (Table 6.1). Our study greatly contributes to the improvement of grafting bitter melon scion-pumpkin rootstock efficiency and may help growers improve germination percentage and germination speed for both scion and rootstock seeds in order to increase the grafting success rate.

Table 6.1 General guidelines for some Cucurbit species and bitter melon response to saline conditions

Salinity (dSm-1)	**Name of Cucurbit varieties**	**Plant response**	**References**
0 to 2	No information	Mostly negligible	(Bernstein 1975)
2 to 4	Cucumber (*Cucumis sativus* L.) Luffa (*Cucumis pepo* L. var *melopepo* Alef.) Bitter melon (*Momodica charantia* L.)	Growth of sensitive plants may be restricted	(Chartzoulakis 1992) (Kotuby-Amacher et al. 2000)
4 to 8	Pumpkin (*Cucurbita maxima* L.) Rockmelon (*Cucumis melo* L.)	Growth of many plants is restricted	(Kotuby-Amacher et al. 2000)
8 to 16	Bitter melon (VINO12) grafted onto Pumpkin rootstocks (Queensland blue, Sampson and Ringer)	Growth of plants may be slightly restricted	**New contributions based on the results of this study**
16 to 26	Pumpkin (Queensland blue, and Sampson)	Growth of plants may be restricted	

Clear differences in seed germination were evident between scions and rootstocks. The germination time of bitter melon was longer than that of the three rootstocks – taking an additional 2-3 days in the off season and 4-5 days in main season. In addition, the low temperature in the off season reduced the germination rates (%) and prolonged the germination time of seeds. These results may be related to differences in seed dormancy or result from the thick hard seed coat of the bitter melon seeds (Seiwa 2000). In fact, the rate of seed germination is not influenced by seed size (Ericksson 1999) but depends on the impact of temperature and humidity on the hard seed coats during incubation (Koomneef et al. 2002).

The survival rate of grafted plants is known to depend on compatibility between the scion and rootstocks. In this study, the SLS grafting method was found to give a better survival rate than the TA method. The survival rate using the SLS method was over 81% for all three rootstocks. The Rg rootstock had the highest grafting survival rate of 91.1% because the seedling diameter of the Rg rootstock is similar to that of the bitter melon seedlings (RHD-SHD = -0.06mm). The hardness of Rg stem is similar to that of bitter melon, therefore, the combination between scion and rootstock, bitter melon – Rg, is easier and more successful than that of bitter melon - Qb and bitter melon - Sp. In our study, the choice of SLS method was to reduce the value of RHD - SHD to the minimum (Table 3.2) due to grafting at the cotyledon. In addition, the SLS grafting method avoids water pooling in the stem following irrigation. With the TA method this can result in the scion producing roots, rather than a successful grafting union (Figure 1.16). Whereas with the SLS method, the grafting union rapidly forms, resulting in a higher grafting success rate.

Our study is novel in that no previous studies have been conducted on the influence of grafting methods on the survival rate of bitter melon. However, similar studies have been undertaken on cucumber and tomato. Cleft grafting methods achieved a higher rate of successful grafting (100%), compared to the tube grafting method (79%) for the tomato plant using the same rootstocks (Marsic and Osvald 2004). The hole-insertion and cleft grafting methods achieved success rates of 88.3% and 91.7%, respectively, for cucumber (Cansev and Ozguz 2010). The success of a grafting method depends on other objective factors, such as species, age of the seedling, grafting time and the subjective factors being the farmer performing the grating.The success of a grafting method will be partly expressed as the survival rate of grafted plants. It has been found to be influenced by main factors, such as quality and age of the seedlings, grafting time, the quality of the joined section and post-grafting management (Singh and Rao 2014). The morphology of seedling development varies widely between species and this stage is usually not long, so it is necessary to control the uniformity of seedling age and stem stiffness (Lovell and Moore 1971, Latimer 1991). Ultimately, selection of the most appropriate grafting method will depend on the main reason for using grafted plants and the growers' experience and skills using the selected technique (Davis et al. 2008).

Our findings revealed that the Sp seedlings were more tolerant to *Pythium aphanidermatum* (PA); the Sp rootstock had the lowest number of seedling deaths (29.1%) compared to the Qb

(96.3%) and Rg (44.8%) rootstocks and the bitter melon scion (62.7%). PA and *Fusarium* wilt are the two major diseases that cause decreased yields of cucumber and watermelon (Pavlou et al. 2002, Boughalleb et al. 2008) and bitter melon (Singh et al. 2012). Previous studies have suggested that *Cucurbita maxima* varieties can resistance to *Fusarium* wilt (Ko 1999, Davis et al. 2008). Moreover, there have been no reports on the occurrence of *Fusarium* wilt on the three rootstocks (Queensland blue, Sampson and Ringer). In fact, *Fusarium* wilt was not found in fields during our study. Therefore, in our study we have only verified the three rootstocks with PA, which was found on bitter melon growing in Vietnam. This study has demonstrated the potential for using grafted plants for the management of bitter melon soil-borne diseases. Future work still needs to be done to look at the effects of *Pythium* root rot on grafted bitter melon plant performance and yield.

The Sp rootstock was also the most resistance to salinity with a survival rate of 100% at 16.0 dSm^{-1}. When the salinity was increased from 16.0 to 26.0 dSm^{-1}, the survival rate was still high at 76.0%. However, all three tested rootstocks, with survival rates of over 60.0%, have potential for producing grafted bitter melon grown on soils with salinity levels at 16.0 dSm^{-1}. Also, as seen in Table 6.1, the Sp rootstock and, to a lesser extent, the Qb rootstock, were able to survive at salinity levels higher than 16.0 dSm^{-1}.

In fact, the limited effect of salinity on growth and reproduction of PA raises concerns of increased mortality resulting from synergistic effects on cucumber seedlings (Al-Sadi et al. 2010). Therefore, further studies are needed to determine the correlation between diseases and salinity in reducing productivity in grafted bitter melon, specifically with three objects used as rootstocks. Based on other studies, metalaxyl is recommended to control PA. Metalaxyl is the most commonly used fungicide for this disease. Similarly, wilt disease caused by *Fusarium oxysporum* is among the most overwhelming disease in tomato (Hassan and Haggag 2006), It also occurred on bitter melon in Vietnam (Hoi et al. 2013). Currently, there are limited reports that relate to the effects of *Fusarium* and salinity on Pumpkin and bitter melon. However, *Trichoderma harzianum* is an effective solution not only for enhancing salt tolerant, metabolic production and biocontrol ability against *Fusarium oxysporum* but also for protecting grafted bitter melon plants under saline conditions (Hassan and Haggag 2006).

6.2 Effect of rootstocks and growing conditions on the development of grafted bitter melon plants and fruit yields

In our study, three novel cucurbit varieties were produced by grafting the Vietnamese VINO 12 bitter melon variety with the Qb, Sp and Rg pumpkin varieties as rootstocks in order to increase the resistance of this bitter melon variety to salinity. Based on fruit yield, this was successfully achieved as two of the rootstocks, Sp and Rg, showed an increased salt resistance compared to the ungrafted and self-grafted VINO 12 bitter melon plants. These two rootstocks can be added to the list of salinity tolerant plants but they also increased the fruit yield when grown under non-saline conditions. Therefore, these new varieties may increase the resilience of the Vietnamese cropping systems where salinity is a major challenge (Dau et al. 2009, Tien 2010).

The Sp and Rg rootstocks increased the number of female flowers produced by the bitter melon plants, which resulted in more fruit and ultimately higher yields, whether they were grown under saline or non-saline conditions. In decreasing order for fruit yield, the Rg rootstock had the best yield, followed by the Sp and finally the Qb rootstocks. Of note, the Rg yield was 45-53% higher than that of the control under saline conditions and 48-64% under non-saline conditions. Therefore, the grafted plants prepared from Rg and Sp rootstocks were tolerant to salinity equal to or higher than 16.0 dSm^{-1}. In contrast, salinity reduced the fruit yield in the control plants, including ungrafted and self-grafted plants. However, salinity (16.0 dSm^{-1}) did not affect the development of all grafted and control plants in that their stem height, number of leaves and laterals, their fruit size (length and diameter) and fruit weight were not affected.

Generally, increasing salinity levels is known to affect fruit yield. Salinity was shown to result in smaller tomato fruit sizes, which lead to a reduced fruit yield (Yurtseven et al. 2005). Similarly, Magan et al. (2008) studied the effects of salinity on two grafted tomato plants and reported a linear reduction in fruit yield with electrical conductivity (EC) with significant differences between experiments done in spring and autumn. Increasing salinity by around 1dSm^{-1} (from 3.2 to 3.3 dSm^{-1}) was enough to reduce fruit yield by 7.2% to 11.8% depending on rootstocks and growing seasons.

Crops growing outside greenhouse and under field conditions must suffer from mechanical stress caused by natural sources, such as wind, temperature and precipitation. Crops also experience these natural mechanical stress caused by irrigation and farm machinery or workers

during cultivation (Latimer, 1991). The greatest contribution from our study is the discovery of three pumpkin varieties for rootstocks. These salt tolerant varieties can be used as rootstocks not only for grafting bitter melon but also for all Cucurbit species. Roy et al. (2014) reported that plant salinity tolerance is determined by genotype. The primary mechanisms for salinity tolerance are shoot ion exclusion, shoot tissue tolerance and 'osmotic' tolerance. Therefore, future studies could include research on salinity tolerance genes which can potentially be isolated and transplanted into other crops.

Interestingly, in our study, the overall fruit yield across all varieties, salinity had no effect on any of the fruit parameters, including yield (Table 6.2). However, the fruit parameters were significantly influenced by whether the plants were grown during the main season or the off season and whether they were grown indoor or outdoor. The fruit parameters was all lower indoor in the off season 2016 compared to indoor in the main season 2107 and except for fruit number, the parameters outdoor in the main season 2017 were also lower than for indoor in the main season 2107 (Table 6.2).

Table 6.2 Comparison of the grafted fruit yields grown under non-saline and saline conditions in different growing seasons

Fruit	Off season		Main season			
	Indoor		Indoor		Outdoor	
	Salinity (16 dSm^{-1})	Non-salinity (0.5-1.6 dSm^{-1})	Salinity (16 dSm^{-1})	Non-salinity (0.5-1.6 dSm^{-1})	Salinity (16 dSm^{-1})	Non-salinity (0.5-1.6 dSm^{-1})
D	5.07 ± 0.12^{c}	5.30 ± 0.17^{bc}	6.60 ± 0.21^{a}	6.72 ± 0.31^{a}	5.68 ± 0.11^{b}	5.48 ± 0.18^{bc}
L	18.18 ± 0.87^{c}	18.06 ± 0.64^{c}	26.86 ± 0.95^{a}	27.06 ± 1.19^{a}	20.75 ± 0.33^{b}	19.96 ± 0.90^{bc}
W	184.66 ± 9.80^{b}	206.87 ± 8.71^{b}	327.74 ± 23.41^{a}	346.47 ± 17.77^{a}	199.92 ± 25.03^{b}	192.46 ± 20.93^{b}
N	31.08 ± 4.25^{c}	40.40 ± 11.52^{c}	82.13 ± 13.98^{ab}	93.33 ± 21.90^{a}	54.67 ± 13.54^{bc}	61.54 ± 15.55^{abc}
Y	5.57 ± 0.95^{b}	7.96 ± 1.64^{b}	26.88 ± 4.20^{a}	30.97 ± 5.62^{a}	11.35 ± 2.44^{b}	11.70 ± 2.46^{b}

Data are the means ± standard deviations (n = 4). Data sharing different superscript letters (a, b, c) in the same row indicate significant difference in the germination rates of one variety at different dates ($P < 0.001$).

Note: D: diameter; L: length; W: weight; N: number; Y: yield.

For example, the yield of fruit grown indoor in the main season 2017 was 3-4 times higher than that of the fruit grown indoor in the off season and 2-2.5 times higher than that of the fruit grown outdoor in the main season 2017. Therefore, the best fruit yield was obtained when the bitter melon plants were grown indoor during the main season. This is likely due to the long

sunlight hours during the main growing season and the controlled environment, including temperature, in the greenhouses. Thus, the increase of fruit yield and quality in grafted bitter melon depend on various factors, such as growing seasons, growing conditions, grafting methods, rootstocks and nutrient supply.

These results (Table 6.2) also confirmed that bitter melon grafted plants can be grown in fields with higher salinity levels. This will facilitate further salinity tolerant trials developing in the two major production areas in Vietnam, the Red river delta and Cuu Long river delta. Especially, future conducted researches of cucurbit plants will have a scientific basis to approach another agricultural production areas in the South of Vietnam with the soil salinity range of 29.25 dSm^{-1} to 33.44 dSm^{-1}, the Mekong river delta. In addtion, the problem of irrigation water for agriculture is becoming difficult, therefore, there should be long - term study on plant that can grow under soil salinizations and use salinity water.

6.3 Effect of rootstocks and growing conditions on grafted bitter melon fruit quality

Fruit quality is closely related to the acceptance by consumers as well as to fruit value (Mowat and Collins 2000). As most of bitter melon is consumed fresh as vegetable, physical properties, such as firmness, taste and flavour are important for marketability. Interestingly, a large component of the bitterness of the fruit taste is due to a high content of saponins, including charantin (Bich et al. 2006) and therefore, the levels of saponins need to be considered. Grafting influenced the apparent fruit quality of bitter melon. Fruit characteristics differed depending on the rootstock cultivars. However, the fruit characteristics of rootstock did not affect the fruit characteristics of scion grafted onto that rootstock (Jang et al. 2013).

Our study investigated the impact of rootstocks and growing conditions on the quality of bitter melon, including physical properties (colour, firmness and moisture), chemical properties (saponins, phenolic compounds, antioxidant capacity). Generally, the three rootstocks and saline conditions (16.0 dSm^{-1}) had no consistent effect on the fruit skin colour, fruit firmness, moisture content, saponins, phenolic compounds and antioxidant activity of the bitter melon fruits. Of these, the fruits grown outdoor during the main season 2017 also had the highest TSC, TPC and antioxidant capacity. Also, under some specific conditions, the Sp rootstock gave the best results across the three antioxidant assays. It is interesting to note that rootstock-scion combination and saline conditions did not affect the firmness of the grafted bitter melon

fruits, as was the case in grafted watermelon (Davis et al. 2005, Colla et al. 2006, Davis et al. 2008).

The growing season and conditions had the most striking influence on TSC, TPC and antioxidant capacity. The TSC and the TPC of the fruits grown outdoors during the main season 2017 were by far the highest amongst all the growing conditions. For example, the values were 2-3 times higher for TSC, 9-10 times higher for TPC and 5-20 times higher for antioxidant activities for the plants grown outdoor during the main season 2017 than for those grown indoor. In fact, if carefully selected, the combination between scion/rootstock, the scion variety and the harvest period, the desired optimal fruit quality can be achieved. In addition, Jang et al. (2013) reported that the fruit quality parameters were also different as affected by the harvest period.

The level of phenolic compounds in our grafted bitter melon fruits was also much higher than that reported for other bitter melon varieties, such as Indian green, Indian white, China green and China white (Horax et al. 2005). Our values were also approximately 2-3 times higher compared to Thai bitter melon fruits grown outdoors (Kubola and Siriamornpun 2008). The content of these bioactive components in the fruits grown outdoors in the main season 2017 (from 171 to 206 EE/g for TSC and from 751 to 856 GAE/g for TPC) were also higher than for bitter melon varieties previously grown in greenhouses in Australia; Tan et al. (2013) reported that the TSC and TPC levels of some bitter melon varieties grown in climate-controlled greenhouses ranged from 46.8 to 93.2mg EE/g and 5.1 to 7.9mg GAE/g dry sample, respectively.

Generally, fruits grown outdoors have higher levels of saponins and phenolic compounds in comparison with those grown indoors (Luthria et al. 2006, Erkan et al. 2008). This effect is most likely due to the fruits grown outdoor being directly exposed to sunlight and having to produce phenolic compounds as protection against ultraviolet radiation. In our study, the greenhouses blocked 60.4% of the light because of their polyethylene covers and the light was more diffuse inside the greenhouses than in the strong direct sunlight, which occurs outside during the Australian summer. As a result of this, the light intensity of the indoor conditions was very different to that of the outdoor. In addition, the phenolic content of bitter melon fruits are determined by various factors such as the cultivar, agronomic management, climatic

factors, developmental stage, harvesting time, storage conditions, and postharvest management (Nagarani et al. 2014, Saeed et al. 2018).

Results from three antioxidant assays (DPPH, ABTS and FRAP assays) also revealed that the rootstocks and the salinity did not significantly affect antioxidant properties in bitter melon fruits. However, as for the TSC and the TPC, the highest antioxidant activities were seen for the fruits grown outdoor during the main season 2017. These results closely mirrored the TPC of the fruits, which is not surprising because antioxidant capacity is known to be closely linked with TPC (Vuong et al. 2015). These results were similar to those observed by Moncada et al. (2013) on the grafted eggplants (*Solanum melongena* L.).

Our study only focused on total saponins and phenolic compounds as components of the bitter melon fruits and not on the many other components, such as macronutrients, dietary fibre, carbohydrates, sugars, lipids, proteins, vitamins (vitamin C, carotenoids), minerals and other individual bioactive compounds. Therefore future studies are needed to investigate the impact of rootstocks, saline conditions and growing conditions on macronutrients, micronutrients and major individual bioactive compounds of the grafted bitter melon fruits.

The various extracts of bitter melon have potential antioxidant activity. The polyphenols found in this fruit can help to protect oxidative damage by acting directly on reactive oxygen species and induce endogenous defense system (Nagarani et al. 2014). Standard constituents of bitter melon are charantin, momordicine, and p-insulin which are steroidal saponins, alkaloids and polypeptides in nature (Tan et al. 2014). Momordicine and charantin are predominantly responsible for the health encouraging effect and the bitterness of bitter melon. Its ethanolic extracts contain high antioxidant activities that are well correlated with phenolic compounds (Saeed et al. 2018).

Diabetes poses a major challenge and the economic pressure to the world population, leading to increasing interest in the use of traditional remedies for the treatment of diabetes mellitus (Tan et al. 2016). Around 228 different compounds were identified from different parts of bitter melon plant and some active ingredients among them are used for the treatment of both Types I and II diabetes (Nagarani et al. 2014). Momordica has potential anti-diabetic properties, which may suggest the inclusion of this plant in anti-diabetic regimens. Xu et al. (2015) suggested that the water-soluble polysaccharide (MCP) was isolated from bitter melon fruit

with the hypoglycemic effects could be incorporated as a supplement in health-care food, drugs and/or combined with other hypoglycemic drugs.

6.4 Relevance of results to conditions in Vietnam

Vietnam has two vast regions, where the soil has been salinised, including the Cuu Long river delta located in the South with 884,000 hectares and the Red river delta located in the North with 132,000 hectares. Salinity of the soil ranges from 4.0 dSm^{-1} – 16.0 dSm^{-1} and these areas have been deserted by growers (Đức et al. 2009, Đức and Đạo 2011). Another challenge for growing bitter melon in these two areas is the big difference in temperature (Figure 6.1). The optimum temperature for bitter melon fruit yield ranges from 25°C to 35°C (Morgan and Midmore 2002, Hoi et al. 2013) and thus, bitter melon can grow throughout the year in the Cuu Long river delta, but can only grow for 6-7 months in the Red river delta.

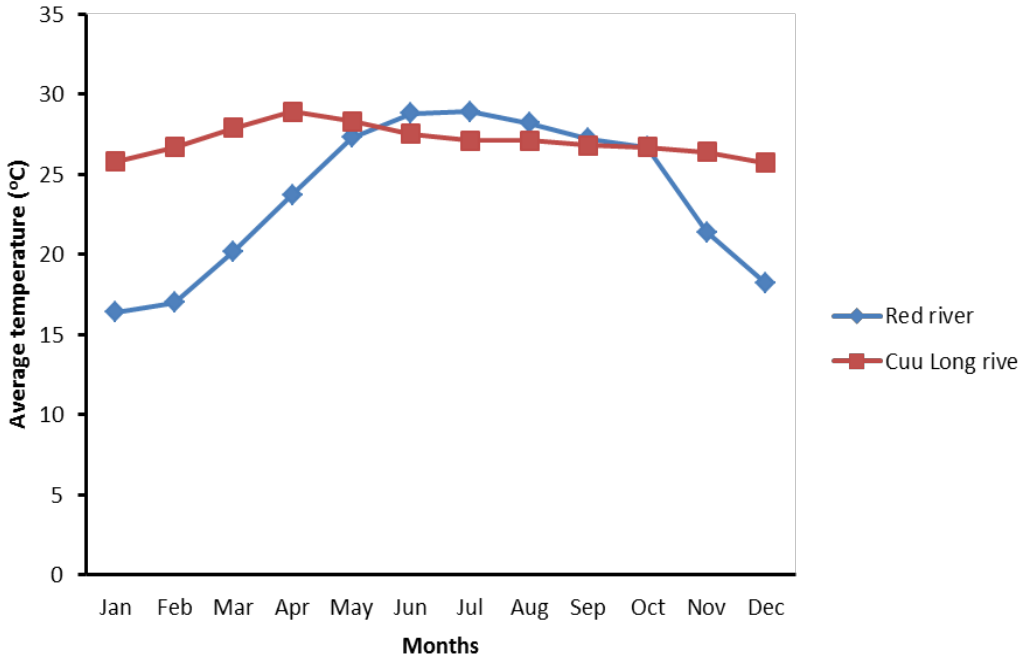


Figure 6.1 Monthly average temperatures for the Red river and Cuu Long river deltas in Vietnam

Findings from this study provide some options to be investigated further for producing bitter melon in sub-optimal growing conditions, such as saline conditions or high disease pressure. With increasing saline conditions, finding crops which can grow well under these conditions, would support growers facing these constraints. This study revealed that Qb, Sp, and Rg are potential rootstocks for grafting other commercial Cucurbit species, such as water melon and cucumber for growing on saline soil, and thus future studies are recommended.

6.5 Conclusion

In conclusion, the hypothesis was supported and the aim and objectives of this project were achieved. Three rootstocks were tested for their potential for grafting to the Vietnamese VINO 12 bitter melon scion, for saline tolerance, *Pythium* resistance, fruit yield and fruit quality. The SLS grafting method was more successful than the TA method and thus, it is recommended for grafting bitter melon. Of the three rootstocks, the Sp variety had the most resistance to Pythium and salinity, the Rg and Sp rootstocks had the highest fruit yield but there was no consistent pattern for fruit quality, although the Qb rootstock gave the best fruit quality under some limited and specific growing conditions. Therefore, the Sp rootstock is recommended to be used as rootstock for resistance to *Pythium* and salinity, while Rg and Qb are suggested to be used as rootstock for fruit yield and fruit quality, respectively, under select conditions.

Assessment of critical criteria in grafted fruits is necessary before developing a practical strategy and extending the applicable scope of all plants belong to Cucurbitaceae, such as watermelon, cucumber, rockmelon, and melon. Based on our research results the use of these rootstocks for growing other cucurbit species under saline soil conditions will be of a great advantage. The actual productivity of bitter melon and other cucurbit plants growing under soil salinization is a valuable and preferred study in the future. This study revealed that grafting using salt tolerant rootstock is a new direction in creating plants that can grow under saline conditions, instead of breeding or improving the new varieties.

Optimum management of global water resources presents one of the most crucial challenges of the 21st century. Agricultural production may soon be limited by fresh water availability because of the agricultural water use is not sustainable in many locales around the world. The main reasons can be the increase of soil salinization and the decrease of available surface water supplies. This situation raises questions about whether there are sufficient water resources to support the agricultural activities on a long-term basis. Thus, finding new salt-tolerant varieties and create salt-tolerant crops is the right direction not only for Vietnam but also for all coastal countries, which are facing of the sea water intrusion and soil salinization.

Finally, the greatest significance in our study is found three pumpkin varieties for rootstocks with resistance to soil-borne diseases (*Fusarium* and *Pythium*) and saline conditions. They are used not only for grafting numerous species belong to Cucurbit family but their genes also might be usefully used to transform crops in order to improve salinity tolerance.

REFERENCES

Abawi, G. and T. Widmer (2000). "Impact of soil health management practices on soilborne pathogens, nematodes and root diseases of vegetable crops." Applied soil ecology **15**(1): 37-47.

Abrol, I., J. S. P. Yadav and F. Massoud (1988). Salt-affected soils and their management, Food & Agriculture Org, 1-13.

Adams, S. R., K. E. Cockshull and C. R. J. Cave (2001). "Effect of Temperature on the Growth and Development of Tomato Fruits." Annals of Botany **88**(5): 869-877.

Akihisa, T., N. Higo, H. Tokuda, M. Ukiya, H. Akazawa, Y. Tochigi, Y. Kimura, T. Suzuki and H. Nishino (2007). "Cucurbitane-type triterpenoids from the fruits of Momordica charantia and their cancer chemopreventive effects." Journal of natural products **70**(8): 1233-1239.

Al-Sadi, A. M., R. S. Al-Masoudi, N. Al-Habsi, F. A. Al-Said, S. A. Al-Rawahy, M. Ahmed and M. L. Deadman (2010). "Effect of salinity on pythium damping-off of cucumber and on the tolerance of Pythium aphanidermatum." Plant Pathology **59**(1): 112-120.

Alan, O., N. Ozdemir and Y. Gunen (2007). "Effect of grafting on watermelon plant growth, yield and quality." Journal of Agronomy **6**(2): 362.

Alexopoulos, A. A., A. Kondylis and H. C. Passam (2007). "Fruit yield and quality of watermelon in relation to grafting." Journal of Food Agriculture and Environment **5**(1): 178.

Ali, M. A., M. A. Sayeed, M. S. Reza, M. S. Yeasmin and A. M. Khan (2008). "Characteristics of seed oils and nutritional compositions of seeds from different varieties of Momordica charantia Linn. cultivated in Bangladesh." Czech J Food Sci **26**(4): 275-283.

Allen, D. J. and D. R. Ort (2001). "Impacts of chilling temperatures on photosynthesis in warm-climate plants." Trends in plant science **6**(1): 36-42.

Aloni, B., R. Cohen, L. Karni, H. Aktas and M. Edelstein (2010). "Hormonal signaling in rootstock–scion interactions." Scientia Horticulturae **127**(2): 119-126.

Balaguera-López, H. E., Cárdenas-Hernández J. F., Álvarez-Herrera, J.G. (2008). Effect of Gibberellic Acid on seed germination (GA3) and growth of Tomato (*Solanum lycopersicum* L.). ISHS Acta Horticulturae, 821(15).

Batu, A. (2004). "Determination of acceptable firmness and colour values of tomatoes." Food and Engineering **61**(3): 471-475.

Behera, T., K. J. John, L. Bharathi and R. Karuppaiyan (2011). Momordica. Wild Crop Relatives: Genomic and Breeding Resources, Springer**:** 217-246.

Behera, T. K., S. Behera, L. K. Bharathi, K. J. John, P. W. Simon and J. E. Staub (2010). "Bitter Gourd: Botany, Horticulture, Breeding." Horticultural reviews **37**(2): 101-141.

Berman, M. E. and T. M. Dejong (1996). "Water stress and crop load effects on fruit fresh and dry weights in peach (Prunus persica)." Tree Physiology **16**(10): 859-864.

Bernstein, L. (1975). "Effects of salinity and sodicity on plant growth." Annual review of phytopathology **13**(1): 295-312.

Bharathi, L. and K. J. John (2013). Momordica Genus in Asia: An Overview, Springer.

Bich, D. H., D. Q. Chung, B. X. Chuong, N. T. Dong, D. T. Dam, P. V. Hien, V. N. Lo, P. D. Mai, P. K. Man, D. T. Nhu, N. Tap and T. Toan (2006). Cay thuoc va dong vat lam thuoc o Viet Nam. Hanoi, Nha xuat ban Khoa hoc va Ky thuat.

Blanco, F. F. and M. V. Folegatti (2005). "Estimation of leaf area for greenhouse cucumber by linear measurements under salinity and grafting." Sci. agric. (Piracicaba, Braz.) **62**(4): 305-309.

Bletsos, F. (2005). "Use of grafting and calcium cyanamide as alternatives to methyl bromide soil fumigation and their effects on growth, yield, quality and Fusarium wilt control in melon." Journal of Phytopathology **153**(3): 155-161.

Bletsos, F., C. Thanassoulopoulos and D. Roupakias (2003). "Effect of grafting on growth, yield, and Verticillium wilt of eggplant." HortScience **38**(2): 183-186.

Boughalleb, N., m. M, B. E. Assadi, E. Bourgi, N. Tarchoun and M. S. Romdhani (2008). "Resistance evaluation of Grafted watermelon (Citrullus lanatus L.) against Fusarium wilt and Fusarium crown and Root rot." Asian Journal of Plant Pathology **2**(1): 24-29.

Budrat, P. and A. Shotipruk (2009). "Enhanced recovery of phenolic compounds from bitter melon (< i> Momordica charantia</i>) by subcritical water extraction." Separation and Purification Technology **66**(1): 125-129.

Burgess, L. W., T. E. Knight, L. Tesoriero and H. T. Phan (2008). Diagnosis manual for plant diseases in Vietnam. Canberra, Australian Centre for International Agricultural Research.

Cansev, A. and M. Ozgur (2010). "Grafting cucumber seedlings on Cucurbita spp.: Comparison of different grafting methods, scions and their performance." Journal of Food, Agriculture and Environment **8**(3&4): 804-809.

Cansev, A. and M. Ozgur (2010). "Grafting cucumber seedlings on Cucurbita spp.: Comparison of different grafting methods, scions and their performance." J Food Agric Env **8**: 804-809.

Cansev, A. and M. Ozguz (2010). "Grafting cucumber seedlings on Cucurbita spp.: Comparison of different grafting methods, scions and their performance." Journal of food, agriculture and environment **8**(3&4): 804-809.

Chandra, P., R. Sao, S. K. Gautam and A. N. Poddar (2010). "Initial population density and its effect on the pathogenic potential and population growth of the root knot nematode Meloidogyne incognita in four species of cucurbits." Asian Journal of Plant Pathology **4**(1): 1-15.

Chang-hua, X., B.-f. KUANG, Y. Xi-mao, T. Mei-li, D. Xian-chao, E. TANG and F. OUYANG (2011). "Effects of Grafting on Bitter Gourd Resistance to Phytophthora Blight, Yield and Quality." China Vegetables **4**: 019.

Chang, C.-I., C.-R. Chen, Y.-W. Liao, H.-L. Cheng, Y.-C. Chen and C.-H. Chou (2006). "Cucurbitane-Type Triterpenoids from Momordica c harantia." Journal of natural products **69**(8): 1168-1171.

Chartzoulakis, K. S. (1992). "Effects of NaCl salinity on germination, growth and yield of greenhouse cucumber." Journal of Horticultural Science **67**(1): 115-119.

Chen, F., M. Wang, Y. Zheng, J. Luo, X. Yang and X. Wang (2010). "Quantitative changes of plant defense enzymes and phytohormone in biocontrol of cucumber Fusarium wilt by Bacillus subtilis B579." World Journal of Microbiology and Biotechnology **26**(4): 675-684.

Chérif, M., J. Menzies, D. Ehret, C. Bogdanoff and R. Belanger (1994). "Yield of cucumber infected with Pythium aphanidermatum when grown with soluble silicon." HortScience **29**(8): 896-897.

Chhabra, R. (1996). Soil Salinity and Water quality. America, A.A Balkema.

Chun-feng, W. (2009). "Effects of Graft with Different Rootstocks on Quality and Disease Resistance of Momordica charantia [J]." Journal of Anhui Agricultural Sciences **2**: 050.

Cohen, R., Y. Burger, C. Horev, A. Koren and M. Edelstein (2007). "Introducing grafted cucurbits to modern agriculture: the Israeli experience." Plant Disease **91**(8): 916-923.

Cohen, R., C. Horev, Y. Burger, S. Shriber, J. Hershenhorn, J. Katan and M. Edelstein (2002). "Horticultural and pathological aspects of Fusarium wilt management using grafted melons." HortScience **37**(7): 1069-1073.

Cohen, R., N. Omari, A. Porat and M. Edelstein (2012). "Management of Macrophomina wilt in melons using grafting or fungicide soil application: pathological, horticultural and economical aspects." Crop Protection **35**: 58-63.

Colla, G., Y. Roupahel, M. Cardarelli and E. Rea (2006). "Effect of salinity on yield, fruit quality, leaf gas exchange, and mineral composition of grafted watermelon plants." HortScience **41**(3): 622-627.

Colla, G., Y. Rouphael, M. Cardarelli, D. Massa, A. Salerno and E. Rea (2006). "Yield, fruit quality and mineral composition of grafted melon plants grown under saline conditions." The Journal of Horticultural Science and Biotechnology **81**(1): 146-152.

Dau, V. T., N. V. Dang, D. H. Nguyen, L. T. Pham, T. T. M. Le, H. T. Phan and L. W. Burgess (2009). "A simplified technique for grafting watermelon onto resistant cucurbit rootstocks for control of Fusarium wilt of watermelon in Nghe An Province, Vietnam." Australasian Plant Disease Notes **4**(1): 114-116.

Davis, A. R., P. Perkins-Veazie, R. Hassell, A. Levi, S. R. King and X. Zhang (2008). "Grafting effects on vegetable quality." HortScience **43**(6): 1670-1672.

Davis, A. R., P. Perkins-Veazie, Y. Sakata, S. López-Galarza, J. V. Maroto, S.-G. Lee, Y.-C. Huh, Z. Sun, A. Miguel, S. R. King, R. Cohen and J.-M. Lee (2008). "Cucurbit grafting." Critical Reviews in Plant Sciences **27**(1): 50-74.

Davis, A. R., P. Perkinz-Veazie, Y. Sakata, S. López-Galarza, J. V. Maroto, S.-G. Lee, Y.-C. Huh, Z. Sun, A. Miguel, S. R. King, R. Cohen and J.-M. Lee (2005). "Rootstock effects on plant vigor and watermelon fruit quality." REPORT-CUCURBIT GENETICS COOPERATIVE **28**: 39.

Dhillon, M. K., R. Singh, J. S. Naresh and H. C. Sharma (2005). "The melon fruit fly, Bactrocera cucurbitae: a review of its biology and management." J Insect Sci **5**: 40.

Dhillon, N. P. S., E. a. Sanguansil, S. P. Singh, M. A. T. Masud, P. Kumar, L. K. Bharathi, H. YetişirRukui, H. D. X. Canh and J. D. M. Creight (2016). "Gourds: Bitter, Bottle, Wax, Snake, Sponge and Ridge." Genetics and Genomics of Cucurbitaceae **20**: 155-172.

Do, L. T. (2000). Nhung Cay Thuoc va Vi Thuoc Viet Nam [Medicinal Plants and Drugs of Vietnam]. Hanoi, Nha Xuat Ban Y Hoc

Dordas, C. (2009). "Foliar application of calcium and magnesium improves growth, yield, and essential oil yield of oregano (Origanum vulgare ssp. hirtum)." Industrial Crops and Products **29**(2-3): 599-608.

Đức, H. Q. and N. V. Đạo (2011). "Đánh giá sự biến động đất mặn và đất phèn vùng đồng bằng sông Cửu Long sau 30 năm sử dụng." Tạp chí Khoa học và Công nghệ Nông nghiệp Việt Nam **1**: 18-24.

Đức, H. Q., N. V. Đạo and T. X. Cường (2009). "Đánh giá sự biến động đất mặn vùng đồng bằng sông Hồng sau 30 năm sử dụng." Tạp chí Nông nghiệp và Phát triển Nông thôn **11**: 8-11.

Đức, H. Q., N. V. Đạo and T. X. Cường (2009). "Đánh giá sự biến động đất mặn vùng đồng bằng sông Hồng sau 30 năm sử dụng." Tạp chí Nông nghiệp & Phát triển Nông thôn **11**: 8-11.

Edelstein, M. (2004). Grafting vegetable-crop plants: pros and cons, International Society for Horticultural Science (ISHS), Leuven, Belgium.

Edelstein, M. (2004). Grafting vegetable-crop plants: pros and cons. VII International Symposium on Protected Cultivation in Mild Winter Climates: Production, Pest Management and Global Competition 659.

Edelstein, M., M. Ben-Hur and Z. Plaut (2007). "Grafted melons irrigated with fresh or effluent water tolerate excess boron." Journal of the American Society for Horticultural Science **132**(4): 484-491.

Edelstein, M., R. Cohen, Y. Burger, S. Shriber, S. Pivonia and D. Shtienberg (1999). "Integrated management of sudden wilt in melons, caused by Monosporascus cannonballus, using grafting and reduced rates of methyl bromide." Plant disease **83**(12): 1142-1145.

El-Sayed, S. F., H. A. Haassan and M.A.Gaara (2015). "Effect of different rootstocks on plant growth, yield and quality of watermelon." Annals of Agric. Sci., Moshtohor **53**(1): 165-175.

El-Tarabily, K., A. Nassar, G. S. J. Hardy and K. Sivasithamparam (2009). "Plant growth promotion and biological control of Pythium aphanidermatum, a pathogen of cucumber, by endophytic actinomycetes." Journal of Applied Microbiology **106**(1): 13-26.

Ericksson, O. (1999). "Seed size variation and its effect on germination and seedling performance in the clonal herb Convallaria majalis." Acta Oecologica **20**(1): 61-66.

Erkan, M., S. Y. Wang and C. Y. Wang (2008). "Effect of UV treatment on antioxidant capacity, antioxidant enzyme activity and decay in strawberry fruit." Posharvest Biology and Technology **42**(2): 163-171.

Fang, E. F. and T. B. Ng (2013). The Bitter Fruit with Sweet Health Benefits: A Comprehensive Synopsis of Recent Research Progress on Medicinal Properties of Momordica Charantia. Antitumor Potential and other Emerging Medicinal Properties of Natural Compounds, Springer**:** 315-334.

Fernández-Garcia, N., V. Martínez, A. Cerdá and M. Carvajal (2004). "Fruit quality of grafted tomato plants grown under saline conditions." The Journal of Horticultural Science and Biotechnology **79**(6): 995-1001.

Flores, F. B., P. Sanchez-Bel, M. T. Estan, M. M. Martinez-Rodriguesz, E. Moyano, B. Morales, J. F. Campos, J. O. Garcia-Alella'n, M. I. Egea, n. Ferna'ndez-Garcia, F. l. Romojaro and M. C. Bolarin (2010). "The effectiveness of grafting to improve tomato fruit quality." Scientia Horticulturae **125**(3): 211-217.

Franco, J. A., C. Esteban and C. Rodriguez (2015). "Effects of salinity on various growth stages of muskmelon cv. Revigal." Jounal of Horticultural Science **68**(6): 899-904.

Gama, P. B. S., I. S. , T. K. and R. Nakazawa (2007). "Physiological response of common bean (*Phaseolus vulgaris* L.) seedlings to salinity stress." African Journal of Biotechnology **6**(2): 079-088.

Gao, J. and H. Liao (2006). "Effect of grafting on watermelon quality." China Cucurbits Veg **5**: 12-14.

Gao, J. J., A. G. Qin and X. C. Yu (2009). "[Effects of grafting on cucumber leaf SOD and CAT gene expression and activities under low temperature stress]." Ying Yong Sheng Tai Xue Bao **20**(1): 213-217.

Giorgi, M., E. Capocasa, J. Scalzo, G. Murri, M. Battino and B. Mezzetti (2005). "The rootstock effects on plant adaptability, production, fruit quality, and nutrition in the peach (cv. 'Suncrest')." Scientia Horticulturae **107**(1): 36-42.

Gómez-Caravaca, A. M., G. Iafelice, A. Lavini, C. Pulvento, M. F. Caboni and E. Marconi (2012). "Phenolic Compounds and Saponins in Quinoa Samples (Chenopodium quinoa Willd.) Grown under Different Saline and Nonsaline Irrigation Regimens." Agricultural and Food Chemistry **60**(18): 4620–4627.

Gruda, N. (2005). "Impact of environmental factors on product quality of greenhouse vegetables for fresh consumption." Critical Reviews in Plant Sciences **24**(3): 227-247.

Gruda, N. (2005). " Impact of Environmental Factors on Product Quality ofGreenhouse Vegetables for Fresh Consumption (Critical Reviews)." Plant Sciences **24**(3): 227-247.

Grünwald, N., F. Workneh, S. Hu and A. Van Bruggen (1997). "Comparison of an in vitro and a damping-off assay to test soils for suppressiveness to Pythium aphanidermatum." European Journal of Plant Pathology **103**(1): 55-63.

Handreck, K. A. and N. D. Black (1994). Growing media for ornamental plants. Randwick NSW Australia, University of New South Wales Press.

Hanson, B. R., S. R. Grattan and A. Fulton (2006). Agricultural Salinity and Drainage. United States of America by the Department of Land, Air and Water Resources, University of California, Davis, California 95616, Regents of the University of California

Division of Agriculture and Natural Resources.

Harinantenaina, L., M. Tanaka, S. Takaoka, M. Oda, O. Mogami, M. Uchida and Y. Asakawa (2006). "Momordica charantia constituents and antidiabetic screening of the isolated major compounds." Chemical and Pharmaceutical Bulletin **54**(7): 1017-1021.

Hassan A-L. A. M., Haggag W. M. (2006). Biocontrol potential of salinity tolerant mutants of Trichoderma harzianum against Fusarium oxysporum. Brazilian Journal of Microbiology, 37(2).

Hatfield, J. L. and J. H. Prueger (2015). "Temperature extremes: Effect on plant growth and development." Weather and Climate Extremes **10**(Part A): 4-10.

Heine, G., G. Tikum and W. J. Hors (2006). "The effect of silicon on the infection by and spread of Pythium aphanidermatum in single roots of tomato and bitter gourd." Journal of Experimental Botany **58**(3): 569–577.

Hien, N. H. and S. H. Widodo (1999). Momordica charantia L. . Leiden, The Netherlands, Backhuys Publisher,.

Hoi, D. V., N. D. Thieu and F. Muller (2013). MUOP DANG. Trainer's Guide – Theory. Hanoi,Vietnam, Department of Primary Industries, NSW-Australia.

Horax, R., N. Hettiarachchy and S. Islam (2005). "Total phenolic contents and phenolic acid constituents in 4 varieties of bitter melons (Momordica charantia) and antioxidant activities of their extracts." Journal of food science **70**(4): C275-C280.

Howell, N. (1981). "The effect of seed size and relative emergence time on fitness in a natural population of Impatiens capensis Meerb.(Balsaminaceae)." American Midland Naturalist: 312-320.

Hu C.M., Zhu Y.L., Yang L.F., Chen S.F., Huang Y.M. (2006). "Comparison of photosynthetic characteristics of grafted and own-root seedling of cucumber under low temperature circumstances" .Acta Bot. Boreali-Occidentalia Sinica 26 : 247-253

Huang, X.-X., B.-C. Wang, G.-L. Zhou, J. Zhu, A.-H. Wang and Y.-H. Deng (2013). "Effects of Grafting on Quality of Momordica charantia." Hubei Agricultural Sciences **4**: 025.

Huang, X., G. Zhou, J. Zhu, B. Wang and C. Lin (2012). "Effects of Different Rootstocks and Grafting Methods on Growth of Momordica charantia." Journal of Changjiang Vegetables **6**: 009.

Huang, Y., Z. Bie, S. He, B. Hua, A. Zhen and Z. Liu (2010). "Improving cucumber tolerance to major nutrients induced salinity by grafting onto Cucurbita ficifolia." Environmental and Experimental Botany **69**(1): 32-38.

Huang, Y., R. Tang, Q. Cao and Z. Bie (2009). "Improving the fruit yield and quality of cucumber by grafting onto the salt tolerant rootstock under NaCl stress." Scientia Horticulturae **122**(1): 26-31.

Jang, Y. M., Ji-Hye ; Lee, Ji-Weon ; Lee, Sang Gyu ; Kim, Seung Yu ; Chun, Changhoo. (2013). Effects of Different Rootstocks on Fruit Quality of Grafted Pepper (Capsicum annuum L.). *Horticultural Science and Technology, 31*(6), 687-699.

Jiebao, C. X. Y. W. P. and Z. Tianlun (1997). "A Brief Report on Experiments of Grafting Balsam Pear on dishcloth Gourd [J]." Acta Agriculturae Jiangxi **4**.

Joseph, B. and D. Jini (2013). "Antidiabetic effects of< i> Momordica charantia</i>(bitter melon) and its medicinal potency." Asian Pacific Journal of Tropical Disease **3**(2): 93-102.

Joyce Griffin Latimer (1991) "Mechanical Conditioning for Control of Growth and Quality of Vegetable Transplants." HortScience 26(12):1456-1461.

Kamonwannasit, S., N. Nantapong, P. Kumkrai, P. Luecha, S. Kupittayanant and N. Chudapongse (2013). "Antibacterial activity of *Aquilaria crassna* leaf extract against *Staphylococcus epidermidis* by disruption of cell wall." Annals of Clinical Microbiology and Antimicrobials **12**(20): 1-7.

Karaca, F., H. Yetişir, İ. Solmaz, E. Candir, Ş. Kurt, N. Sari and Z. Güler (2012). "Rootstock potential of Turkish Lagenaria siceraria germplasm for watermelon: plant growth, yield and quality." Turk J Agric For **36**: 167-177.

Keller, A. C., J. Ma, A. Kavalier, K. He, A. M. Brillantes and E. J. Kennelly (2011). "Saponins from the traditional medicinal plant Momordica charantia stimulate insulin secretion in vitro." Phytomedicine **19**(1): 32-37.

Kempers, R., D. A. M. Prior, A. J. E. Vanbel and K. J. Oparka (1993). "Plasmodesmata between sieve element and companion cell of extrafascicular stem phloem of Cucurbita maxima permit passage of 3 kDa fluorescent probes." The Plant Journal **4**(3): 567-575.

Kimura, S. and N. Sinha (2008). "Grafting tomato plants." CSH Protoc **2008**: pdb prot5083.

King, S. R., A. R. Davis, X. Zhang and K. Crosby (2010). "Genetics, breeding and selection of rootstocks for Solanaceae and Cucurbitaceae." Scientia horticulturae **127**(2): 106-111.

Klomann, S. D., A. S. Mueller, J. Pallauf and M. B. Krawinkel (2010). "Antidiabetic effects of bitter gourd extracts in insulin-resistant db/db mice." British journal of nutrition **104**(11): 1613-1620.

Ko, K.-D. (1999). "Response of cucurbitaceous rootstock species to biological and environmental stresses." PhD Diss., Seoul Nat'l Univ., Korea.

Koike, S. T., P. Gladders and A. Paulus (2006). Vegetable diseases: A colour handbook, CRC Press.

Koomneef, M., L. Bentsink and H. Hilhorst (2002). "Seed dormancy and germination." Current Opinion in Plant biology **5**(1): 33-36.

Kotuby-Amacher, J., R. Koenig and B. Kitchen (2000) "SALINITY AND PLANT TOLERANCE." Utah State University extension.

Koutsika-Sotiriou, M. and E. Traka-Mavrona (2002). "The cultivation of grafted melons in Greece, current status and prospects." Acta Horticulturae: 325-330.

Kubola, J. and S. Siriamornpun (2008). "Phenolic contents and antioxidant activities of bitter gourd (Momordica charantia L.) leaf, stem and fruit fraction extracts in vitro." Food Chemistry **110**(4): 881-890.

Kubota, C., M. A. McClure, N. Kokalis-Burelle, M. G. Bausher and E. N. Rosskopf (2008). "Vegetable grafting: History, use, and current technology status in North America." HortScience **43**(6): 1664-1669.

Läuchli, A. and S. R. Grattan (2007). "Plant Growth And Development Under Salinity Stress." Advances in Molecular Breeding Toward Drought and Salt tolerant Crops: 1-32.

Lee, J.-M., C. Kubota, S. Tsao, Z. Bie, P. H. Echevarria, L. Morra and M. Oda (2010). "Current status of vegetable grafting: Diffusion, grafting techniques, automation." Scientia Horticulturae **127**(2): 93-105.

Lee, J.-M., C. Kubota, S. J. Tsao, Z. Bie, P. H. Echevarria, L. Morra and M. Oda (2010). "Current status of vegetable grafting: Diffusion, grafting techniques, automation." Scientia Horticulturae **127**(2): 93-105.

Lee, S.-G. (2006). Production of high quality vegetable seedling grafts. XXVII International Horticultural Congress-IHC2006: Global Horticulture: Diversity and Harmony, an Introduction to IHC2006 759.

Leo Sabatino, G. I., Fabio D'Anna, Eristanna Palazzolo, Giuseppe mennella, Giuseppe L. Rotino (2017). "Hybrids and allied species as potential rootstocks for eggplant: Effect of grafting on vigour, yield and overall fruit quality traits." Science Horticulturae **228**: 81-90.

Leoni, S., R. Grudina, M. Cadinu, B. Madeddu and M. Carletti (1989). The influence of four rootstocks on some melon hybrids and a cultivar in greenhouse. II International Symposium on Protected Cultivation of Vegetables in Mild Winter Climates 287.

Liao, C. T. and C. H. Lin (1996). "Photosynthetic responses of grafted bitter melon seedlings to flood stress." Environmental and Experimental Botany **36**(2): 167-172.

Liu, J.-Q., J.-C. Chen, C.-F. Wang and M.-H. Qiu (2009). "New cucurbitane triterpenoids and steroidal glycoside from Momordica charantia." Molecules **14**(12): 4804-4813.

Lombello, R. A. and C. l. A. F. Pinto-Maglio (2007). "Cytomolecular Studies in Momordica charantia L. (Cucurbitaceae), a Potential Medicinal Plant." The Japan Mendel Society **72 (4)**(Cytologia): 415-418.

Louws, F. J., C. L. Rivard and C. Kubota (2010). "Grafting fruiting vegetables to manage soilborne pathogens, foliar pathogens, arthropods and weeds." Scientia horticulturae **127**(2): 127-146.

Lowell P. and Moore K. (1971). "A comparative study of the role cotyledon in seedling development." Journal of Experimental Botany 22 (70):153-162.

Lovatt, C. J. (1999). "Timing Citrus and Avocado Foliar Nutrient Applications to Increase Fruit Set and Size." Hort Technology **9**(4): 607-612.

Lu, A. and C. Jeffre. (2011). Flora of China. Beijing, Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis.

Luthria, D. L., SudarsanMukhopadhyay and D. T.Krizek (2006). "Content of total phenolics and phenolic acids in tomato (Lycopersicon esculentum Mill.) fruits as influenced by cultivar and solar UV radiation." Food Composistion and Analysis **19**(8): 771-777.

M. Rai, S. P., and S. Kumar (2008). Cucurbit research in India: a retrospect. Cucurbitaceae 2008, Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Avignon (France).

Magan, J. J., M. Gallardo, R. B. Thopson and P. Lorenzo (2008). "Effects of salinity on fruit yield and quality of tomato grown in soil-less culture in greenhouses in Mediterranean climatic conditions." Agricutural Water Management **95**(9): 1041-1055.

Manera, F. J., P. Legua, P. Melgarejo, J. M. Brotons, F. Hernández and J. J. Martínez (2013). "Determination of a colour index for fruit of pomegranate varietal group "Mollar de Elche"." Scientia Horticulturae **150**: 360-364.

Marsic, N. K. and J. Osvald (2004). "The influence of grafting on yield of two tomato cultival (*Lycopersicon esculentum* Mill.) grown in a plastic house." Acta Agriculturae Slovenica **83**(2): 243-249.

Masayuki, O., T. Kenkoh and S. Hidekazu (1993). "Effect of Hypocotyl Morphology on Survival Rate and Growth of Cucumber Seedlings Grafted on Cucurbita spp." Japan Agricultural Research Quarterly **26**: 259-259.

McLellan, M. R., R. L. Lind and R. W. Kime (1995). "Hue angle determinations and statistical analysis for multiquadrant hunter L, a, b data." Food quality **18**(3): 235-240.

Metternicht, G. I. and J. A. Zinck (2003). "Remote sensing of soil salinity: potentials and constraints." Remote Sensing of Environment **85**(1): 1-20.

Mitchell, J. P., C. Shennan, S. R. Grattan and D. M. May (1991). "Tomato Fruit Yields and Quality under Water Deficit and Salinity." Journal of the American Society for Horticultural Science **116(2)**: 215-222.

Moncada, A., A. Miceli, F. Vetrano, V. Mineo, D. Planeta and F. D'Anna (2013). "Effect of grafting on yield and quality of eggplant (< i> Solanum melongena</i> L.)." Scientia Horticulturae **149**: 108-114.

Moorman, G. and S. Kim (2004). "Species of Pythium from greenhouses in Pennsylvania exhibit resistance to propamocarb and mefenoxam." Plant Disease **88**(6): 630-632.

Morgan, W. and D. Midmore (2002). "Bitter melon in Australia." RIRDC Publication (02/134).

Mowat, A. and R. Collins (2000). "Consumer behaviour and fruit quality: supply chain management in an emerging industry." An International Journal **5**(1): 45-54.

Munns, R. and M. Tester (2008). "Mechanisms of Salinity Tolerance." Annual Review of Plant Biology **59**: 651-681.

Nagarani G., Abirami A., and Siddhuraju P.(2014) "Food prospects and nutraceutical attributes of Momordica species: A potential tropical bioresources – A review." Food Science and Human Wellness 3: 117-126.

Navarro, J.M, Botella, M.A, Martinez, V. (2015). "Yield and fruit quality of melon plants grown under saline conditions in relation to phosphate and calcium nutrition." The Journal of Horticultural Science and Biotechnology **74**(5): 573-578.

Nerson, H. (2007). "Seed production and germinability of Cucurbit crops." Seed science and Biotechnology **1**(1): 1-10.

Nguyen, V. T., Q. V. Vuong, M. C. Bowyer, I. A. van Altena and C. J. Scarlett (2015). "Effects of different drying methods on bioactive compound yield and antioxidant capacity of *Phyllanthus amarus*." Drying Technology **33**: 1006-1017.

Nieves Ferna'ndez-Garcia, V. M. n., and Miceala Carvajal (2004). "Effect of salinity on growth, mineral composition, and water relations of grafted tomato plants." Journal Plant Nutrien **167**: 616-622.

Nisini, P. T., G. Colla, E. Granati, O. Temperini, P. Crino and F. Saccardo (2002). "Rootstock resistance to fusarium wilt and effect on fruit yield and quality of two muskmelon cultivars." Scientia Horticulturae **93**(3): 281-288.

Oda, M. (2006). Vegetable seedling grafting in Japan. XXVII International Horticultural Congress-IHC2006: Global Horticulture: Diversity and Harmony, an Introduction to IHC2006 759.

Okoli, B. E. (1984). "Wild and cultivated cucurbits in Nigeria." Economic Botany **38**(3): 350-357.

Oronje, M. L., M. Hagen, M. Gikungu, M. Kasina and M. Kraemer (2012). "Pollinator diversity, behaviour and limitation on yield of karela (Momordica charantia L. Cucurbitaceae) in Western Kenya." African Journal of Agricultural Research **7**(11): 1629-1638.

Palada, M. and L. Chang (2003). "Suggested cultural practices for bitter gourd." AVRDC International Cooperators' Guide: 03-547.

Palada, M. C. and L. C. Chang (2003). "Suggested cultural practices for bitter gourd." AVRDC International Cooperators' Guide **3**(547): 1-5.

Patel, R., N. Mahobia, N. Upwar, N. Waseem, H. Talaviya and Z. Patel (2010). "Analgesic and antipyretic activities of Momordica charantia Linn. fruits." Journal of advanced pharmaceutical technology & research **1**(4): 415.

Pavlou, G. C., D. J. Vakalounakis and E. K. Ligoxigakis (2002). "Control of root and stem rot of cucumber, caused by Fusarium oxysporum f. sp. radicis-cucumerinum, by grafting onto resistant rootstocks." Plant Disease **86**(4): 379-382.

Petropoulos, S. A., E. M. Khah and H. C. Passam (2012). "Evaluation of rootstocks for watermelon grafting with reference to plant development, yield and fruit quality." Int. J. Plant Prod **6**(4): 481-492.

Pham, T. V. (2001). Nghien cuu thuoc dieu tri benh dai thao duong tu qua cua cay muop dang - Momordica charantia L. Doctor Pharmacy, National Institute of Medicinal Materials [NIMM].

Pham, T. V. and T. V. Nguyen (1999). "Mot so ket qua nghien cuu buoc dau ve mat thuc vat cua cay muop dang trong o Viet Nam [Botanical studies on bitter melon growing in Vietnam]." Tap Chi Duoc Lieu [Journal of Materia Medica - Hanoi] **IV**.

Pham, T. V., M. K. Pham, N. T. Doan, D. T. Nguyen and T. K. Vu (2011). "Nghien cuu thanh phan hoa hoc cua cay muop dang va chung minh tac dung ha duong mau cua nhom glycosid tren tho gay dai thao duong [Chemical Composition of Momordica charantia L. and Hypoglycemic Effect of Its Glycosides on Alloxan-diabetic Rabbits]." Tap Chi Duoc Lieu [Journal of Materia Medica - Hanoi] **VI**: 48-54.

Pina, A. and P. Errea (2005). "A review of new advances in mechanism of graft compatibility–incompatibility." Scientia Horticulturae **106**(1): 1-11.

Proietti, S., Y. Rouphael, G. Colla, M. Cardarelli, M. De Agazio, M. Zacchini, E. Rea, S. Moscatello and A. Battistelli (2008). "Fruit quality of mini-watermelon as affected by grafting and irrigation regimes." Journal of the Science of Food and Agriculture **88**(6): 1107-1114.

Pogonyi, A., Z. Pek, L. Helyes and A. Lugasi (2005). "Effect of grafting on the tomato's yield, quality and main fruit components in spring forcing." Acta Alimentaria **34**(4): 453-462.

Qing, Z., B. Ji and M. Zudeb (2017). "Predicting soluble solid content and firmness in apple fruit by means of laser light backscattering image analysis." Food Engineering **82**(1): 58-67.

Raman, A. and C. Lau (1996). "Anti-diabetic properties and phytochemistry of< i> Momordica charantia</i> L.(Cucurbitaceae)." Phytomedicine **2**(4): 349-362.

Rengasame, P. (2006). "World salinization with emphasis on Australia." Journal of Experimental Botany **57**(5): 1017–1023.

Rengasamy, P. (2010). "Soil processes affecting crop production in salt-affected soils." Functional Plant Biology **37(3)**: 613-620

Ribeiro, J. N. S. and C. S. B. Costa (2015). "The effect of temperature regulation on seed germination of the tropical tree Myrsine parvifolia A. DC near its southern limit." South African Journal of Botany **98**: 128-133.

Rivero, R. M., J. M. Ruiz and L. Romero (2003). "Role of grafting in horticultural plants under stress conditions." Journal of food agriculture and environment **1**: 70-74.

Rizvi, S. I. and N. Mishra (2013). "Traditional Indian medicines used for the management of diabetes mellitus." Journal of diabetes research **2013**.

Rouphael, Y., D. Schwarz, A. Krumbein and G. Colla (2010). "Impact of grafting on product quality of fruit vegetables." Scientia Horticulturae **127**(2): 172-179.

Roy J. S, Negrão S. and M. Tester (2014) “Salt resistant crop plants” ScienDirect 26: 115-124.

Saeed F., Afsaal M., Niaz B., Arshad M. U., Tufail. T, Hussain M. B. and Javed A. (2018) “Bitter melon (Momordica charantia) a natural healthy vegetable. International Journal of Food Properties 21 (1): 1270-1290.

Sakata, Y., T. Ohara and M. Sugiyama (2005). The history and present state of the grafting of cucurbitaceous vegetables in Japan. III International Symposium on Cucurbits 731.

Santa-Cruz, A., M. M. Martinez-Rodriguez, F. Perez-Alfocea, R. Romero-Aranda and M. C. Bolarin (2002). "The rootstock effect on the tomato salinity response depends on the shoot genotype." Plant Science **162**(2002): 825-831.

Sathish Kumar, D., K. Vamshi Sharathnath, P. Yogeswaran, A. Harani, K. Sudhakar, P. Sudha and D. Banji (2010). "A medicinal potency of Momordica charantia." International Journal of Pharmaceutical Sciences Review and Research **1**(2): 95-100.

Schaefer, H. and S. S. Renner (2010). "A three-genome phylogeny of Momordica (Cucurbitaceae) suggests seven returns from dioecy to monoecy and recent long-distance dispersal to Asia." Molecular Phylogenetics and Evolution **54**(2): 553-560.

Schaefer, H. and S. S. Renner (2011). "Phylogenetic relationships in the order Cucurbitales and a new classification of the gourd family (Cucurbitaceae)." Taxon **60**(1): 122-138.

Schwarz, D., Y. Rouphael, G. Colla and J. H. Venema (2010). "Grafting as a tool to improve tolerance of vegetables to abiotic stresses: Thermal stress, water stress and organic pollutants." Scientia Horticulturae **127**(2): 162-171.

Seiwa, K. (2000). "Effects of seed size and emergence time on tree seedling establishment: importance of developmental constraints." Oecologia **123**(2): 208-215.

Semiz, A. and A. Sen (2007). "Antioxidant and chemoprotective properties of Momordica charantia L.(bitter melon) fruit extract." African journal of Biotechnology **6**(3).

Shrivastava, P. and R. Kumar (2015). "Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation." Saudi Journal of Biological Sciences **22**(2): 123-131.

Singh, P. K. and K. M. Rao (2014). "Role of grafting in cucurbitaceous crops." Agricultural Reviews **35**(1): 24-33.

Singh, S. K., B. Conde and M. Hodda (2012). "Root-Knot Nematode (Meloidogyne incognita) on Bitter Melon (Momordica charantia) near Darwin, Australia." Australasian Plant Disease Notes **7**(1): 75-78.

Sivritepe, N., H. O. Sivretepe and A. Eris (2003). "The effects of NaCl priming on salt tolerance in melon seedling grown under salin conditions." Science Horticulturae **97**(3&4): 229-237.

Soladoye, M., E. Chukwuma and F. Owa (2012). "An 'Avalanche'of Plant Species for the Traditional Cure of Diabetes mellitus in South-Western Nigeria." J. Nat. Prod. Plant Resour **2**(1): 60-72.

Soyler D. and Khawar K. M. (2007). Seed Germination of Caper (Capparis ovata var. Herbacea) Using α Naphthalene Acetic Acid and Gibberellic Acid. *International Journal of Agriculture & Biology, 09*(1), 35-37.

Stefania De Pascale, A. M., Vincenzo Fogliano, Patrizia Ambrosino & Alberto Ritieni (2001). "Irrigation with saline water improves carotenoids content and antioxidant activity of tomato " The Journal of Horticultural Science and Biotechnology **67** (4): 447-453.

Tan, M.-J., J.-M. Ye, N. Turner, C. Hohnen-Behrens, C.-Q. Ke, C.-P. Tang, T. Chen, H.-C. Weiss, E.-R. Gesing and A. Rowland (2008). "Antidiabetic activities of triterpenoids isolated from bitter melon associated with activation of the AMPK pathway." Chemistry & biology **15**(3): 263-273.

Tan, S. P., T. C. Kha, S. E. Parks and P. D. Roach (2016). "Bitter melon (Momordica charantia L.) bioactive composition and health benefits: A review." Food Reviews International **32**(2): 181-202.

Tan, S. P., S. E. Parks, C. E. Stathopoulos and P. D. Roach (2014). "Greenhouse-grown bitter melon: production and quality characteristics." J Sci Food Agric **94**: 1896-1903.

Tan, S. P., S. E. Parks, C. E. Stathopoulos and P. D. Roach (2014). "Extraction of Flavonoids from Bitter Mellon." Food and Nutrition Sciences **5**: 458-465.

Tan, S. P., S. E. Parks, C. E. Stathopoulos and P. D. Roach (2014). "Greenhouse-grown bitter melon: production and quality characteristics." Journal of the Science of Food and Agriculture **94**(9): 1896-1903.

Tan, S. P., Q. V. Vuong, C. E. Stathopoulos, S. E. Parks and P. D. Roach (2014). "Optimized Aqueous Extraction of Saponins from Bitter Melon for Production of a Saponin-Enriched Bitter Melon Powder." Journal of food science **79**(7): E1372-E1381.

Taylor, L. (2002). Technical Data Report for Bitter Melon (Momordica charantia). Austin, Sage Press, Inc.

Thaipong, K., U. Boonprakob, K. Crosby, L. Cisneros-Zevallos and D. H. Byrne (2006). "Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts." Journal of Food Composition and Analysis **19**: 669-675.

Thiruvengadam, M., N. Praveen and I.-M. Chung (2012). "In vitro regeneration from internodal explants of bitter melon (Momordica charantia L.) via indirect organogenesis." African Journal of Biotechnology **11**(33): 8218-8224.

Tho, N., N. Vromant, N. T. Hung and L. Hens (2008). "Soil salinity and sodicity in a shrimp farming coastal area of the Mekong Delta, Vietnam." Environmental Geology **54**(8): 1739-1746.

Thomas, T. D. (2008). "The effect of in vivo and in vitro applications of ethrel and GA3 on sex expression in bitter melon (Momordica charantia L.)." Euphytica **164**(2): 317-323.

Tien, B. N. (2005). Danh Luc Cac Loai Thuc Vat Viet Nam [The List of plants in Vietnam]. Hanoi, Nha Xuat Ban Khoa Hoc Ky Thuat.

Tien, N. M. (2010). Đất mặn và đất phèn Việt Nam. Hà Nội, Nhà xuất bản Nông nghiệp.

Traka-Mavrona, E., M. Koutsika-Sotiriou and T. Pritsa (2000). "Response of squash (Cucurbita spp.) as rootstock for melon (Cucumis melo L.)." Scientia Horticulturae **83**(3): 353-362.

Treeby, M. T., R. E. Henriod, K. B. Bevington, D. J. Milne and R. Storey (2007). "Irrigation management and rootstock effects on navel orange [Citrus sinensis (L.) Osbeck] fruit quality." Agricultural Water Management **91**(1): 24-32.

Turhan A., Ozmen N., Kuscu H., Serbeci M. S., and Seniz V. (1012) "Influence of rootstocks on yield and fruit characteristics and quality of watermelon" Horticulture, Environment and Biotechnology53 (4): 336-341.

Van Tang Nguyen, J. A. S. C. J. S. (2017). "Physicochemical, Antioxidant, and Cytotoxic Properties of Xao Tam Phan (Paramignya trimera) Root Extract and Its Fractions." Chemistry and Biodiversity **14**: 1-9.

Vo, C. V. (2012). Tu Dien Cay Thuoc Viet Nam [A Dictionary Medicinal Plants of Vietnam]. Ho Chi Minh City, Nha Xuat Ban Y Hoc.

Voutsela, S., G. Yarsi, S. A. Petropoulos and E. M. Khan (2012). "The effect of grafting of five different rootstocks on plant growth and yield of tomato plants cultivated outdoors and indoors under salinity stress " African Journal of Agricultural Research **7**(41): 5553-5557.

Vrcek, I. V., V. Samobor, M. Bojic, M. Medic-Saric, M. Vukobratovic, R. Erhatic, D. Horvat and Z. Matotan (2011). "The effects of grafting on the antioxidant properties of tomato (Solanum lycopersicum L.)." Spanish Journal of Agriculturea Research **9 (3)**.

Vuong, Q. V., S. Hirun, P. D. Roach, M. C. Bowyer, P. A. Phillips and C. J. Scarlett (2013). "Effect of extraction conditions on total phenolic compounds and antioxidant activities of *Carica* papaya leaf aqueous extracts." Journal of Herbal Medicine **3**(3): 104-111.

Vuong, Q. V., N. Zammit], B. R. Munro, S. Murchie, M. C. Bowyer and C. J. Scarlett (2015). "Effect of Drying Conditions on Physicochemical and Antioxidant Properties of *Vitex agnus-castus* Leaves " Food Processing and Preservation **39**(6): 2562-2571.

Walters, T. W. and D. S. Decker-Walters (1988). "Balsam-pear (Momordica charantia, Cucurbitaceae)." Economic Botany: 286-288.

Wang, H. Y., C. L. Chen and J. M. Sung (2003). "Both warm water soaking and matriconditioning treatments enhance anti-oxidation of bitter gourd seeds germinated at sub-optimal temperature." Seed science and technology **31**(1): 47-56.

Xiangbo, C., Z. Wullui, L. Hailong, X. Chen, W. Zhong and H. Li (1998). "The test of grafting bitter gourd on Luffa." China Veg **3**: 19-21.

Xingxue, H., W. Bincai, Z. Guolin, Z. Jin, W. Aihua and D. Yaohua (2013). "Effects of Grafting on Quality of Momordica charantia." Hubei Agricultural Sciences **4**: 025.

Xingxue, H., Z. Guolin, Z. Jin, W. Bincai and L. Chufa (2012). "Effects of Different Rootstocks and Grafting Methods on Growth of Momordica charantia." Journal of Changjiang Vegetables **6**: 009.

Xu X., Shan B., Liao C-H., Xie J-H., Wen P-W. and Shi J-Y. (2015). "Anti-diabetic properties of Momordica charantia L. polysaccharide in alloxan-induced diabetic mice" International Journal of Biological Macromolecules 81: 538 – 543.

Yamaguchi, T. and E. Blumwald (2005). "Developing salt-tolerant crop plants: challenges and oppotunities." Trends in Plant Science **10**(12).

Yetisir, H. and N. Sari (2003). "Effect of different rootstock on plant growth, yield and quality of watermelon." Animal Production Science **43**(10): 1269-1274.

Yetisir, H. and N. Sari (2003). "Effect of Hypocotyl Morphology on Survival Rate and Growth of Watermelon Seedlings Grafted on Rootstocks with Different Emergence Performance at Various Temperatures." Academic Journals **28**(2004): 231-237.

Yetışır, H., N. Sari and S. Yücel (2003). "Rootstock resistance to Fusarium wilt and effect on watermelon fruit yield and quality." Phytoparasitica, **31**(2): 163-169.

Yetisir, H. and V. Uygur (2010). "Responses of grafted watermelon onto different gourd species to salinity stress." Journal of plant nutrition **33**(3): 315-327.

Yoshioka, Y., H. Horie, M. Sugiyama and Y. Sakata (2009). "Quantifying cucumber fruit crispness by mechanical measurement." Breeding Science **59**(2): 139-147.

Yuan, B. Z., J. Sun and S. Nishiyama (2004). "Effect of Drip Irrigation on Strawberry Growth and Yield inside a Plastic Greenhouse." Biosystems Engineering **87**(2): 237-245.

Yurtseven, E., G. D. Kesmez and A. Ünlükara (2005). "The effects of water salinity and potassium levels on yield, fruit quality and water consumption of a native central anatolian tomato species (Lycopersiconesculantum)." Agricultural Water Management **78**(1-2): 128-135.

Zehatab-Salmasi, S. (2006). "Study of cardinal temperatures for pumpkin (*Cucurbita pepo*) seed germination." Journal of Agronomy **5**(1): 95-97.

Zhang, Z. K., Y. Zhang, Z. J. Huang, S. H. Liu and S. Q. Liu (2010). "Effects of grafting on cucumber rhizospheric soil microbial characteristics and enzyme activities under copper stress." Ying Yong Sheng Tai Xue Bao **21**(9): 2317-2322.

Zhen Dong, Chen Jia Zuo, Liang Jiang Lü, Che Rong Ping, Wang Xiao Feng, Chen Ru Kui and Huang (2013). "Effects of wild luffa rootstocks on fruit quality and photosynthetic physiology of bitter gourd." Journal of Southern Agriculture **44**(7): 1095-1099.

ZhenDong, C., L. JiaZuo, C. JiangLü, W. RongPing, C. XiaoFeng and H. RuKui (2013). "Effects of wild luffa rootstocks on fruit quality and photosynthetic physiology of bitter gourd." Journal of Southern Agriculture **44**(7): 1095-1099.

Zhu, Y., Y. Dong, X. Qian, F. Cui, Q. Guo, X. Zhou, Y. Wang, Y. Zhang and Z. Xiong (2012). "Effect of superfine grinding on antidiabetic activity of bitter melon powder." International journal of molecular sciences **13**(11): 14203-14218.