THE EFFECT OF SALINITY ON THE GERMINATION AND EARLY SEEDLING GROWTH OF WINGED BEANS (*Psophocarpus tetragonolobus* (L.) DC.)

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ABSTRACT

The winged bean has been considered as a traditional vegetable but has the potential to be commercialized in the Malaysian food market. Salinity is one of the major problems that decreases the production of crops throughout the world. An experiment was conducted in Faculty of Sustainable Agriculture, Universiti Malaysia Sabah to evaluate the effect of salinity on the germination parameters and early seedling growth of winged beans under different salinity conditions. The treatments consist of six different levels of salinity which consists of 0%, 0.25%, 0.50%, 1.0%, 2.0% and 4.0% of NaCl. The NaCl powder is diluted in 1L of distilled water. The seeds were germinated in a germination box and uses coarse sand as the planting medium. Each germination box was irrigated daily with treatment solutions as required by the plants. Result was taken at the 10^{th} day after sowing. The treatments were arranged in a Completely Randomized Design manner with three replicates each. The parameters observed in this study are germination percentage, seed vigor index (SVI), length of plumule, length of radicle and number of leaves. This study showed that there were significant different between all the parameters of the winged bean to the different salinity levels. Winged bean seeds treated with 0% NaCl resulted in the highest means with values of 68% for germination percentage, 38.5 for SVI, 0.87 for number of leaves, 56.78mm for plumule length and 43.06mm for radicle length ($p<0.01$). This study also exposed that as the salinity increases, the mean values for germination percentage and early seedling growth parameters decreased. Also, the winged beans were able to survive up until the T3 (0.50% NaCl solution) even though they resulted with the least germination percentage 7.67%. Winged bean seeds treated with T4 onwards did not germinate and did not show any growth. Therefore, it can be concluded that winged bean are able to survive up to 0.5% NaCl solution and salinity level beyond that is unsuitable for growing winged bean.

Key word: Winged bean, salinity, germination, seed vigor index

KAJIAN MENGENAI KESAN KEMASINAN TERHADAP PERCAMBAHAN DAN PERTUMBUHAN AWAL BIJI BENIH KACANG BOTOL (*Psophocarpus tetragonolobus* (L.) DC.)

ABSTRAK

Kacang botol telah dikategorikan sebagai sayur tradisional dan mempunyai potensi untuk di komersialkan di pasaran makanan Malaysia. Kemasinan merupakan salah satu masalah besar yang telah mengurangkan jumlah produksi tumbuh-tumbuhan di seluruh dunia. Satu kajian telah diadakan di Fakulti Pertanian Lestari, Universiti Malaysia Sabah bagi menilai kesan kemasinan terhadap parameter percambahan dan pertumbuhan awal biji benih kacang botol di bawah kondisi kemasinan yang berbeza. Rawatan yang diberikan terhadap biji benih kacang botol adalah sebanyak enam tahap kemasinan yang berbeza iaitu 0%, 0.25%, 0.50%, 1.0%, 2.0% dan 4.0% NaCl. Serbuk NaCl dilarutkan ke dalam 1L air suling. Biji benih kacang botol dicambah dalam kotak percambahan dan menggunakan pasir kasar sebagai medium tanaman. Setiap kotak percambahan disiram setiap hari menggunakan rumusan rawatan dalam kuantiti yang diperlukan oleh biji benih tersebut. Rawatan ini akan diatur secara rekabentuk penuh rawak dan mempunyai tiga replikasi. Parameter yang di perhatikan dalam kajian ini adalah peratus percambahan, indeks tenaga biji benih, panjang pucuk (plumul), panjang akar (radikel) dan bilangan daun. Kajian ini menunjukkan bahawa kacang botol yang di beri rawatan kemasinan 0% memberi keputusan purata yang tertinggi dengan nilai 68% bagi peratus percambahan, 38.5 bagi indeks tenaga biji benih, 0.87 bagi bilangan daun, 56.78mm bagi panjang plumul dan 43.06mm bagi panjang radikel ($p<0.01$). kajian ini juga menunjukkan bahawa apabila tahap kemasinan meningkat, nilai purata bagi peratus percambahan parameter pertumbuhan awal biji benih kacang botol berkurang. Kacang botol mampu bertahan sehingga rawatan T3 (0.50% rumusan NaCl) walaupun mempunyai keputusan peratus percambahan yang paling sedikit (7.67%). Kacang botol yang dirawat dengan T4 dan seterusnya tidak bercambah dan tidak tumbuh. Kesimpulannnya, kacang botol dapat bertahan pada tahap kemasinan sebanyak 0.5% NaCl dan tahap kemasinan lebih daripada itu tidak sesuai bagi pertumbuhan kacang botol.

Kata kunci: Kacang botol, kemasinan, percambahan, indeks vigor biji benih

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LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

%	Percentage
ANOVA	Analysis of Variance
Cl^-	Chloride ion
Corp.	Corporation
CRD	Completely Randomized Design
FSA	Faculty of Sustainable Agriculture
g/mol	gram per mol
GP	germination percentage
ISTA	International Seed Testing Association
L	litre
m	metre
M	molar mass
mL	milli litre
mM	milli Molar
mm	millimeter
Na	Sodium
NaCl	Sodium chloride
NaOCl	Sodium hypochlorite
SAS	Statistical Analysis System
Sdn. Bhd.	Sendirian Berhad
SVI	seed vigour index
t/ha	tonne per hectare
UMS	Universiti Malaysia Sabah

LIST OF FORMULAE

$$\frac{x}{(x+y)} \text{X } 100 = \% \text{ concentration of NaCl Solution}$$

where,

x = amount of NaCl needed (g)

y = amount of water needed (g)

CHAPTER 1

INTRODUCTION

1.1 Background

Winged bean which is scientifically identified as the *Psophocarpus tetragonolobus* (L.) DC., are locally known as "kacang botol". To date, winged bean is considered as the traditional or indigenous vegetable but has the potential to be commercialized. It is also considered as the underutilized pulse crops of Asia, according to a global research on underutilized crops by Williams and Haq (2000). Underutilized species are unpopular and rarely consumed. They are normally undervalued consumer preference compared to introduced, exotic fruits and vegetables (Rubaihaiyo, 2002). However, winged bean is an essential crop in Asia and in the Western Pacific (Eagleton, 2002). There is no clear fact on the origin of winged bean but it is believed to be from the parts of Africa (Madagascar or Mauritius) and later spread to Asia (Natural Resources Institute, 1987).

The access towards continuous supply of winged bean in Malaysia is very limited. Its mass production is highly constrained because of its planting method which requires trellising. This increases the production cost of the winged beans (Rahman, 1998). Other problems of commercially cultivating the winged beans are shortage of seed supplies and the genetic variability of the existing supplies (Natural Resources Institute, 1987). However, the production of winged bean should be commercialised as Malaysia have the suitable agroclimatic condition to cultivate them. The quantity of imported vegetables in Malaysia increased about 16,000 metric tonne from the year 2009 to 2010 (Unit Perangkaan, Jabatan Pertanian Malaysia, 2012). This figure can be further reduced by producing our own vegetables.

One of the programs of the Agro-Food Policy (2011-2020) is to ensure that there is enough food supply for Malaysia (Unit Perangkaan, Jabatan Pertanian Malaysia, 2012).

Different plants have different mechanism in response to salt stress. Among the crops that are able to produce an adequate amount of yields at higher salinity level are cotton and barley. This is possible because certain crops are able to make osmotic adjustments which enables them to extract more water from the saline soil (USDA-NRCS, 2011).

The most viable criteria in used in selecting salt tolerance in plants are germination and seedling characteristics. The germination percentage, rate and seedling growth are the most suitable criteria for selection of cultivars. Sodium chloride (NaCl) is the most commonly used treatment in a seed germination experiment (Khodarahmpour *et al.*, 2012). The NaCl induced stress stimulates the seed response towards salinity in the germination experiments. Toxic accumulation of NaCl in the plant tissues causes the ionic stress. As the concentration of NaCl increases, the germination rate will decrease (Murillo-Amador *et al.*, 2002).

1.2 Justification of Study

The increasing demand of the production of winged bean urges the study of winged bean cultivation through various aspects. By far, there are lack of research that studied the effect of salinity on the germination and early seedling growth of winged bean.

1.3 Objective

The objective of this study is to evaluate the effect of salinity on the germination parameters and early seedling growth of winged bean under different salinity conditions.

1.4 Hypothesis

The proposed hypothesis for this study are:

H_0: There is no significant difference on the germination parameters and early seedling growth of the winged bean under different salinity conditions.

H_a: There is a significant difference on the germination parameters and early seedling growth of the winged bean under different salinity conditions.

CHAPTER 2

LITERATURE REVIEW

2.1 *Psophocarpus tetragonolobus* (L.) DC

2.1.1 Botany

Psophocarpus tetragonolobus (L.) DC or commonly known as winged bean belongs in the family Leguminosae (Fabaceae). It is a creeping herbaceous perennial plant which can grow up to 2m high. The flowers of the winged bean are hermaphrodite and are usually pollinated by insects. They bears seeds that are rich in proteins and vitamins with tuberous roots which contains about 12 to 15 percent more protein than other root crops such as yam and cassava (National Academy of Sciences, 1975). The winged bean is cultivated mostly for its edible pods and tuberous roots. This plant grows in an environment with full sunlight exposure and the water requirement for planting this plant is moderate.

2.1.2 Morphology

The name winged bean is given due to its appearance. Figure 2.1 shows the morphological parts of winged bean. Winged bean is a plant which produces pea-like beans and the edges are shaped with four wings. It is usually harvested immature. Harvesting during this stage allows both the pods and the beans within to be eaten. The winged bean has a sweet flavour like other pea varieties and the leaves of the winged bean have a mild spinach-like flavour. The flowers of the winged bean are of different colours. They can be white, blue, red and yellow. It is also among the edible parts of the plant. The flower of the winged bean is said to taste like mushroom (Christman, 2003).

Figure 2.1 Illustrated parts of the winged bean.
Source: (Simeonsma and Piluek, 1993)

Winged bean is a climbing legume of South and Southeast Asia. It is also an indeterminate, perennial legume which requires system support in order for it to grow successfully and to produce more yields (Rahman, 1998). All parts of the winged bean are edible and some grows it for its beans and green pods (Eagleton, 2002).

2.1.3 Production of Winged Bean

Winged bean can be grown in any soil condition as long as it receives good drainage. It is a tropical crop in nature and grows thrivingly in the subtropical regions. However, winged bean plant is highly sensitive to photoperiod. It is best to grow winged bean at any place with day length of 11 to 13 hours (Salunkhe and Kadam, 1998). The winged bean seeds are planted in flat beds or on ridges at a distance of approximately 60cm. The distance between rows can be up to 60 to 120cm, depending on the cultivar and location.

Winged bean seedlings are able to show signs of twining a few weeks after the germination process. At this stage of growth, it is crucial for the vines to be supported either by staking or simply using a string (Rahman, 1998). Winged beans cultivated for tubers usually require pruning and removal of flower in order to increase the tuber yield.

The common diseases and pests that often attack the winged bean crop are false rust (caused by *Synchytrium psophocarpi*),leaf spot (caused by *Cercospora psophocarpi*), root-knot nematode (*Meloidogyne* spp.) and cowpea aphid (*Aphis craccivora*) (Salunkhe and Kadam, 1998).

The pods of winged beans are ready to be harvested after ten weeks of planting and can be continuously harvested for a few months. Each of the vines are able to produce around 25 pods per week and the yield of pods varies from 5 to 20 t/ha (Salunkhe and Kadam, 1998).

2.1.4 Nutritional Value and Beneficial Uses

The winged bean is an edible legume that is considered as a cash crop because almost all parts of the winged bean are edible and hence increases it marketable values. All parts of the winged beans are highly nutritious except for its stems and roots. They are also considered to be more palatable than soybean, a crop that is most frequently compared to winged beans (Natural Resources Institute, 1987). The most common characteristics of all parts of the winged bean is the relatively high protein content. However, protein is said to be the richest in the seeds and tubers (Board of Science and Technology for International Development, 1981).

The pods of winged bean trees are the most popular part of the plant. These pods were consumed in various ways from eating them raw or as salads into adding them into soups or curries. The winged bean pods is said to taste like green pea. The people of Papua New Guinea often steam the pods into oil drums or baked in an open fire as it is too fibrous to be eaten raw (Board of Science and Technology for International Development, 1981). The composition of nutrients in the immature pods does not differ significantly from that found in green pods of other leguminous plants and the average protein content is 2.4g per 100g of its edible portion. Table 2.1 shows the nutritional compositions of winged bean according to their parts and their ripeness level. Table 2.2 shows the mineral contents in different parts of winged beans.

Table 2.1 Nutrient composition of different parts of winged bean

	Flowers	Leaves	Immature Pods	Unripe Seeds	Ripe Seeds	Tubers
Water[a]	84.2-87.5	64.2-85.0	76.0-93.0	35.8-88.1	8.7-24.6	54.9-65.0
Energy (mJ)	0.17	0.20	0.19	0.10-0.71	1.61-1.89	0.63
Protein	2.8-5.6	5.0-7.6	1.9-4.3	4.6-10.7	29.8-39.0	3.0-15.0
Fat	0.5-0.9	0.5-2.5	0.1-3.4	0.7-10.4	15.0-20.4	0.4-1.1
Carbohydrate (total)	3.0-8.4	3.0-8.5	1.1-7.9	5.6-42.1	23.9-42.0	27.2-30.5
Fiber		3.0-4.2	0.9-3.1	1.0-2.5	3.7-16.1	1.6-17.0
Ash	0.8	1.0-2.9	0.4-1.9	1.0	3.3-4.9	0.9-1.7

[a]Values expressed as g per 100g fresh weight shows the rates reported by authors
Source: (National Academy of Science, 1981)

Table 2.2 Mineral Content in Different Parts of the Winged Beans[a]

	Leaves	Immature Pods	Ripe Seeds	Tubers
Potassium	80-436	205-381	1110-1800	550
Phosphorus	52-98	26-69	200-610	30-64
Sulfur	-	-	380	21
Calcium	113-260	53-330	80-370	25-40
Magnesium	54	58	110-255	23
Sodium	2.5-18	3.0-3.4	14-64	33
Iron	2.0-6.2	0.2-2.3	2.0-18.0	0.5-3.0
Manganese	1.5	2.2	4-25	10
Zinc	1.4	0.2	3.1-5	1.3
Copper	0.5	0.6	1.3	1.3

[a]Values expressed as g per 100g fresh weight shows the rates reported by authors
Source: (National Academy of Science, 1981)

Since winged bean is part of the Leguminosae family, it has the ability to fix nitrogen in the soil. This species has a symbiotic relationship with certain soil bacteria in which these bacteria will form nodules on the roots and fix the atmospheric nitrogen. The dried seed of the winged bean contains about 35% protein which is higher than that of soybean and the green pods are about 2% protein, raw leaves 5% and dried roots 25% (Christman, 2003). This allows them to be used as green manure in the intercropping system. In other agricultural fields, winged bean stems and leaves are used as forage for cattle (Christman, 2003).

According to a New York Times article by Brody (1982), winged bean is recognized as a potent 'weapon' against malnutrition. Also from the reported article, an Indonesian researcher once produced a substitute for coffee and tobacco by roasting the seeds of winged bean and drying the tobacco leaves respectively (Brody, 1982). In Indonesia, soy bean seeds were replaced by winged bean seeds in the production of local food called 'tempeh' and tofu. Due to its high protein content, winged bean 'milk' and flour are used as dietary treatments for children with lack of protein (Christman, 2003).

2.2 Salinity

Salinity is a measure of the content of salts in soil or water. Salt are highly soluble in both surface and groundwater and can be easily transported through the water movement. Excessive amount of salt in water supply or in the ground affects agricultural activities, drinking water system and the ecosystem health (Department of Sustainability, Environment, Water, Population and Communities, 2012).

2.2.1 Effect of Salinity on Plants

Salinity in soil usually occurs when soluble salts (NaCl) are elevated in soil and water. In the arid or semi-arid areas, natural processes such as weathering of mineral rocks is said to be among the reason why soil are affected with salt (Hannick, 2005). There is often inadequate rainfall or drainage that can mobilize the salt down through the soil so it is able to leach away from the plants. In addition to this, human intervention is also the cause of secondary salinity. Human activity that cuts down trees with deep roots catalyzes the rise of underground water table (as shown in Figure 2.2) and hence moving the salts up to the soil surface (Munns, 2002).

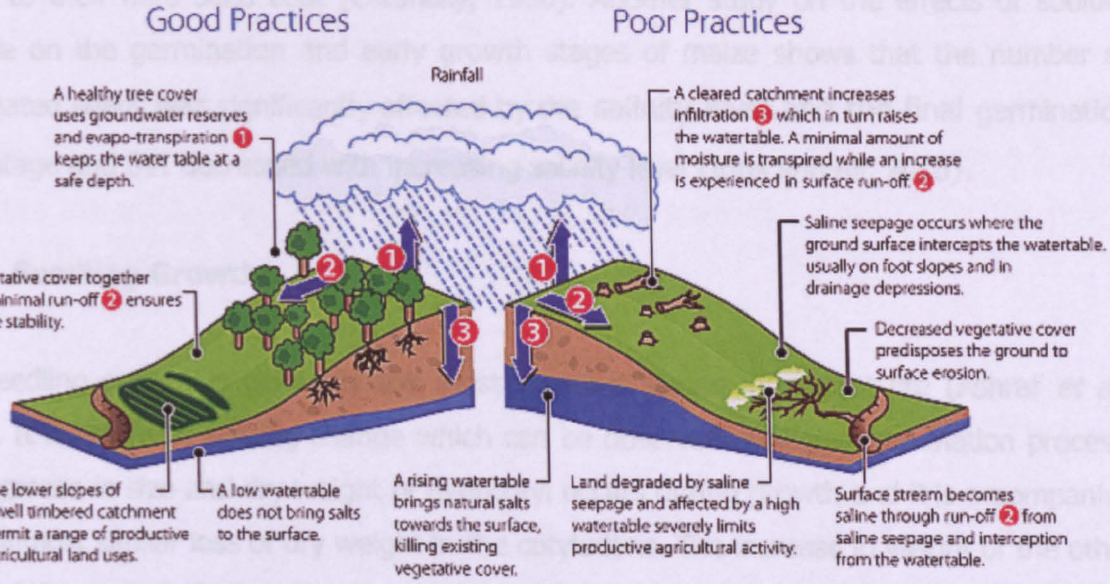

Figure 2.2 How deforestation affects the rising of underwater table.
Source: Blue Planet, 2015

Salt, when enters inside the plants excessively, causes ionic stress. This is because Na (and Cl^-) inhibits the metabolic processes including protein synthesis. Munns (2002) reported that Na can rise to toxic levels in older leaves and hence causing them to die. This will result in the reduction of available leaf area for photosynthesis and the plants will not be able to sustain growth or crop yield. Salt tolerance in plants is defined when the plant itself shows little growth reduction at concentrations of 300mM NaCl or more (Tester and Davenport, 2003). The effects of salinity depend on the species and on the stage of the plant's development (such as germination or vegetative growth) (Hannick, 2005).

2.3 Germination

The term germination describes a series of process which begins with the uptake of water by the inactive dry seed and terminates with the elongation of the embryonic axis (Bewley and Black, 1994). Among the visible sign of a complete germination is the penetration of structures surrounding the embryo by the radicle (Bewley, 1997).The winged bean seeds shows hypogeal type of germination in which the cotyledons remain below the ground. The cotyledon will later become the source of reserves for the seedling or receive materials from the endosperm (Mayer and Poljakoff-Mayber, 1982). Germination percentage is an estimate of the viability of a population of seeds (Saupe, 2009). According to a study by Csizinsky in 1980, the percentage of germination in winged bean seeds is very low. This is due to their hard seed coat (Csizinsky, 1980). Another study on the effects of sodium chloride on the germination and early growth stages of maize shows that the number of germinated seeds was significantly affected by the salinity level and the final germination percentage and SVI decreased with increasing salinity level (Idris and Ali, 2015).

2.4 Seedling Growth

The seedling growth is generally the most sensitive stages in a plant life (Ashraf *et al.*, 1986). It is the most striking change which can be observed after the germination process. The increase in size and dry weight of hypocotyl occurs during growth and it is accompanied by an almost similar loss of dry weight in the cotyledons. The increase in weight of the other parts of the embryo (epicotyl, plumule and radicle) begins later and takes place at a slower rate (Manha, 1998).

Therefore, the study of seed germination will affect the growth of the seedling. Further observations on the seedling growth are conducted in order to study in detail the effects of salinity towards the growth pattern of the seeds. Among the parameters included in observing the seedling growth are the length of the radicle and plumule.

2.5 Seedling Vigour Index (SVI)

In order to supplement germination and viability tests, seed vigour is an important quality parameter. It can be used to identify the performance of seed lot in a field or storage. The ISTA congress of 1977 defined seed vigour as "the sum total of those properties of the seed which determines the level of activity and performance of the seed or seed lot during germination and seedling emergence" (Hampton, 1993). The seed vigour index (SVI) is used to describe the vigour level of a seed lot. Seed lot with higher SVI is considered to be more vigorous (Abdul-Baki and Anderson, 1973).

2.6 Conclusion

In conclusion, the botany and morphology of the winged beans is reviewed to introduce the crop used in this study. The significance of winged bean in terms of its nutritional values and beneficial uses is reviewed in order to highlight the importance of winged beans to the society.

A gentle introduction towards salinity have been reviewed in order to provide a basic idea on how salinity occurred and its effects towards the plants. It is clear that there were lack of study on the effects of salinity on winged bean plants as most of the studies regarding salinity were carried on other crops. Some aspects on the effects of salinity on winged beans will be the germination percentage and seedling growth. Thus, the review strongly highlights the importance of this study.

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