EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI (AMF) INOCULUM ON VEGETATIVE GROWTH OF *Allium Cepa* var. *ascalonicum* L.

TAN SENG YIAP

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF AGRICULTURE SCIENCE WITH HONOURS

CROP PRODUCTION PROGRAMME
FACULTY OF SUSTAINABLE AGRICULTURE
UNIVERSITI MALAYSIA SABAH

2018

UMS
UNIVERSITI MALAYSIA SABAH

PUMS 99:1

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN TESIS

JUDUL: KESAN INOKULUM KULAT MYKORHIZA ARBUSCULAR (AMF) TERHADAP PERTUMBUHAN VEGETATIF *Allium cepa* var. *ascalonicum* L.

IJAZAH: DEGREE OF BACHELOR OF AGRICULTURE SCIENCE WITH HONOURS

SAYA: TAN SENG YIAP (HURUF BESAR) SESI PENGAJIAN: 2017/2018

Mengaku membenarkan tesis *(LPSM/Sarjana/Doktor Falsafah) ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:-

1. Tesis adalah hak milik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. Sila tandakan (/)

[ ] SULIT (Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di AKTA RAHSIA RASMI 1972)

[ ] TERHAD (Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)

[/] TIDAK TERHAD

Disahkan oleh:

(TANDATANGAN PENULIS)

Alamat Tetap: 3-1, Jalan Perak 3, Taman Bersatu, 81000, Kulai, Johor

NURULAIN BINTI ISMAIL
PUSTAKAWAN KANAN
UNIVERSITI MALAYSIA SABAH
(TANDATANGAN PUSTAKAWAN)

Associate Professor Dr. Kartini Saibeh
(NAMA PENYELIA)

TARIKH: 15-1-2018

TARIKH: 15 Januari 2018

Catatan:
*Potong yang tidak berkenaan.
*Jika tesis ini SULIT dan TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT dan TERHAD.
*Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana Secara Penyelidikan atau disertai bagi pengajian secara kerja kursus dan Laporan Projek Sarjana Muda (LPSM).

DECLARATION

I hereby declare that this dissertation is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that no part of this study has been previously or currently submitted for a degree at this or any other university.

TAN SENG YIAP
BR14110079
05 JANUARY 2018

VERIFIED BY

1. Associate Professor Dr. Kartini Saibeh
SUPERVISOR

Signature and stamp

ASSOC. PROF. DR. KARTINI SAIBEH
LECTURER DS54
FACULTY OF SUSTAINABLE AGRICULTURE
UMS SANDAKAN

ACKNOWLEDGEMENT

Foremost, I would like to express my deep and sincere gratitude to my research supervisor Assoc. Prof. Dr. Kartini Saibeh for giving me the opportunity to finish this dissertation. Her guidance helped me a lot in writing this thesis and I am thankful to have this great mentor for my degree study.

My thanks must go also to my ex-supervisor Assoc. Prof. Dr. Markus Atong throughout this study. I would never forget his encouragements, insightful comments, and guidance which have helped me tremendously in completing this dissertation.

On the other hand, I am extremely grateful to my parents for their loves, caring, understandings, continuing supports, and all their sacrifices in supporting me to complete this research work. I would like to express my special thanks to my friends Shirley Lee, Robin Lim and Jinn Yang who have supported me to complete the research work directly or indirectly.

ABSTRACT

An experiment was conducted to study the effect of arbuscular mycorrhizal fungi (AMF) inoculum on vegetative growth of *Allium cepa* var. *ascalonicum* L.. The experiment design used was completely randomized design (CRD). Data was analysed by using one-way analysis of variance (ANOVA) and the differences between means was tested by using Duncan's Multiple Range test. For the first part of this study, the AMF inoculums were prepared by using host plants namely *Eleusine indica* L., *Chloris barbata* Sw., and *Mimosa pudica* L.. *Zea mays* L. used as a trap plant. Significant ($p<0.05$) differences in the percentage of AMF colonization (%) and soil phosphorus concentration (ppm) were observed in *Z. mays*. AMF inoculum of *M. pudica* was found to be the most responsive as it showed to have highest percentage of AMF colonization (87.5±7.1 %) and lowest soil phosphorus concentration (21,742±3,674 ppm). For the second part of this study, there were no significant differences in plant height, root length, number of leaves and number of tillers of *Allium cepa* var. *ascalonicum* L. were observed in all treatments during week 1 to week 4. A significant ($p<0.05$) difference in vegetative growth response of *Allium cepa* var. *ascalonicum* L. were observed in fresh weight (88.0± 8.1 g), dry weight (80.3±7.53 g), percentage of AMF colonization (88.2±5.8 %) and soil phosphorus concentration (22,697±3,585 ppm) in *Allium cepa* var. *ascalonicum* L. during week 4. It can be concluded that the 1.0 kg AMF inoculum treatment was more efficient than other treatments in increasing the dry weight of *Allium cepa* var. *ascalonicum* L..

KESAN INOKULUM KULAT MYKORHIZA ARBUSCULAR (AMF) TERHADAP PERTUMBUHAN VEGETATIF *Allium cepa* var. *ascalonicum* L.

ABSTRAK

Satu kajian telah dijalankan untuk mengkaji kesan tentang penggunaan AMF inokulasi terhadap pertumbuhan vegetative *Allium cepa* var. *ascalonicum* L... Reka bentuk eksperimen yang digunakan adalah reka bentuk sepenuhnya rawak (CRD). Data dianalisis dengan menggunakan analisa varians satu arah (ANOVA) dan perbezaan antara cara diuji dengan menggunakan ujian Multiple Range Duncan. Dalam bahagian pertama kajian ini, inokulum AMF disediakan dengan menggunakan tumbuhan tuan rumah iaitu *Eleusine indica* L., *Chloris barbata* Sw., dan *Mimosa pudica* L.. *Zea mays* L. digunakan sebagai tumbuhan perangkap. Perbezaan ($p < 0.05$) yang signifikan dalam peratusan penjajahan AMF (%) dan kepekatan fosforus tanah (ppm) telah diperhatikan dalam *Z. mays*. Inokulum AMF *M. pudica* didapati majoriti responsif kerana ia menunjukkan peratusan tertinggi penjajahan AMF (87.5 ± 7.1%) dan kepekatan fosforus tanah adalah terendah (21,742 ± 3,674 ppm). Bagi bahagian kedua kajian ini, tidak terdapat perbezaan signifikan dalam ketinggian tumbuhan, panjang akar, bilangan daun dan bilangan penanam *Allium cepa* var. *ascalonicum* L. telah diperhatikan dalam semua rawatan sepanjang minggu 1 hingga minggu 4. Perbezaan signifikan ($p < 0.05$) dalam tindak balas pertumbuhan vegetatif *Allium cepa* var. *ascalonicum* L. telah diperhatikan dalam berat segar (88.0 ± 8.1 g), berat kering (80.3 ± 7.53 g), peratusan kolonisasi AMF (88.2 ± 5.8%) dan kepekatan fosforus tanah (22,697 ± 3,585 ppm) terhadap *Allium cepa* var. *ascalonicum* L. pada minggu 4. Kesimpulannya, rawatan inokulum AMF 1.0 kg adalah lebih berkesan dalam meningkatkan berat kering *Allium cepa* var. *ascalonicum* L. apabila berbanding dengan rawatan lain.

TABLE OF CONTENT

Content **Page**

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

%	Percentage
°C	Degree Celsius
AMF	Arbuscular Mycorrhizal Fungi
ANOVA	Analysis of Variance
cm	Centimeters
CRD	Completely Randomised Design
CT1	Control 1
Fe	Iron
FSA	Faculty of Sustainable Agriculture
g	Gram
HCL	Hydrochloric acid
Kg	Kilogram
KOH	Potassium Hydroxide
m^2	Square meter
ml	Milliliter
mm	Millimeter
MI1	Mycorrhizal Inoculum 1
MI2	Mycorrhizal Inoculum 2
MI3	Mycorrhizal Inoculum 3
N	Nitrogen
N_20	Nitrous oxide
NK1	NPK Treatment 1
P	Phosphorus
ppm	Parts per million
pH	Potential of Hydrogen
R1	Replicate 1
R2	Replicate 2
R3	Replicate 3
R4	Replicate 4
R5	Replicate 5
SPSS	Statistical Product and Service Solutions
UMS	Universiti Malaysia Sabah
Zn	Zinc

CHAPTER 1

INTRODUCTION

1.1 Background

Soil contains a lot of microorganisms which have an important role in improving the fertility of the soil. One of the soil profitable microorganisms is mycorrhizae. Mycorrhizae are mutualisms formed between fungi and plant roots which recognized as a group of soil fungi that infect the roots of most plants. It is about 95% of all the world's plant species form a mycorrhizal relationship with fungi and the plants would not survive without them (Jeffries *et al.*, 2003). The most common one is the arbuscular mycorrhizal fungi (AMF) (Newton *et al.*, 2010).

AMF as soil microorganisms which are obligate, symbionts and belong to the phylum Glomeromycota (Schüßler *et al.*, 2001). This type of fungi represents a key link between plants and soil mineral nutrient and they able to collect growing interest as a natural fertilizer. AMF form the mutualistic symbioses relationship with more than 80% of know land plant species and including of several agricultural crops. They have the role of providing the host plant with mineral nutrients and water, in exchange for produce photosynthetic products (Smith and Read, 2008). Crops benefit from AMF through enhanced uptake of nutrients with low mobility, especially phosphorus (Smith and Read, 2008).

AMF have their greatest effect when a host plant associated with them, which is of deficient in phosphorus (Koide, 1992). To get the maximum agricultural benefit, inoculation of the soil with a suitable type of AMF is necessary. Hence, the only way to culture and maintain AMF production, the use of AMF inoculum that is produced by additional application of these fungi should become commercially feasible. The plants that naturally facilitate the higher colonization of AMF can be generally considered to be used as trap plants (Koide, 1992). Trap plants to culture the AMF for inoculum practice with utilization as natural biofertilizer were selected in order to increase agricultural crops as well as to minimize the use of chemical fertilizer and thereby reduce the environmental pollution.

There are about 12 AMF inoculum producers in the European Union, with the producers in the United Kingdom, Czech Republic, Germany, Switzerland, Spain and France and more than 20 others worldwide (Vostáka *et al.*, 2008). Large-scale multiplication of AMF aiming to produce mycorrhizal inoculum for field applications is generally carried out in substrate-based (nursery beds, pots, poly bag, concrete tanks), substrate-free (i.e., aeroponic boxes) and in vitro systems. Commercial inoculum produced using these systems are available in several countries, especially in Asia and Europe. However, the costs associated with the technology of inoculum production, including the establishment of single cultures of AMF species, shipping and handling and development of the carrier substrate are borne by farmers and nursery owners making the technology expensive. Culturing AMF is conventionally labor-intensive, requiring large-scale production of plants in pots or nursery beds, from which the AMF inoculum can be harvested (IJdo *et al.*, 2011).

Allium cepa var. *ascalonicum* L. is a high-value vegetable crop for its popularity in many spicy dishes use as matured bulbs or as a green vegetable. *Allium cepa* var. *ascalonicum* L. is highly mycorrhizal-dependent plants. Previous studies do indicate that the *Allium* species is highly responsive to mycorrhization, resulting in improved plant growth and yield under normal as well as stressed conditions. A significant correlation between natural AMF colonization and *Allium* species yields in conventionally managed farmlands has recently been reported (GALVÁN *et al.* 2009). In conventional agriculture, large amounts of fertilizers are used to increase the yield of *Allium* species. However, large amounts of synthetic fertilizer are used in *Allium* species cultivation which resulted in susceptible to pests and diseases (GALVÁN *et al.* 2009).

1.2 Justification

One of the most important effects of AMF is to increase the crop yield, especially in soils with the characteristic of low fertility. AMF is reported to have a widespread occurrence in the plant kingdom with many benefits to plant growth. Various study on AMF have been conducted but it still lacks of study on techniques to produce it efficiently. *Zea mays* L. act as trap plant which suitable used to produce AMF inoculum. The percentage of AMF colonization (%) and soil phosphorus concentration (ppm) are important to study the interaction between the trap plant and AMF. The *Allium cepa* var. *ascalonicum* L. has an inefficient root system and cannot maintain adequate uptake of mineral nutrients such as phosphorus (P), which has a negative effect on yields. This research was conducted to study the effect of AMF inoculum on vegetative growth of *Allium cepa* var. *ascalonicum* L.. There are three potential AMF host plants which *Eleusine indica* L., *Chloris barbata* Sw. and *Mimosa pudica* L. can be found in the area of Faculty of Sustainable Agriculture (FSA) which act as a potential source of AMF inoculum. The AMF inoculum has a lot of benefits and can be a potential source of natural fertilizer which should be introduced to the farmer.

1.3 Objective

i. To study the percentage of AMF colonization and soil phosphorus concentration of the *Zea mays* L. (trap plant) inoculated from the *Eleusine indica* L. (host plant), *Chloris barbata* Sw. (host plant) and *Mimosa pudica* L. (host plant).

ii. To study the effect of AMF inoculum on vegetative growth of *Allium cepa* var. *ascalonicum* L.

1.4 Hypothesis

i. Null hypothesis, H_0: There is no significant difference of AMF inoculum on vegetative growth of *Allium cepa* var. *ascalonicum* L.

ii. Alternative hypothesis, H_a: There is a significant difference of AMF inoculum on vegetative growth of *Allium cepa* var. *ascalonicum* L.

CHAPTER 2

LITERATURE REVIEW

2.1 Mycorrhizae

Mycorrhizae have existed together with the first plants appeared on the earth with more than 450 million years ago (Schüßler *et al.*, 2001). They form a close symbiotic relationship with plant roots. They are called mycorrhizae from the Greek "mukés", meaning fungus, and "rhiza," meaning roots. Mycorrhizae have the ability to associate with plant roots and draw nutrients from the soil by forming a network of filaments. This fungus-plant relationship stimulates plant growth and promotes the root development (Singh *et al.*, 2011).

There are approximately 95% of vascular plants belonging to genera that form mycorrhizal relationships, and it is about that 80% of plant species associated with arbuscular mycorrhizal fungi. About 5% of the plant families that typically do not associate with mycorrhizal fungi. The following plants or plant group do not respond to mycorrhizal fungi including the Amaranthaceae, the pigweed family, and Brassicaceae, the mustard family (Jeffries *et al.*, 2003).

2.1.1 Types of Mycorrhizae

Normally, there have several forms of mycorrhizae with different forms of hyphal arrangement or associated microscopic structures. Mycorrhizae are traditionally classified into four groups of mycorrhizal fungi which are the ectomycorrhizae and arbuscular mycorrhizae, ericoid mycorrhizae and orchid mycorrhizae. The most common mycorrhize fungi are arbuscular mycorrhizal fungi (Singh *et al.*, 2011).

2.1.2 Arbuscular Mycorrhizal Fungi (AMF)

AMF as soil microorganism which is obligate, symbionts and belong to the phylum Glomeromycota (Schüßler *et al.*, 2001). This type of fungi represents a key link between plants and soil mineral nutrient and they able to collect growing interest as natural fertilizers. AMF form the mutualistic symbioses relationship with more than 80% of know land plant species and including of several agricultural crops. They have the role of providing the host plant with mineral nutrients and water, in exchange for produce photosynthetic products (Smith and Read, 2002).

The AMF mycelium can get the nutrients from soil volumes that are inaccessible to roots (Smith *et al.*, 2000). Fungal hyphae are much thinner than roots, therefore, able to penetrate the smaller pores (Allen, 2011). Then, the carbohydrates and mineral nutrients exchanged inside the roots across the interface between the plant and the fungus. Through the AMF, the nutritional supply of plant can be improved. The AMF interaction provides other benefits to plant such as drought. AMF is known to alleviate heavy metal toxicity in the host plants and to tolerate high metal concentrations (such as Fe, Cu and Zn) in the soil. Metal transporters play a central role in heavy metal homeostasis (Göhre and Paszkowski, 2006).

Furthermore, AMF will direct effect on the ecosystem as they improve the soil structure and aggregation (Rillig and Mummey, 2006) and drive the structure of plant communities and productivity (van der Heijden *et al.*, 1998). The need to benefit from AMF as a natural fertilizer, with a view to sustainable agriculture, is becoming increasingly urgent since the appropriate management of these symbiotic fungi could potentially decrease the use of agrochemicals. The main strategy adopted to achieve this goal is the inoculation of AMF propagules (inoculum) into a target soil (Berruti *et al.*, 2015).

2.2 AMF Association in Weeds (Host Plant)

In the present investigation, most of the weeds were found in association with AMF. Weeds act as the host plant for AMF, without host plant, the AMF cannot survive. Weeds rhizosphere and roots revealed the varied number of spores and percentage of root colonization. This could provide an estimation of the AMF status of the weeds, as well as measurement of the mycorrhizal colonization of each contributing species (Smith and Read, 2002). In addition, AMF species differ in the services that they provide for host plants (e.g. nutrient uptake, protection against pathogens, and water uptake (Klironomos, 2000). Members of Asteraceae, Fabaceae, Poaceae, Compositae and Euphorbiaceae had significantly higher colonization with optimum spore count (Table 2.1). Heavy colonization may result from the rapid colonization which is likely to occur when large numbers of propagules are present in the soil (Wilson and Trinick, 1983).

There are several factors to consider when selecting a host plant for the AMF. Obviously, one must select a plant that supports colonization by AMF. Though most dicotyledonous plants become colonized by AMF, certain families such as the mustards and other crops such as spinach and sugar beet do not become colonized by AMF and should not be used as host plants. Good hosts are typically members of the grass family, for example corn, sorghum-sudangrass, and others (van der Heijden *et al.*, 1998).

Table 2.1 Showing the presence of AMF in some weeds, of their percentage of root colonization and spore number

Scientific name	Family	% Root colonization	AMF spores / 50g of soil
**Chloris barbata* Sw.	Poaceae	81.3	320
**Eleusine indica* L.	Poaceae	74.2	331
**Mimosa pudica* L.	Fabaceae	88.4	207
Chloris gayana Kuntch	Poaceae	90.3	302
Eragrostis gangetica	Poaceae	81.4	214
Andropagon halepense L.	Poaceae	79.6	234
Acalypha indica L.	Euphorbiaceae	73.1	216
Euphorbia hirta L.	Euphorbiaceae	61.6	235
Euphorbia geniculata Orteg.	Euphorbiaceae	53.1	143
Ageratum conyzoides L.	Asteraceae	76.4	194
Bidens pilosa L.	Asteraceae	69.2	219
Alternanthera sessilis L	Amaranthaceae	19.7	92
Desmodium triflorum	Fabaceae	56.5	189

Sources: Airsang and Lakshman, 2014

2.3 Plant Coexistence Mediated by AMF

Recently, the researchers have investigated the influence of AMF on plant coexistence by comparing the outcome of plant competition with AMF presence versus absence. The presence of AMF is known to have a strong effect on the direction of succession (Allen *et al.*, 1997).

The immigration and subsequent presence of AMF could thus affect plant coexistence by enabling a mycorrhizal-dependent plant (one whose growth responds significantly to AMF infection) to become more competitive because of its increased uptake of limiting soil nutrients. When two competing plants differ in mycorrhizal dependency, the coexistence of those plants will be promoted in the presence of AMF when the inferior competitor is more mycorrhizal dependent, but coexistence will be inhibited if the superior competitor is more mycorrhizal dependent. At a high concentration of AMF inoculum, infection by AMF might become detrimental rather than beneficial because heavily infected plants might experience a large carbon removal that outweighs any benefit (Allen, 1990).

In highly fertile soils, mycorrhizal-dependent plants would be selected against, whereas infertile soils would support a highly mycorrhizal-dependent plant community (Johnson, 1993). Fertilization of soil reduces the abundance and diversity of AMF (Eom *et al.*, 1999) and could select for AMF that is less beneficial (Johnson, 1993).

2.4 Effect of Phosphorus to AMF

An important consideration in AMF production is the level of available P in the media in which the host plant are grown. Plants growing in high P situations limit colonization of their roots by AMF. In effect, they are deciding to limit the "cost" (in terms of sugar) of the symbiosis in the absence of benefit (in this case, improved uptake of phosphorus) since the roots can function well enough on their own in the high nutrient situation. The reduction of colonization then limits the fungus' ability to acquire sugars for growth and reproduction. This phenomenon is also important in the greenhouse production phase for the growth of inoculated vegetable seedlings (Grant *et al.*, 2005).

The chemical fertilizer which contains high amount of phosphorus can inhibit the mycorrhizal colonization and growth. It was showing that the benefits of AMF are greatest in the land where soil test of phosphorus is low (Grant *et al.*, 2005). As the plant available of soil phosphorus levels increases, the plant tissue phosphorous will increases too. Thus, the plant carbon investment in mycorrhizae is not economically beneficial to the plant (Grant *et al.*, 2005). Encouragement of mycorrhizae symbiosis may increase early uptake of phosphorus, improving crop yield potential without starter P-fertilizer applications (Grant *et al.*, 2005).

2.5 AMF Inoculum

AMF inoculum can be obtained after a known isolate of AMF and a host/trap plant (i.e., a plant that can be massively colonized by many AMF species) are grown together in an inert medium optimized for AMF propagation. Finally, the infected root fragments alone of a known AMF host plant that has been separated from a trap plant culture can also serve as a source of inoculum (IJdo *et al.*, 2011).

Large-scale multiplication of AMF aiming to produce AMF inoculum for field applications is generally carried out in substrate-based (nursery beds, pots, poly bag, concrete tanks), substrate-free (i.e., aeroponic boxes) and in vitro systems. Commercial inoculum produced using these systems are available in several countries, especially in Asia and Europe. However, the costs associated with the technology of inoculum production, including the establishment of single cultures of AMF species, shipping and handling and development of the carrier substrate are borne by farmers and nursery owners making the technology expensive. Culturing AMF is conventionally labor-intensive, requiring large-scale production of plants in pots or nursery beds, from which the AMF inoculum can be harvested (IJdo *et al.*, 2011).

2.6 Challenges of AMF Inoculum Production and Application

The production of AMF inoculum on a large-scale remains very challenging even though new methods for massive production (IJdo *et* al., 2011) and seed coating technology

(Vosátka *et al.*, 2013) have been developed in recent years. The main obstacle to the production of an AMF inoculum lies in the obligate symbiotic behavior of AMF, that is, their need to have a host plant for growth and completion of their life cycles. This means that the propagation step must include a phase of cultivation with the host plant that is usually time and space-demanding (van der Heijden *et al.*, 2015).

2.7 *Zea mays* L. (Trap plant)

To isolate AMF from environments, the choice of appropriate trap plants is critical. The spore morphology based technique is inevitably biased because of the preference of AMF. AMF inhabit various ecosystems with a wide range of host plant species. However, as these fungi are obligate symbionts with living roots, the hosts play an important role in mycorrhizal development, spore formation and distribution of AMF. Usually, AMF diversity in farm or degraded soils has been shown to be lower than in soils supporting a diverse flora of native plants (Morton *et al.*, 1993).

AMF trap cultures can be very helpful in unveiling fungal community members that are undetected in initial extraction of spores from field soil (Morton *et al.*, 1995). Although many AMF is thought to have a broad host range, the appropriate test plants for trap cultures should be evaluated to ensure maximum detection of fungal species in specific soils or site types. The *Z. mays* will be a suitable trap plant to encourage multiplication of the AMF species (Morton *et al.*, 1993).

According to Kabir *et al.*, 1999, *Z. mays* show the higher percentage of mycorrhizal response in terms of colonization and arbuscules. Maximum in spore count also achieved by using *Z. mays* as a trap plant (Kabir *et al.*, 1999). Besides that, the mycorrhizal inoculation by trap culture in *Z. mays* resulted in longer shoots and roots than other plants. Furthermore, increase in dry weight with higher percentage also was observed for *Z. mays* which concluded that all the results prove that *Z. mays* is more suitable trap plant for AMF spore propagation and trap culture technique can be used effectively (Kabir *et al.*, 1999).

2.7.1 Botanical Description of *Zea mays* L.

The *Z. mays* have a profusely branched, fine root system. The permanent root system has adventitious and props roots. Adventitious roots develop in a crown of roots from nodes below the soil surface (Figure 2.1). Normally four to six adventitious roots are formed per band. Numerous root hairs occur on young plants. Root hairs increase root surface area that is exposed to the soil and play an important role in the absorption of water and nutrients. The leaf of *Z. mays* is a typical grass leaf and consists of a sheath, ligules, auricles and a blade. The leaf blade is long, narrow, undulating and tapers towards the tip and is glabrous to hairy. The leaf is supported by a prominent mid-rib along its entire length. Stomata occur in rows along the entire of the leaf surface (Plessis, 2003).

The stem of *Z. mays* is cylindrical, solid and is clearly divided into nodes and internodes. It may have eight to 21 internodes. The internodes directly below the first four leaves do not lengthen, whereas those below the sixth, seventh and eighth leaves lengthen to approximately 25, 50 and 90 mm, respectively. Tillers may develop from nodes below the soil surface. Male and female flowers are borne on the same plant as separate inflorescence (Plessis, 2003).

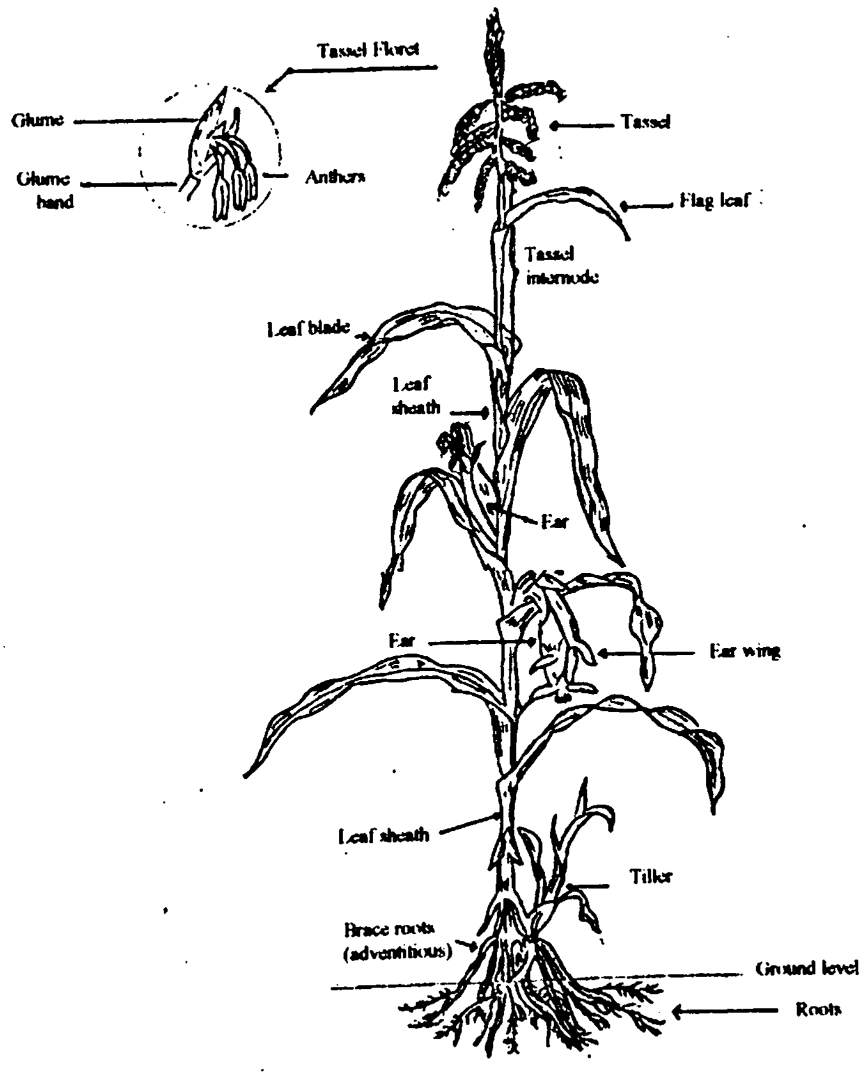

Figure 2.1 Morphology of *Zea mays* L.
Source: Seed Program Specific Work Instruction (SWI), 2015

2.8 *Allium cepa* var. *ascalonicum* L. (Trial plant)

The herbaceous biennial *Allium cepa* is the most widely cultivated taxon in the family Alliaceae. *Allium cepa* is highly mycorrhizal-dependent (Deressa and Schenk, 2008). The previous studies have indicated that the *Allium* species is highly responsive to mycorrhization, resulting in improved plant growth and yield under normal as well as stressed conditions. Maximum in spore count also achieved by using *Allium cepa* var. *ascalonicum* L. as a trial plant. Research on Allium species and their interactions with AMF has a long history that dates back to 1884 when Mollberg described in roots *Allium cepa* var. *ascalonicum* L. what we currently known as AMF. Allium species, in particular shallot, are excellent models as a trial plant used for mycorrhizal research because they have a simple rooting system, slow growth, and high response to AMF (Koide and Mosse, 2004).

2.8.1 Botanical Description of *Allium cepa* var. *ascalonicum* L.

Allium is the onion genus, with about 1250 species, making it one of the largest plant genera in the world. The shallot *Allium cepa* var. *ascalonicum* L. is native to South West Asia and cultivated worldwide. The name *ascalonicum*, used for the species name *Allium cepa* var. *ascalonicum* L. comes from Ascalon, the ancient Palestinian city where the shallot is believed to have originated (Herbst, 2001). Shallots are variously placed in the flowering plant family Alliaceae or Liliaceae. Alliaceae, a family of herbaceous plants, are monocots and part of the order Asparagales. The family has been widely but not universally recognized; in the past, the plants involved were often treated as belonging to the family Liliaceae—and still are by some botanists (Herbst, 2001).

The Liliaceae, or the lily family, is a family of monocots in the order Liliales. Plants in this family have linear leaves, mostly with parallel veins, and flower parts in threes. *Allium cepa* var. *ascalonicum* L. belong to perennial herb and having the adventitious and fibrous roots. The bulbs show uniform in size, skin color and shape. Shapes range from spherical to nearly cylindrical and include flat and cone-like bulbs (Figure 2.1). Skin variations are considered as skin color, which may be yellow, brown, white, red or purple. Stem The plant may grow to 0.3 meters (PFAF, 2008). The flowers form as an umbel (Figure 2.2). They are insect pollinated. The leaves are small and hollow (PFAF, 2008).

REFERENCES

Abdissa, Y., Tekalign, T. and Pan, L. M. 2011. Growth, Bulb Yield and Quality of Onion (*Allium cepa* L.) as Influenced by Nitrogen and Phosphorus Fertilization on Vertisol. *African Journal of Agricultural Research* **6(14):** 3252-3258

Airsang, R. V. and Lakshman, H. C. 2014. Prevalence of Arbuscular Mycorrhizal Fungal Association in Some Weeds Growing in University Botanical Garden. *The International Journal of Science and Technoledge* **2(6):** 165-171

Allen, E. B. and Allen, M. F. 1990. *The Mediation of Competition by Mycorrhizae in Successional and Patchy Environments.* Berlin: Springer

Allen, M. F., Klironomos, J. N., and Harney, S. 1997. The Epidemiology of Mycorrhiza Fungal Infection during Succession. *Plant Relationships Part B.* Berlin: Springer

Allen, M. F. 2011. Linking Water and Nutrients through the Vadose Zone: A Fungal Interface between the Soil and Plant Systems: Linking Water and Nutrients through the Vadose Zone: a fungal interface between the soil and plant systems. *Journal Arid Land* **3:** 155–163

Amijee, F., Tinker, P. B. and Stribley, D. P. 1989. The Development of Endomycorrhizal Root Systems. *New Phytologist* **111:** 435-446

Anonymous. 2012. A Simple Method for Making your Own Mycorrhizal Inoculum. http://Sunseed.org.ik. Accessed on 28 April 2017.

Berruti, A., Lumini, E., Balestrini, R., and Bianciotto, V. 2015. Arbuscular Mycorrhizal Fungi as Natural Biofertilizers: Let's Benefit from Past Successes. *Frontiers in Microbiology* **6**

Brundrett, M., Bougher, N., Dell, B., Grove, T., and Malajczuk, N. 1996. *Working with Mycorrhizas in Forestry and Agriculture Australian.* Australia: Centre for International Agricultural Research Australia

Brundrett, M. 2009. Mycorrhizals in Natural Ecosystem. *Advances in Ecological Research* **21:** 171-313

Danihelka, J., Chrtek, J. Jr. and Kaplan, Z. 2012. Checklist of Vascular Plants of the Czech Republic. *Preslia* **84:** 647–811

Deressa, T. G., Schenk, M. K. 2008. Contribution of Roots and Hyphae to Phosphorus Uptake of Mycorrhizal Onion (*Allium cepa L.*). *Journal of Plant Nutrition and Soil Science* **171:** 810–820

Eom, A. H., Hartnett, D. C., Wilson, G. W., and Figge, D. A. 1999. The Effect of Fire, Mowing and Fertilizer Amendment on Arbuscular Mycorrhizas in Tallgrass Prairie. *The American Midland Naturalist* **142(1):** 55-70

Galván, Guillermo, A., István, P., Karin, B., Jacqueline, B., Thomas, W., Kuyper, Olga, Scholten, E., and Chris, K. 2009. Molecular Diversity of Arbuscular Mycorrhizal Fungi in Onion Roots from Organic and Conventional Farming Systems in the Netherlands. *Mycorrhiza* **19(5):** 317-328

Göhre, V. and Paszkowski, U. 2006. Contribution of the Arbuscular Mycorrhizal Symbiosis to Heavy Metal Phytoremediation. *Planta* **223(6):** 1115–1122

Grant, C., Bittman, S., Montreal, M., Plenchette, C. and Morel, C. 2005. Soil and Fertilizer Phosphorus: Effects on Plant Phosphorus Supply and Mycorrhizal Development. *Canadian Journal of Plant Science* **85(1):** 3-14

Harley, J. L. and Smith S. E. 1983. *Mycorrhizal Symbiosis*. London: Academic Press

Herbst, S. T. 2001. The New Food Lover's Companion. Barron's Educational Series

Hayman, D. S. and Mosse, B. 1971. Plant growth responses to vesicular–arbuscular mycorrhiza. *New Phytologist* **70:** 19-27

IJdo, M., Cranenbrouck, S. and Declerck, S. 2011. Methods for Large-Scale Production of Arbuscular Mycorrhizal Fungi: Past, Present, and Future. *Biotropica* **12:** 56–64

Jaime, Hsiang, T. and Mcdonald, M. R. 2008. Effects of Glomus Intraradices and Onion Cultivar on *Allium* White Rot Development in Organic Soils in Ontario. *Canadian Journal of Plant Pathology* **30:** 543–553

Janos, D. P. 2007. Plant Responsiveness to Mycorrhizas Differs from Dependence upon Mycorrhizas. *Mycorrhiza* **17:**75–91

Jeffries, P., Gianinazzi, S., Perotto, S., Turnau, K., and Barea, J. M. 2003. The Contribution of Arbuscular Mycorrhizal Fungi in Sustainable Maintenance of Plant Health and Soil Fertility. *Biology and fertility of soils* **37(1):** 1-16

Johnson, N. C. 1993. Can Fertilization of Soil Select Less Mutualistic Mycorrhizae? *Ecological applications* **3(4):** 749–757

Kabir, Z., Halloran I. P and Hamel, C. 1999. Combined Effects of Soil Disturbance and Fallowing on Plant and Fungal Components of Mycorrhizal Corn (*Zea mays L.*). *Soil Biology and Biochemistry* **31:** 307-314

Kandowangko, Novri, Y., Giat, S., Nenny, N., and Robert. 2009. Proline and Abscisic Acid Content in Droughted Corn Plant Inoculated with *Azospirillum* sp. and Arbuscular Mycorrhizae Fungi. *Journal of Biosciences* **16(1):** 15-20

Klironomos, J. N. 2000. Host-specificity and Functional Diversity Among Arbuscular Mycorrhizal Fungi. *Microbial Biosystems: New Frontiers* **1:** 845-851

Koide, R. T, Mosse, B. 2004. A History of Research on Arbuscular Mycorrhiza. *Mycorrhiza* **14:** 145–163

Lidermann, R. G. and Davis, E. A. 2004. Evaluation of Commercial Inorganic and Organic Fertilizer Effects on Arbuscular Mycorrhizae Formed by Glomus Intraradices. *Horttechnology* **14:**196-202

Menge, J. A., Steirle, D., Bagy, A., Johnson, D. J. and Leonard, R. T. 1978. Phosphorus Concentrations in Plants Responsible for Inhibition of Mycorrhizal Infection. *New Phytologist* **80:** 575-578

Morton, J. B., Bentivenga, S. P. and Wheeler, W. W. 1993. Germplasm in the International Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM) and Procedures for Culture Development, Documentation and Storage. *Mycotaxon* **48:**491-528

Murphy, J. and Riley, J. R. 1962. A Modified Single Solution Method for the Determination of Phosphate in Natural Waters. *Analytica Chimica Acta* **27:** 31-36

Newton, A. C., Fitt, B. D., Atkins, S. D., Walters, D. R. and Daniell, T. J. 2010. Pathogenesis, Parasitism and Mutualism in the Trophic Space of Microbe–Plant Interactions. *Trends in microbiology* **18(8):** 365-373

Plants for a Future (PFAF). 2008. *Allium cepa ascalonicum*, common name shallot. Plants For A Future

Plessis, J., 2003. *Maize Production.* South Africa: Department of Agriculture Republic of South Africa

Rillig, M. C., and Mummey, D. L. 2006. Mycorrhizas and Soil Structure. *New Phytologist* **171(1):** 41–53

Schüβler, Arthur, Daniel, S., and Christopher, W. 2001. A New Fungal Phylum, the Glomeromycota: Phylogeny and Evolution. *Mycological Research* **105(12):** 1413-1421

Shinde, S. K., Shinde, B. P. and Patale, S. W. 2013. The Alleviation of Salt Stress by the Activity of AM Fungi in Growth and Productivity of Onion (*Allium cepa* L.) Plant. *International Journal of Life Science and Pharma Research* **3(1):** 1-11

Singh, Tanuja and Parihar, P. 2010. *Soil microbiology.* India: AGROBIOS

Smith, S. E. and Read, D. J. 2002. *Mycorrhizal Symbiosis.* London: Academic Press

Smith, F. A., Jakobsen, I. and Smith, S. E. 2000. Spatial Differences in Acquisition of Soil Phosphate between Two Arbuscular Mycorrhizal Fungi in Symbiosis with Medicago Truncatula. *New Phytologist* **147:** 357–366

Thomas, S. J. 2000. Soil Test Phosphorus: Bray and Kurtz P-1. In: Method of P Analysis for Soil, Sediments, Residues and Waters. *Southern Cooperative Series Bulletin* **396:** 13-14

Van der Heijden, M. G., Klironomos, J. N., Ursic, M. and Moutoglis, P. 1998. Mycorrhizal Fungal Diversity Determines Plant Biodiversity, Ecosystem Variability and Productivity. *Nature* **396(6706):** 69

Van der Heijden, M. G. A., Martin, F. M., Selosse, M. A. and Sanders, I. R. 2015. Mycorrhizal Ecology and Evolution: The Past, the Present, and the Future. *New Phytol* **205:** 1406–1423

Vostáka, M. J., Albechtrova and Pattern, R. 2008. The International Market Development for Mycorrhizal Technology. *Springer* **3(4):**419-438

Vosátka, M., Látr, A., Gianinazzi, S. and Albrechtová, J. 2013. Development of Arbuscular Mycorrhizal Biotechnology and Industry: Current Achievements and Bottlenecks. *Symbiosis* **58:** 29–37

Wilson, J. M. and Trinick, M. J. 1983. Infection Development and Interactions between Vesicular-Arbuscular Mycorrhizal Fungi. *New Phytologist* **93:** 543-553