UNIVERSITAT POLITECNICA DE VALENCIA

Master in Plant Genetics and Breeding

Screening for Drought Tolerance in Eggplants relatives and Interspecific Hybrids (*Solanum* spp.)

Master Thesis Presented by :

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D. Dr. Jaime Prohens Tomas

Experiment Director :

Dna. Ana Maria Fita Fernandez

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RESUMEN

El escenario que impone el cambio climático obliga a desarrollar cultivos mejor adaptados a la sequía. La berenjena (*Solanum melongena* L.) es uno de los 35 cultivos catalogados como más importantes para la seguridad alimentaria mundial y, como tal, está incluido en el Anexo 1 del Tratado Internacional sobre los Recursos Fitogenéticos para la Alimentación y la Agricultura. Aunque la berenjena no es un cultivo muy sensible a la sequía todavía se puede mejorar su tolerancia haciendo uso de sus parientes silvestre (*Solanum* spp.). El objetivo del presente trabajo fue evaluar la tolerancia a la sequía de la berenjena y algunos de sus parientes silvestres, así como híbridos entre ellos, para determinar el interés de introgresar genes de tolerancia a la sequía desde dichos parientes silvestres. Los experimentos se realizaron en el laboratorio de solanáceas del COMAV, UPV durante 55 días. Se utilizaron las especies *S. melongena*, *S. insanum*, *S. dasyphyllum*, *S. linnaeanum*, *S. anguivi* y sus híbridos. Se realizaron dos tratamiento de sequía (déficit de agua y PEG 7%) y un control. Se evaluó la tasa de crecimiento de las plantas (altura de la planta y el área foliar), la biomasa, el color, la tasa de fotosíntesis, la tasa de transpiración, la conductancia estomática, concentración de CO2 intercelular y la eficiencia en la utilización de agua. El resultado de la investigación concluyó que el tratamiento sequía indujo una reducción media del 17% en la altura de la planta, un 30% en el área foliar y el 32% de la biomasa seca en el conjunto de genotipos evaluados. Por otra parte, se demostró que *S. anguivi*, *S. insanum*, y los híbridos *S. melongena* x *S. anguivi*, *S. melongena* x *S. insanum*, y *S. melongena* x *S. dasyphyllum* eran más tolerantes a las condiciones de sequía. Por lo tanto estas especies e híbridos podrán ser utilizados en posteriores programas de mejora para la resistencia a la sequía en berenjena.

Palabra clave: *Solanum* spp, híbridos interespecíficos, sequía, selección

Abstract

The scenario posed by climate change forces to develop better adapted to drought. Eggplant is one of the 35 crops judged to be most important for food security and as such is included in the Annex 1 of the International Treaty on Plant Genetic Resources for Food and Agriculture. Even though the eggplant is not very sensitive crop to drought its tolerance can still be improved using their wild relatives (*Solanum* spp.). The aim of this study was to evaluate the drought tolerance of eggplant and some of their wild relatives, as well as hybrids between them, to determine the interest of introgress genes for drought tolerance from these wild relatives. Experiments were performed in the Solanaceae laboratory of COMAV, (UPV) for 55 days. Species *S. melongena*, *S. insanum*, *S. dasyphyllum*, *S. linnaeanum*, *S. anguivi* and their hybrids were used. Two drought treatment (water deficit and PEG 7%) and one control were applied. The analysis was focused on plant growth rate (plant height and leaf area), biomass, green-color level, photosynthesis rate, transpiration rate, stomatal conductance, intercellular CO2 concentration and water-used efficiency. The research result concluded that drought treatment induced an average reduction of 17% in plant height, 30% in leaf area, and

32% in dry shoot biomass. Moreover, it showed that *S. anguivi*, *S. insanum*, and the hybrids *S. melongena* x *S. anguivi*, *S. melongena* x *S. insanum*, y *S. melongena* x *S. dasyphyllum* were more tolerant to drought condition. Therefore these species and hybrids can be used in future breeding programs for drought resistance in eggplant.

Keyword : *Solanum* spp, interspecific hybrids, drought, selection

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ABBREVIATION

WUE : Water-use efficiency

FAO : Food and Agricultural Organization

CIMMYT : Centro Internacional de Mejoramiento de Maiz y Trigo

CRI : Crop Research Institute

IITA : International Institute of Tropical Agriculture

ICARDA : International Center for Agricultural Research in the Dry Area

IRRI : International Rice Research Institute

TPE : Target Population of Environment

RFLP : Restriction Fragment Length Polymorphism

RAPDs : Random Amplification of Polymorphic DNA

CAPS : Cleaved Amplified Polymorphic Sequences

PCR : Polymerase Chain Reaction

AFLP : Amplified Fragment Length Polymorphism

SSRs : Simple Sequence Repeats

SNPs : Single Nucleotide Polymorphism

QTL : Quatitative Traits Locus

PEG : Polyethylene Glycol

IRGA : Infra-red Gas Analysis

1. INTRODUCTION

1.1. The Economic Importance of Drought

1.1.1. The Drought and the crops

Based on the special report of emission scenario (SRES), it has been predicted that levels of CO_2 will be rise from 370 ppm to 550 ppm by 2050 (Nakic 'enovic' and Swart, 2000). The human-caused is believed to have a main role in this increase more than glacial-interglacial cycles (Intergovernmental Panel on Climate Change, 2001, 2007). The rising of CO_2 in combination with other changes in the atmosphere, will increase the global surface temperature. The global warming will rise the evaporation from wet surface and plant. It will enlarge the drought-prone areas worldwide. Henceforth, in the future, plant will experience an acute heat and drought stress that will affect to ecosystem productivity and biodiversity (Cias *et al.*, 2005; Thomas *et al.*, 2004). Drought usually occurs in arid or semi-arid regions (see Figure 1) since they have the lowest rainfall. However, it also could happen in rainfed area. Subsaharan-Africa and Central Asia are the most drought-vulnerable regions (Meehl *et al.*, 2007; CGIAR, 2013).

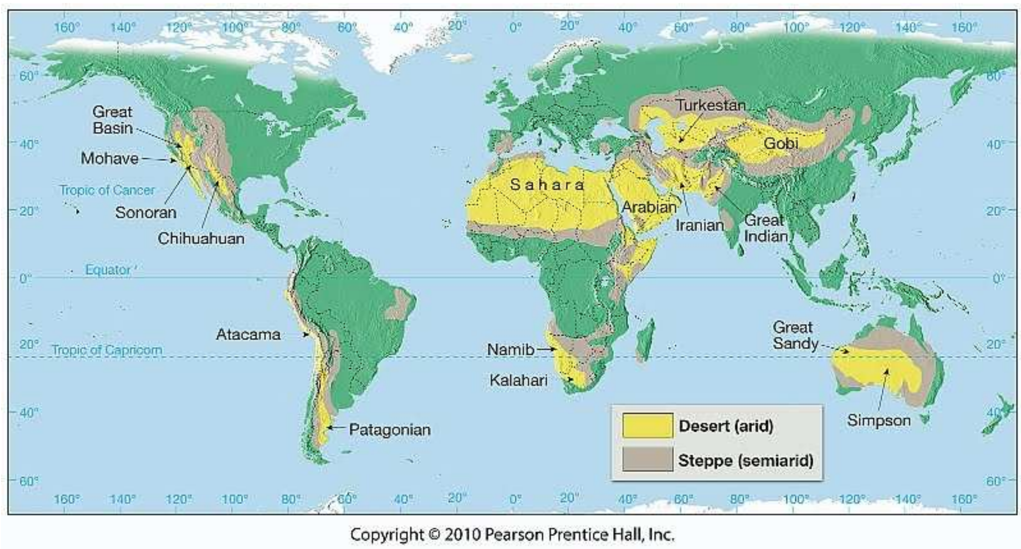

Figure 1. Distribution of arid and semi-arid regions around the world (courtesy: Pearson Prentice Hall, Inc.)

The term "drought" means the insufficient of water availability by rainfall and/or irrigation to meet the crops transpiration. It is the most important wordwide problem in agriculture since it cause severe losses of crops yield of many species (Tuberosa, 2012). Moreover, this stress could emerged the disease and pest problems, impairs crop growth rate (reduces leaf size, stem elongation, root proliferation), reduces water use efficiency (WUE), decrease the rate of photosynthesis, decline the biomass accumulation, and diminish crop productivity (Li *et al*., 2009 ; Farooq *et al*.,2009).

The alteration of crops yield may affect to its contribution for feeding the world's population. In 2009, FAO assumed that 1,02 billion people in the world were undernourished. This condition occured due to food insecurity caused by many factors including the limited natural resources and climate change. (FAO, 2009; WFP, 2009; World Bank, 1986). The rising of drought episodes will reduce the supply of food that will impact to the increasing of hunger people in the world. Therefore, finding drought-tolerance crops with high yield is very important to overcome these problems.

1.1.2. Drought mitigation through technology

Human suffering and massive economic losses caused by drought is spreading around the world, especially in the development country. Nowadays, technology helps the mitigation of drought. There are many projects conducted for overcome drought problems. Improving water-use efficiency in agriculture becomes important due to the water scarcity. This will require an integrated approach of water resources management to provide efficient, equitable and sustainable use of the resources (ISAAA, 2008). Instead of traditional irrigation-, the drip- and micro- irrigation system (see Figure 2) represent the best water management solution for avoid such wastage by applying water directly to the roots of plants. As well as saving water, this system will rise the productivity of crops (Balch, 2014). This irrigation system usually is integrated with remote sensing which improve the irrigation efficiency. In addition, some companies also launched the data analytic equipment and farm data management software which given weather information, water balance information, soil moisture etc., to help the farmers for manage the land and water against the drought condition (Fehrenbacher, 2015).

Figure 2. Drip- and micro- irrigation system improve the irrigation efficiency (courtesy : shoreline sprinkling)

The idea of purifying water from the ocean has appear as an attempt for overcome the insufficient of water available. Oasys Water, the membrane-based desalination process water technology from US company, have sucessfully turned the five times level of salt from seawater into drinking water (Balch, 2014).

Nevertheless, probably not many people know about these technologies. Moreover, the irrigation system, desalination system and other technologies usually still expensive for the farmers from the developing country. Therefore, breeding for drought-tolerance crops is still an appropriate way for fighting the drought. However, it requires the identification of genetic variability to drought among crop varieties, or among sexually compatible species and then, introducing the drought-tolerance traits along with suitable agronomic characteristics. Eventhough it is a slow process, the breeding has and continue to have some success.

1.2. Breeding for Drought-Tolerance

1.2.1. Plant breeding for drought-tolerance traits

Every year, the drought episodes are rising due to global climate change. There are many efforts have been attempted to produce the drought-tolerance plant with high yield through conventional breeding, marker-assisted selection or genetic engineering (transgenic approach). Any of these approaches needs genetic variation at any specific level (Ashraf, 2010). Moreover, it should be considered that the plant breeding for drought-tolerance traits is rather complicated since several other stresses can influence the crops simultaneously. Drought-tolerance plants mean plants having the ability for growing, flowering and displaying the economic yield under suboptimal water supply (Beck *et al*., 2007; Farooq *et al*., 2009).

Since the green revolution started in 1940, the traditional breeding obtained a phenomenal increasing in wheat and rice yield in many parts of the world, particularly in South Asia. Thus, during the 20th century, plant breeding has already largely contributed to tackle the challenging food at global level (Rajaram, 2005). Until now, a number of plant breeding program for developing drought tolerance crops have been successfully done by international research center such as CIMMYT, CRI (Crop Research Institute), IITA, ICARDA, IRRI (Banziger *et al*., 2004; MacLean *et al*., 2002; Ashraf, 2010).

Before conducting plant breeding, we have to define nomenclature, mechanism of the crops under drought condition and identify functional basis of plants (Tuberosa, 2012). Basically, plants have different mechanism under drought condition that goes from individual plant physiological processes to ecosystem level (Chaves *et al*., 2003; Izanloo *et al*., 2008; Xu *et al*., 2009; Xu *et al*., 2010).

First strategy lies on drought escape. It includes of short life cycle utilizing the soil moisture in the most favorable season and a survive drought period in a metabolically inactive stage such as seed or dessicated vegetative tissues (Geber *et al*., 1990).

Second mechanism is drought avoidance via morpho-physiological features (e.g. early flowering, osmotic adjustment, deep roots) which enable the plant, or parts thereof, to keep the hydration. It is based on minimizing the tissue dehydration (Levit, 1972; Schulze *et al*., 1986; Jackson *et al*., 2000; Tuberosa, 2012).

Third is drought tolerance, including of the features that permit the plant to retain, at least partially, proper functionality in a severe dehydration (ex: remobilization of stem water-soluble carbohydrate (WSC), accumulation of molecular protectants, etc) (Levit et *al*., 1972; Morgan, 1984; Tuberosa, 2012).

Fourth is drought resistance via changing metabolic pathway for life survival under severe stress such as rise the antioxidant metabolism (Bartoli *et al*., 1999; Peñuelas *et al*., 2004).

Fifth is drought abandon with removing a part of individual such as shedding elder leaves under deficit water (Chaves *et al*., 2003).

The last strategy is drought-prone biochemical-physiological traits for plant evolution under long-term drought condition by genetic mutation and genetic modification (Hoffman *et al*., 1999; Sherrard, 2009; Maherali, 2010).

Nevertheless, the target population of environment (TPE) must be classified and considered that genotype x environment interaction have influence on the differences of TPE (Tuberosa, 2012). All these plant strategy could provide the basic knowledge about the plant mechanism resources before conducting plant breeding.

The potential traits which genetically have higher yield, higher heritability and drought-resistance are the main selected target for improving yield in drought-limited condition (Blum, 1988, 2011; Monneveux and Ribaut, 2006). Nevertheless, since drought tolerance is a quantitative trait, every single gene may have important role in adaptation on drought condition (Forster *et al*., 2004). A good phenotyping has a pivotal role for selecting drought-tolerance traits. The collection of accurate, precision, relevant and meaningful data from a biological and agronomic standpoint, under the current conditions in farmers field within TPE, could decrease the genotype-phenotype gap and minimize the experimental "noise" introduced by uncontrolled environmental and experimental variability (Sadras, 2002; Saint Pierre *et al.*, 2012; Tuberosa, 2012).

The appropriate molecular markers on genetic studies (e.g. RFLP, RAPDs, CAPS, PCR, AFLP, SSRs, SNPs, Isozyme) can provide useful tools for selecting both cultivated varieties and wild relatives. Up to date, QTL mapping have helped to understand the genetic basis of physiological, morphological, and developmental of plant growth under water-limited conditions (Sari-Gorla *et al.*, 1999; Ashraf, 2010; Tuberosa, 2012).

1.2.2. Morpho-physiological mechanism associated to drought-tolerant plants

Many of morphological and physiological mechanisms were associated to drought-tolerance traits. These morpho-physiological traits could categorized as drought-responsive traits which expresses only under severe drought conditions, and/or constitutive traits which expresses in low and intermediate of drought levels (Lafitte and Edmeades, 1995; Blum, 2006). Despite the existence of morpho-physiological traits that can used for improve yield, only a few of them have commonly tested and proposed for plant breeding. Plant height, root length, leaf area, and fresh and dry biomass are reliable indicator for morphological plant response to drought condition. Meanwhile, stomatal conductance, transpiration rate, photosynthesis rate and water use efficiency are physiological plants responses (Farooq *et al.*, 2009[b]; Hafeez *et al.*, 2015). These characters were investigated to find drought-tolerant plants in this research (see material and methods).

The reduction of plant growth rate, such as plant height, total leaf area, root length, and plant biomass, occurs through physiological process in drought-prone environment. Decreasing of photosynthesis become major source physiological problems of this reduction as impact of the closure of stomata which limited gas exchange in leaf (Wahid and Rasul, 2005; Farooq *et al.*, 2009[b]). Plants with a better control stomatal function are more tolerant to drought stress since it can make an efficiency of gas exchange which will lead to the increasing of water-use efficiency and photosynthetic capacity (Silva *et al.*, 2013).

Water-use efficiency (WUE) can be analyzed ranging from instantaneous measurement into integrative ones at the plant and crop level (see Figure 3). At leaf level, WUE is divided into Intrinsic WUE and Instantaneous WUE which can be calculated by gas exchange method. Intrinsic WUE describes about the ratio between photosynthetic rate (A) and stomatal conductance (g_s), whereas Instantaneous WUE explaines the assimilation of net CO_2 by photosynthetic (A) divided with water transpired in the same time period (E). On the other hand, at plant level, WUE defines as the assimilated dry matter named biomass (WUE biomass) or accumulation dry matter partitioned the economical product, such as grain (WUE yield) (Tambussi *et al*., 2007; Medrano *et al*., 2015).

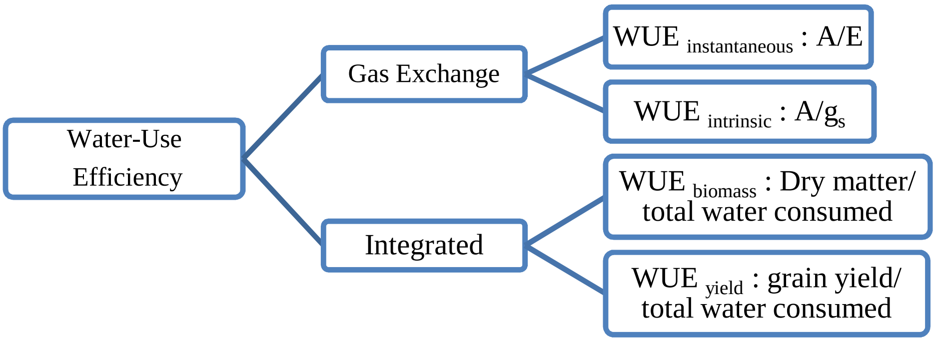

Figure 3. Scheme of Water Use Efficiency definition.

Each genotypes has different WUE under drought conditions. Passioura (1977), defined WUE yield as formula :

$$Y = T \times WUE \times HI$$

where T is the amount of water transpired by the crop and evaporated from the field, and HI is the harvest index (the ratio between yield (Y) and total biomass). The variables are interdependence to each other. Improving water-use efficiency (WUE) in order to increase yield (Y) may be partially equated to reduce the water absorbtion from the soil. Furthermore, it has to be kept in mind that agronomist and farmers are interested in yield. Therefore, WUE should not be equated to the drought tolerance, since there is possibility that WUE could negatively associated with the yield (Y) (Tuberosa, 2012). Meanwhile, Richards (1991) proposed the other formula related to the crops which grown in water-limited location :

$$\text{WUE (biomass)} = TE/(1 + E_s/T)$$

where TE is transpiration efficiency, E_s is the water lost due to the evaporation, and T is water lost by the transpiration. This formula is useful for identifying the agronomic and breeding strategies (Tuberosa, 2012).

Drought stress also induced different distribution of mass to the plants organs. The previous research implied that a significant decrease occured in leaves but not in shoot or roots. Furthermore, it explained that drought-tolerant plants usually have higher roots mass than sensitive one (Erice *et al*., 2010; Krisnamurthy *et al*., 2011). A well-developed roots system will provide plants to adapt better in drought condition (Bacon *et al*., 2002; Yu *et al*., 2007). The faster growing and deeper roots will increase water harvest and help to stabilize yield under drought condition (King *et al*., 2009).

Morpho-physiological traits are important tools which could be use for select drought-tolerant plant. However, those characters should associated with yield, and having greater heritability than yield. So, it may assist in development and adaptations of new genotypes with higher yield that are able survive on water scarcity.

1.3. The Eggplant

1.3.1. The Origin and Distribution of Eggplant

Eggplant (*Solanum melongena* L.) (2n = 24) has been classified as :

Kingdom	: Plantae
Division	: Tracheophyta
Subdivision	: Spermatophytina
Class	: Magnoliopsida
Order	: Solanales
Family	: Solanaceae
Genus	: Solanum
Species	: *Solanum melongena L.*

The name "*Solanum melongena*" comes from a sixteenth-century Arabic term for one kind of eggplant. United states, Australia, New Zealand and Canada named it "Eggplant" due to their fruits that resembled goose or hens egg. This plant called "Aubergine" in British English derived from French *aubergine*. It known as "Brinjal" in Indian and South African (New World Encyclopedia, 2013). Eggplant as one of species on the *Solanaceae* family or nightshade family, has choosen and developed as human food plants, others include of the New world crops tomato (*Solanum lycopersicum* L.), potato (*Solanum tuberosum* L.) and chilli pepper (*Capsicum* spp.). This plant contains vitamins, minerals, fibre and an important phytonutrients (Raigon *et al*., 2008; Rotino *et al*., 2014). Instead of food, they were used as traditional medicine in history. Moreover,its leaves and flowers can be poisonous if it consumed in large quantities due to their solanine (Rotino *et al*., 2014).

The origin and evolution of eggplant are still under debate. Relationship among wild species, semi-cultivated and cultivated are still controversial. Genetic studies on the relationship within eggplant and its closely allied have only determined the position of them but not their origin and progenitor. Several hypotheses have developed about the eggplant evolution and biogeography (Lester and Hasan, 1991; Mace *et al*., 1999; Daunay *et al*., 2001; Weese and Bohs, 2010). Some taxonomists argue that *S. incanum* and *S. undatum* are the candidate progenitor of *S. melongena*. *Solanum incanum*, native to north Africa and middle east, gave rise to *S. undatum* as it spread to east asia (Lester and Hasan, 1991). Alternatively, some believed that *S. undatum* as true wild species, whereas *S. melongena* domesticated directly from *S.insanum* in India (De Candolle, 1886; Prain, 1903). The advanced study described that probably there is differences between *S. incanum* in Africa and Asia (Karihaloo, 2009).

The investigation of eggplant domestication process has proposed three theories. First theory explained that cultivated eggplant originated from India and spread to Western Asia and Europe (see Figure 4) brought by Arabic traders (Mace *et al*., 1999; Doganlar *et al*, 2002[a];2002[b]; Daunay, 2008; Weese and Bohs, 2010, Meyer *et al*., 2012). The evidence of eggplant domestication was recorded in Sanskrit literature, dated to 300 BC (Khan, 1979; Wang *et al*., 2008). Second theory implied that the landraces of eggplant were cultivated in China and distributed to northeast and southeast into Japan, mainland Southeast Asia and Malesia, and Eastern Asia (see Figure 4) (Wang *et al*., 2008; Ali *et al*., 2011; Meyer *et al*., 2012). It recorded in Chinese literature, Tong yue, dated to 59 BC. The earliest domestic relatives of eggplant had round and green fruit. The domestication process has changed the quality of fruit : size, shape and taste.

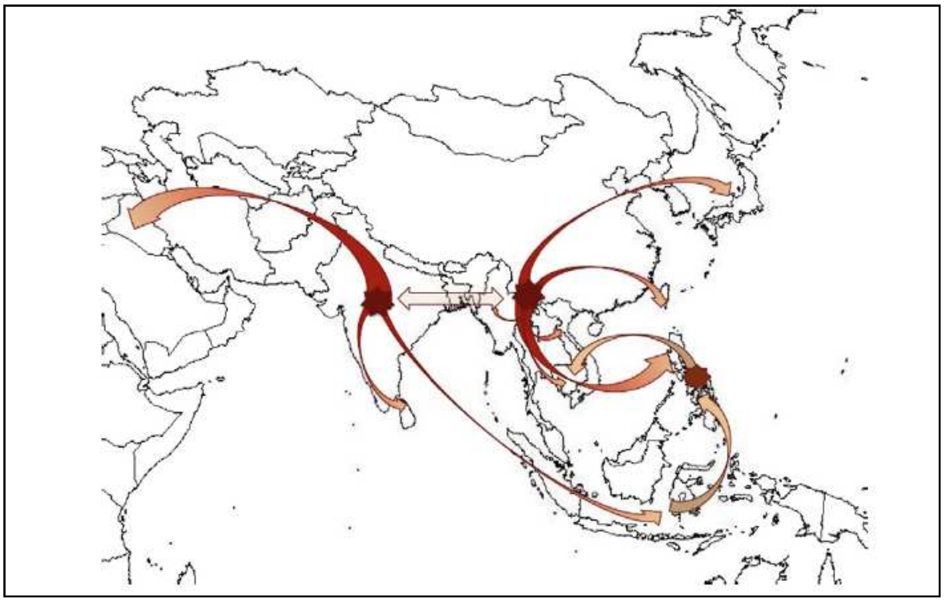

Figure 4. Proposed scheme of eggplant distributions. First, landraces originating from India were proposed spread to west to western Asia and Europe. Second, landraces occurs to China distributed to northeast and

southeast into Japan. Furthermore, third proposed domestication event, *Solanum melongena* subsp. *ovigerum* originated in Malesia which spread into Indochina only (Meyer *et al*., 2012)

The third theory arose from AFLP analysis conducted by Meyer *et al*., 2012. It described that there was domestication process for *S. melongena* subsp. *ovigerum* in Malesia which has restricted spreading only into Indochina (Figure 4). However, it is generally concurred that Asia is the center of diversity of eggplants (Meyer *et al*., 2012; Knapp *et al*., 2013).

1.3.2. Drought Responses in the Eggplant

Solanum melongena is the third most important crops worldwide. It has been widely cultivated for centuries in Asia, Africa, Europe and Near East. Eventhough it commonly sold in American, European and Australian markets. Over of 90% of eggplant production is concentrated into seven countries including of China, Egypt, Turkey, India and Japan (Lucier and Jerardo, 2006). In 2015, the production of eggplant exhibited an increase with 49,418,212 tonnes (FAO, 2015).

Nonetheless, the production of eggplant may decrease due to diseases and stresses. Drought is one of abiotic stress which has potential to make severe losses in eggplant. It can decrease both the quality and productivity of crops. The reduction in leaf area, dry matter, weight, volume, height and diameter of fruits, which impact to decrement of fresh yield hence of water stressed in the eggplant were reported in the previous study (Kirnak *et al.*, 2002; Chaves *et al*., 2003; Madramooto and Rigby, 1991; Lovelli *et al*., 2007; Mitchell *et al*., 1991; Tan and Blake, 1993; Smittle *et al*. 1994, Hartz, 1997).

However, eggplant has the ability to survive better under drought condition than other crops. Likely, a better stomatal control and a better photosyntesis maintenance are the pivotal factors which maintain plant physiological state in water stress condition (Behboudian, 1997a; Behboudian, 1997b; Ludlow 1976). Nonetheless, there were variation of tolerance levels which eggplant could stand or not to the drought condition. For instance, the tolerance levels variations were found on the eggplant accession from certain landraces that tolerance to drought in Indonesia (Sudarmonowati, 2012).

1.4. The Wild Relatives as Genetic Resources for Tolerance Genes

1.4.1. Eggplants, their allied and their wild relatives

Solanum melongena belongs to the subgenus *Leptostemonum*, the largest subgenus in the Solanum with 450 species diffuse worldwide. Unlike most of genus, eggplant and its relatives belong to the Old World. The majority of wild relatives of eggplant derived from

Africa. *Solanum melongena* is differentiated from their wild relatives which usually have small, round, yellow fruits and the plant are very abundantly prickly (Weese and Bohs, 2010; Daunay and Hazra, 2012; Knapp *et al*.,2013).

There are three cultivated eggplants whithin the large leptostemonum clade including of *S. melongena* L, *S. macrocarpon*, and *S. aethiopicum* L. Both of *S. melongena* and *S. macrocarpon* belongs to sections *Melongena* Mill. (Dunal), whereas *Solanum aethiopicum* L. belongs to sections *Oliganthes* Dunal (Bitter) (Daunay *et al*., 2001; Daunay and Hazra, 2012; Knapp *et al*., 2013).

<u>Common Eggplant</u>

Solanum melongena L. (Figure 1.4., J,K,L) is known as common eggplant, or brinjal eggplant. Currently, it is one of the most important crop which grown worldwide. According to Lester and Hasan, 1991, *Solanum melongena* divided into groups E-H which refers to *S. insanum* L., *S. cumingii* Dunal, *S. ovigerum* Dunal and *S. melongena* L. They were wild and weedy plants, landraces and derived cultivar which found in Asia and India. However, a new classification made groups E-F into *S. insanum* L., meanwhile groups G-H refers to *S. melongena* L (Knapp *et al*., 2013)

Solanum incanum L., is known as putative wild anchestor of *Solanum melongena*. It is native to Africa. It can cross compatible with *Solanum melongena*. Different to *Solanum melongena*, *S. incanum* has small green, yellow or even white fruit, prickly stem and leaves. Lester and his colleagues considered *S.incanum* into groups A-D which refers to *S.campylacanthum* A. Rich, *S.Panduriforme* E.Mey, *S.delagoense*, *S.incanum* L. sensuu stricto, and *S. lichtensteinii*. Nonetheless, Knapp and colleagues considered *S. incanum* L. groups A-B refers to *S. campylacanthum* A. Rich, group C refers to *S. incanum* L., whereas group D refers to *S. lichtensteinii* Willd (Lester and Hasan, 1991; Mace *et al*., 1999; Knapp *et al*., 2013).

<u>Scarlet Eggplant Complex</u>

Solanum aethiopicum L., commonly known as scarlet eggplant, is native from south africa. It has been introduced primary to the Brazil, then to the West Indies and South Africa (Lester and Niakan, 1986; Daunay *et al*., 2001; Weese and Bohs, 2010). It has small white corolla and usually bright scarlet fruits that resemble to *Capsicum* peppers. *Solanum anguivi* (Figure 5, A,B,C), as known for the wild progenitor of *S. aethiopicum*, could produce the fully fertile hybrids with the *S. aethiopicum*. *Solanum anguivi, Solanum aethiopicum* and their intermediate fertile formed scarlet eggplant complex (Lester and Niakan, 1986; Lester and Thitai, 1989; Plazas *et al*., 2014)

Gboma Eggplant Complex

Solanum macrocarpon L, the Gboma eggplant, is native from the humid tropics of central Africa. It has deeply lobed leaves and very large calyces (Daunay *et al*., 2001; Weese and Bohs, 2010). *Solanum macrocarpon* was cultivated from the wild S. *dasyphyllum* Schum and Thonn. The cross breeding of both species also gain the fully interfertile hybrids. *Solanum macrocarpon* L., *S. dasyphyllum* and their intermediate fertile are usually called as Gboma Eggplant complex (Bukenya and Carasco, 1994; Plazas *et al*., 2014)

Both Scarlet and gboma eggplant are an important genetic resources for common eggplant breeding. All three cultivated eggplants can be intercrossed giving intermediate fertile hybrids. (Daunay *et al*., 1991; Oyelana and Ugborogho, 2008; Prohens *et al.*, 2012, Khan *et al.*, 2013; Plazas *et al*., 2014). Nevertheless, scarlet eggplant and gboma eggplant are distantly related with and not involved in evolution of common eggplant (Whalen, 1984; Plazas *et al*., 2014).

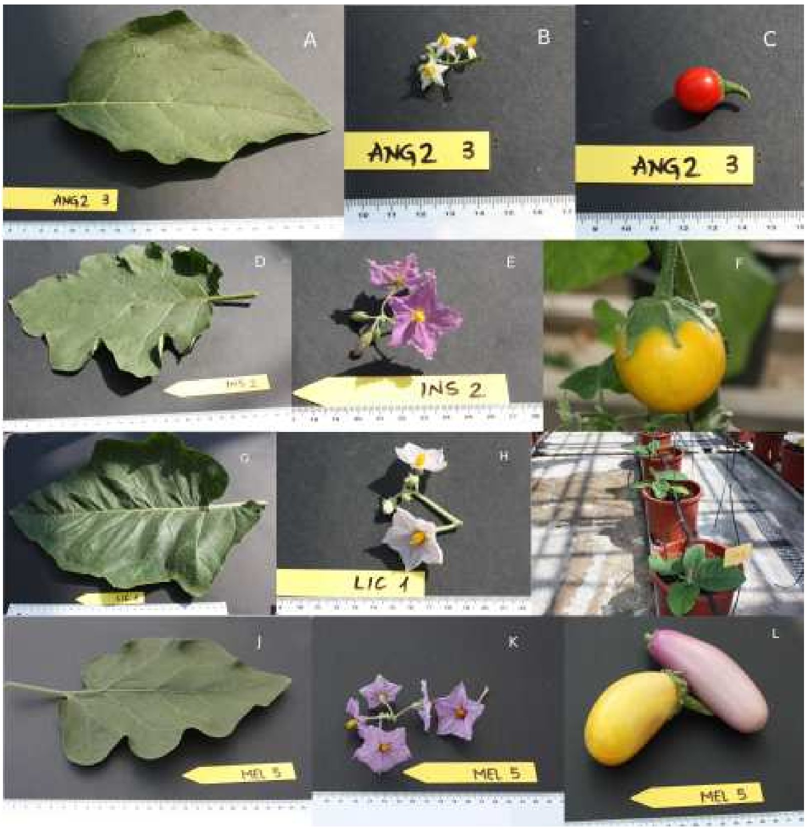

Figure 5. A,B, C are photos of *S. anguivi* leaves, fruit, and flower. D,E,F are photos of *S. Insanum* leaves, flower and fruit. G, H, and I are images of *S. lichtensteinii*. Whereas J, K, and L are photos of *S. melongena* (Zamkova, 2015).

Other than three cultivated and their wild relatives already mentioned, there are a few of wild relatives of *Solanum melongena* which usually used in breeding since they have close relationship with *Solanum melongena*. In this following, the explanation about the wild relatives :

Solanum insanum L. (Figure 5, D,E,F) distributes from India to South East Asia, and also found in Madagascar and Mauritius. It usually mistaken with *Solanum incanum*. According to Lester and Hasan (1991), this species was a variety from *Solanum melongena*. Nevertheless, in the new classification, Knapp *et al.*, 2013, *Solanum insanum* has considered as wild plant, which is almost certainly the wild progenitor of *Solanum melongena* (Daunay and Hazra, 2012; Knapp *et al*., 2013). In addition, the group E (wild) and F (weedy) of Lester and Hasan (1991) have been unified and belongs to this taxa (Daunay *et al*., 2001).

Solanum lichtensteini Willd. (Figure 5, G,H,I) is spreading from South Africa to Angola, DR Congo and Tanzania. This species is morphologically similar with *Solanum incanum*. Nevertheless, it can be differentiated by its ridged young stem and its geographic. The dwarf form also found in upland dry areas of South Africa. This species is placed to *Solanum incanum* group D (Lester and Hasan, 1991). It also sister to *S. linnaeanum* (Weese and Bohs, 2010)

Solanum linnaeanum Hepper & P. is likely native to South Africa then diffuse to mediterranean region. In spain, the fruit from *S. linnaeanum* don't seems for feeding any animals. Weese and Bohs, 2010, have found the clearly relationship of *S. linnaeanum* and the eggplants wild relatives. This species has almost glabrous leaves which different from the other eggplants relatives. It is also a good candidate to make ILs that beneficial for eggplant breeding resources (Knapp *et al*., 2013).

Solanum tomentosum L. is known as snake apple which belongs to Section *Oliganthes*. It occurs on roadside, undisturbed soil, and rocky grassland in coastal belt of South Africa, except Malawi and Zambia. It is a shrub which grows up to 60 cm high. This species usually use as medicine to threat syphilis, sore throat, toothache and for treatments of boils. It also potential for antimicrobial activities (Schmelzer and Gurib-Fakim, 2008; Aliero and Afolayan, 2006).

1.4.2. The introgression of tolerance traits from wild relatives into cultivated eggplant

In eggplants, the wild germplasm resources are important tools to get the potential genetic variability and allelic variation of many potential agronomic traits. They are good resources for tolerance to disease and pest resistance, abiotic and biotic stresses. For instance, several previous studies that inform resistance to root-knot nematodes in *Solanum aethiopicum* (Hebert, 1985; Prohens, 2012), resistance to salinity in *Solanum linnaeanum* (Daunay *et al*., 1991; Rotino *et al*., 2014), or high tolerance to salt in *S. torvum* (Bletsos *et al*., 2003; Rotino *et al*., 2014). The wild relatives usually used in an interspecific hybridisation to introduce the potential traits from wild relatives to cultivated eggplants for crop improvement. However, since there are certain fertilization barriers, the capability of eggplants to cross over with other genera or subgenera was very low (Rotino *et al*., 2014).

Most of publications investigated about interspecific crosses in eggplants and wild relatives assumed that there were inconsistency result. It occured because of ambigous or miss-applied *Solanum* species nomenclature, heterogenous, or not specified criteria used for imply the succes or failure of crosses. In the conventional breeding, sterility, reduced fertility and/or infertility were displayed as a common phenomenon in the interspecific hybrid which may associated to self-incompatibility due to wild parents, being eggplants self-compatible. Therefore, the conventional breeding should be provided by biotechnology approach to accomplished the fertility progenies. Many biotechnology attempts such as somaclonal variation, somatic hybridizations, in vitro embryo rescue, genetic engineering (transformation), and molecular marker have been conducted to further enlarge the genetic variability. However, despite genetic engineering very useful for plant breeding, there are still many people don’t believed in genetic modified crop production (Devi *et al*., 2015; Kashyap *et al*., 2002; Daunay and Hazra, 2012; Rotino *et al*., 2014).

The reviewed on wide scale inter-specific hybridization experiments between *Solanum melongena* with *Solanum* species exhibited that there were 27 species belongs to section *Melongena* (11 species), section *Oliganthes* (15 species) and section *Nycterium* (*S.lidii*) which have successfully breeding with eggplants (using minimum threshold 10% of pollen stainability or more). To put it another way, most of *Solanum* species having partially fertile hybrids with eggplants, including of *S. dasyphyllum, S. anguivi, S. tomentosum, S. linneanum, S. macrocarpon, S. aethiopicum*. By contrast, only limited having fertile hybrids with eggplants, such as *S. lichtensteinii, S. incanum* and *S. insanum* (Daunay, 2008; Daunay and Hazra, 2012; Rotino *et al*., 2014).

The interspecific hybridisations between wild relatives with cultivated eggplants may result the introgression undesired susceptible traits, for example, susceptible S. torvum to fruit anthracnosis. Therefore, a susceptible traits in given wild relatives should be systematically looked and eliminated from eggplant breeding (Daunay, 2008).

According to drought-tolerance traits, many of researchers has worked in a varies of plants, including of eggplant and their wild relatives, to find the best genotypes which can survive in dry environment. The previous studies implied that *Solanum macrocarpon* and *Solanum eleagnifolium* possesed tolerance to drought (Daunay *et al*., 1991; Fita *et al*., 2015). COMAV as a research center in UPV, Spain, has been investigated onto drought-tolerance experiments. They observed to the cultivated eggplant, and their wild relatives about their tolerance to drought. The eggplants and the wild relatives also were crossed over to obtain a new genotypes withstand drought climate. However, the further investigation were needed to acquire information about those genotypes. Therefore, in these experiments, the simple analysis was conducted to find the best drought-tolerant *Solanum* spp.

2. OBJECTIVES

According to the briefly explanation previously in Introduction, eggplant and it wild relatives have ability to resist in the drought-prone environment. Nevertheless, the existence of level variance has discovered. Therefore, this research has aimed for :

1. To compare the growth development on the eggplant, the wild relatives and their hybrid (*Solanum* spp.) on the drought condition.
2. To analyze the genotype of *Solanum* spp. which more tolerant to the drought condition.

3. MATERIAL AND METHODS

3.1. Plant Material

There were 17 accessions which were obtained from the Solanaceae laboratory (see table 1), COMAV, used for this research. Each accession has 15 seeds that grown in the petri dish before transplant to the pots (see Figure 6)

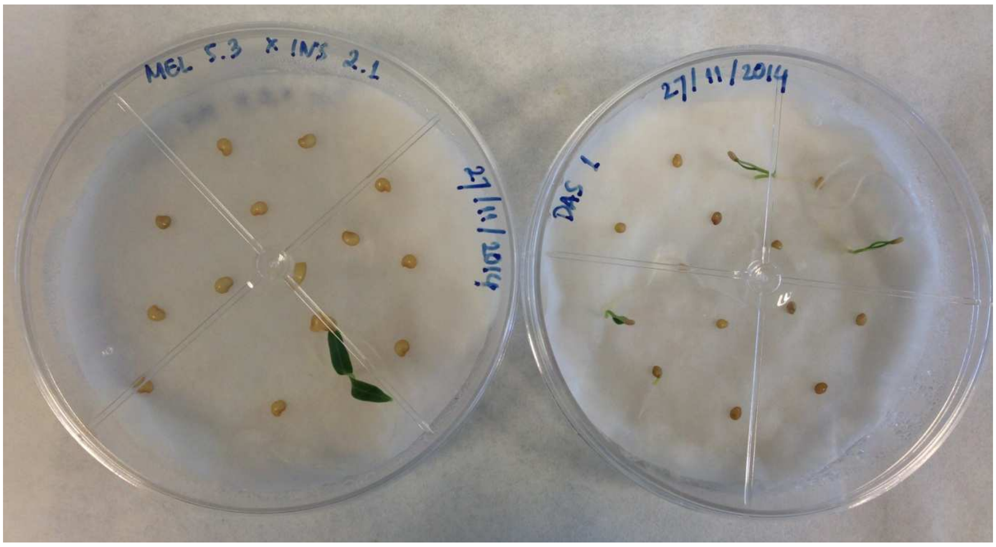

Figure 6. Eggplant's seeds were grown in the petridish

Table 1. Names of the 17 accessions used for the experiments

No	Entry	Species	Seeds
1.	Mm664	*Solanum incanum*	15
2.	DAS1	*Solanum dasyphyllum*	15
3.	Tom 1.1	*Solanum tomentosum*	15
4.	Ang 2.2	*Solanum anguivi*	15
5.	Ins 2.1	*Solanum insanum*	15
6.	Mel 5.1	*Solanum melongena*	15
7.	An-s.26 (1)	*Solanum melongena*	15
8.	An-s.26 (2)	*Solanum melongena*	15
9.	Lic 1.2	*Solanum lichtensteinii*	15
10.	Lin 1.2	*Solanum linneanum*	15
11.	Mel 5.3 x Inc. 1.1	*Solanum melongena x Solanum incanum*	15
12.	Mel 5.3 x DAS 1.3	*Solanum melongena x Solanum dasyphyllum*	15
13.	Mel 5.3 x Ins 2.1	*Solanum melongena x Solanum insanum*	15
14.	Mel 5.3 x Ang 2.2	*Solanum melongena x Solanum anguivi*	15
15.	Mel 5.2 x Lic 1.1	*Solanum melongena x Solanum lichtensteinii*	15
16.	Mel 5.1 x Lin 1.2	*Solanum melongena x Solanum linneanum*	15
17.	Tom 1.1 x Mel 5.1	*Solanum tomentosum x Solanum melongena*	15

From all the seeds, only a few of seeds grown up and were used for the experiment (table 2)

Table 2. Number of plants germinated per acession that could be used for the experiment

No	Entry	Species	Number of plant
1	Ins 2.1	*Solanum insanum*	15
2	Das 1	*Solanum dasyphyllum*	5
3	Mel 5.1	*Solanum melongena*	15
4	Lin 1.2	*Solanum linneanum*	10
5	Ang 2.2	*Solanum anguivi*	8
6	Mel 5.2 x das 1.3	*Solanum melongena x Solanum dasyphyllum*	9
7	Mel 5.3 x Ins 2.1	*Solanum melongena x Solanum insanum*	15
8	Mel 5.3 x Ang 2.2	*Solanum melongena x Solanum anguivi*	5
9	Mel 5.2 x lic 1.1	*Solanum melongena x Solanum lichtensteinii*	5

Note : Not all the seeds grown up in petri dish, only Das, Ins, An-s, Mel. x Inc., Mel x Das and Mel x Ins. Meanwhile, Ang, Lin, Mel, and Mel x Ang germinated in the soil. Nevertheless, since we used ≥ 5 plants of eggplants, so we only used 9 accession for doing the experiment (see table 2)

3.2.Experimental Design

3.2.1. Cultural Practices

The research were conducted in the laboratory of Solanaceae, COMAV, UPV during the beginning of December 2014 until the middle of March 2015 (14 weeks). The seeds and the plants were grown and treated in the growth control room in COMAV, UPV.

Firstly, seeds of the accession of eggplant, wild relatives and their hybrid were grown in the petri dish. Afterwards, they were transplanted to the pot (±3 cm) (see figure 7). In addition, the seeds which didn’t grow in petri dish were also transplanted into the pots which filled by soil. Next, they were irrigated daily until having three-to-four leaves stage prior to the drought treatment.

Figure 7. seedling from eggplants seeds which transplants to the pots

Thus, a number of plants with three-to-four leaves stages were transplanted again to the pot before doing the treatment (see figure 8).

Figure 8. Image of three-to-four stages of eggplant’s plant which transplant to the pots

Both of the seeds, seedling and the plants were placed in the climatic chamber with 16h light/8h dark with 25̊ C temperature.

3.2.2. The Deficit-Irrigated treatment

Two drought treatment (water deficit and PEG 7%) and one control were applied to a number of grown genotype plants for 55 days (the middle of January 2015 – the middle of March 2015). In the control treatment, the plants were irrigated with proper amount of water depend on the genotype in twice a week (see Table 3). Plants under deficit irrigation treatment were watered a half of the control plants. The plants under PEG solution were irrigated with PEG 7% (Polyethylene glycol, mol wt.8, Sigma-Aldrich) once or twice per week avoiding plant toxicity. As it was impossible to germinate 15 seeds of each accession, so PEG treatment was only employed to Ins 2.1, Mel 5.1 and Mel 5.3 x Ins 2.1. Well-watered plants as control, got 100% of irrigation, water-deficit plants, only got 50% of irrigation, whereas PEG 7% plants got 85% of irrigation. All the plants were also fertilized every 2 weeks after plant emergence. The plants were placed in a climatic chamber with 16h light/8h darkness photoperiod with 25̊ of temperature. The measurement for both control and treatment were done on february and on march.

Table 3. Number of plants evaluated in each treatment and the average amount of water per plant applied twice a week during 55 days

No	Genotype	type of treatment	Number plant	average amount of water (ml)
1	Ins 2.1	Control	5	76,39
1	Ins 2.1	Deficit Irrigation	5	42,36
1	Ins 2.1	PEG	5	63,89
2	Das 1	Control	2	75,00
2	Das 1	Deficit Irrigation	3	41,91
3	Mel 5.1	Control	5	72,06
3	Mel 5.1	Deficit Irrigation	5	40,44
3	Mel 5.1	PEG	5	63,89
4	Lin 1.2	Control	5	63,24
4	Lin 1.2	Deficit Irrigation	5	36,03
5	Ang 2.2	Control	4	64,71
5	Ang 2.2	Deficit Irrigation	4	36,76
6	Mel 5.2 x das 1.3	Control	5	77,78
6	Mel 5.2 x das 1.3	Deficit Irrigation	4	43,06
7	Mel 5.3 x Ins 2.1	Control	5	75,00
7	Mel 5.3 x Ins 2.1	Deficit Irrigation	5	41,67

7	Mel 5.3 x Ins 2.1	PEG	5	63,89
8	Mel 5.3 x Ang 2.2	Control	2	72,06
8	Mel 5.3 x Ang 2.2	Deficit Irrigation	3	40,44
9	Mel 5.2 x lic 1.1	Control	2	71,43
9	Mel 5.2 x lic 1.1	Deficit Irrigation	3	42,86

3.3. Drought Tolerance Assesment

Plant height, foliar length and width (from three leaves) were measured with ruler to calculate Leaf Area (LA) as the length per width divided by 2. Plant fresh and dry weight were measured. Water used efficiency (biomass) were measured as the ratio of dry weight and total amount of water used.

Green colour levels were determined using colorimeter Minolta CR-300 in three replicas per each plant.

Then, after 8 weeks of treatments, all of the plants were measured with an Infrared Gas Analyzer (Li-Cor 6400, Nebraska, USA) for photosynthetic rate (A), transpiration rate (E), stomatal conductance to H_2O (gs) and Intercellular CO_2 concentration (Ci). All of measurements were done in the morning, out of growth climatic chamber, with a 900 PAR and with CO_2. Afterwards, water-used efficiency (intrinsic and instantaneous) were calculated from ratio between photosynthetic rate (A) and stomatal conductance (g) or ratio between photosynthetic rate (A) and transpiration rate (E).

Figure 9. (left) Image of Li-cor 6400 portable photosynthesis system. The eggplant leaves were measured out of growth climatic chamber in the morning. (right) Illustration when the leaves were measure using Li-cor 6400 portable photosynthesis (the eggplants leaves measurement was not recorded).

3.4.Data Analysis

All the data which obtained from the measurement were put into microsoft excell and ANOVA was performed with statgraphics.

4. RESULTS AND DISCUSSION

4.1. Plant Growth Parameter

Water stress limits plant growth rate including of decline in plant height, leaf area, number of branches, and dry weight of shoots and roots (Byari and Al-Rabighi, 1995; Antholin and Sanchez-Diaz, 1992; Aranjuelo *et al*., 2007). Nevertheless, the drought-tolerant plants have the ability to minimize their impaired growth rate. They still can growing, flowering and displaying the economic yield under water scarcity (Beck *et al*., 2007; Farooq *et al*., 2009[a]). Eggplants, their wild allied and their hybrids have variable levels of drought-tolerance as can be seen in the following results.

4.1.1. Leaf Area and Plant height

ANOVA analysis for Leaf Area and plant height (table 4) indicated that there were significant differences among genotypes and treatments. The general effect of the drought treatment was to reduce LA and plant height. In any case there was significant genotype x treatment interactions meaning that all the genotypes were affected to a certain extend by the water deficit.

Table 4. Multifactor ANOVA results table showing the effects of the *Solanum* accessions and treatments (water deficit and control) and the genotype over Leaf Area and Plant Height

		Mean squares	
	df[1]	Leaf Area (cm^2)	Plant Height (cm)
Main effects			
Treatment (T)	1	5903^{***}	50^{***}
Accession (A)	8	3401^{***}	31^{***}
Interactions			
TxA	8	343^{ns}	1^{ns}
Error	54	514	4

[1] Degrees of freedom; ^{ns} non-significant, *,** ,*** significant at P-value < 0.05, 0.01, and 0.001 respectively

Despite of lack interraction of genotype x treatment, the individual analysis on the leaf area and plant height measurements showed that different responses against drought condition. *Solanum melongena* (Mel), *S. dasyphyllum* (Das) and *S. linneanum* (Lin), had significant reduction in their plant height and leaf area, whereas *S. melongena x S.lichtensteinii* hybrid (Mel x Lic) only has significant decrease in leaf area.

Regarding the LA (leaf area) and plant height data, *Solanum melongena* (Mel), *S. linneanum* (Lin) *S. dasyphyllum* (Das) and *Solanum melongena x Solanum lichtensteinii* hybrid (Mel x Lic) were more sensitive to drought condition (Figure 10).

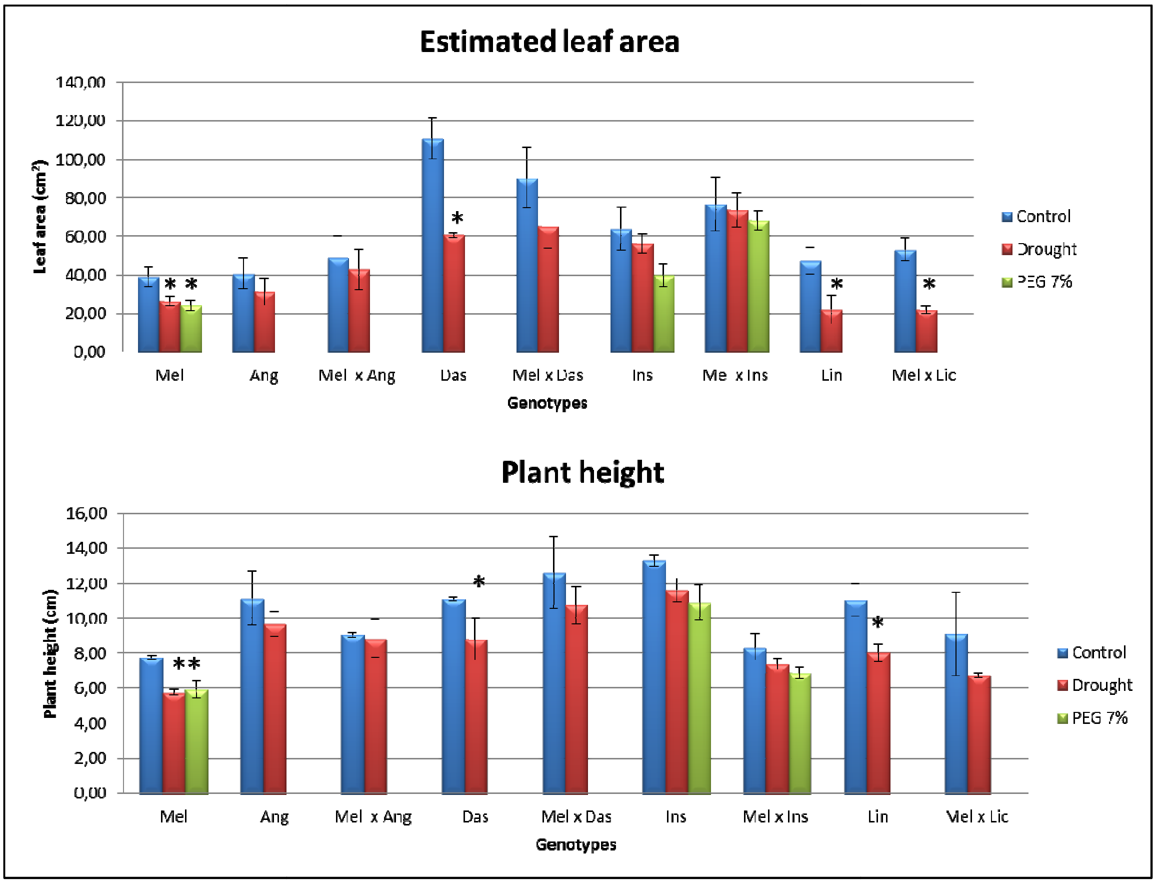

Figure 10. Bar diagram showing estimation measurement for leaf size area and plant height for every accession assayed under control and water deficit conditions. * indicate significant differences between treatment values within an accession at P-value < 0.05

Solanum anguivi and *S. insanum* were poorly affected by drought treatment, in our experiment were observed. Their hybrids with *S. melongena* perform well being the *S. melongena* x *S. anguivi* (Mel x Ang) hybrid the genotype less affected by the drought conditions. Interestingly, the reduction in LA (Leaf Area) and plant height in the hybrid *S. melongena* x *S. dasyphyllum* (Mel x Das) hybrid was less intense than in any of its parents (figure 12).

Figure 11. Comparation of eggplant growth between control, drought and PEG 7% in the genotypes *Solanum melongena x Solanum insanum* hybrid (Mel x Ins) hybrid. Leaf size, plant height and number of leaves were decrease in both water-deficit treatment and PEG 7%. Leaves scarcities were also occured in both treatments.

Figure 12. Comparation between control and water-deficit treatment on *Solanum melongena x Solanum dasyphyllum* (Mel x Das) hybrid. Leaves size and plant height decreasing occured in water-deficit

4.1.2. Biomass Analysis

Drought stress can reduce production of plant biomass. Nonetheless, the reduction of biomass applied differently in plants organs (Krisnamurthy *et al*., 2011). To put it another way, water stress is effective to decreasing leaf biomass, but not shoot or roots biomass. Moreover, roots tend to be less reduced than other organs and usually drought-tolerance plants have higher root biomass than susceptible ones (Spollen *et al*., 1993; Erice *et al*., 2010). A well-developed roots system will allow plants to exploit deep soil water in drought-prone environment. Therefore, the accumulation of dry matter will re-allocating to the roots rather than in other plant organs (Xu *et al*., 2010; Esmailpour *et al*., 2015).

In this experiment, ANOVA analysis for fresh weight roots, dry weight roots, fresh shoot biomass and dry shoot biomass (table 5) concluded that there were significant differences among genotypes. The general effect of drought treatment was not really significant on reduces roots weight. Despite lack interraction of genotype x treatment, each of genotype has different response to drought condition (figure 13)

Table 5. Multifactor ANOVA results table showing the effects of the *Solanum* accessions and treatments (water deficit and control) and their genotype over fresh weight roots, dry weight roots, fresh shoot biomass and .

		Mean squares			
	df[1]	Fresh weight roots	Dry weight roots	Fresh Shoot biomass	Dry Shoot biomass
Main effects					
Treatment (T)	1	$45{,}54^{**}$	$0{,}01^{ns}$	$277{,}56^{***}$	$3{,}27^{**}$
Accession (A)	8	$28{,}\ 11^{***}$	$0{,}36^{*}$	$86{,}98^{***}$	$0{,}89^{*}$
Interactions					
TxA	8	$8{,}71^{ns}$	$0{,}01^{ns}$	$9{,}91^{ns}$	$0{,}19^{ns}$
Error	54	16,85	0,10	14,74	0,40

[1] Degrees of freedom; ^{ns} non-significant, *, **, *** significant at P-value < 0.05, 0.01, and 0.001 respectively

Furthermore, the roots weight increased in genotypes with good response in drought condition, *Solanum anguivi* (Ang), *S. insanum* (Ins) and *S. melongena x S. insanum* (Mel x Ins) hybrid (figure 13). Interestingly, *S. melongena x S. dasyphyllum* (Mel x Das) hybrid which performed better than any of the parents also showed an increase in dry root weight under stress.

The individual analysis of shoot biomass showed that the drought do not really influence the reduction of accumulation dry matter in eggplants leaves. A good response in drought was performed well by *S. melongena x S. anguivi* hybrid (figure 14 and figure 15). Probably, it happened because *Solanum melongena x Solanum anguivi* hybrid could maintain their photosynthesis to accumulate dry matter eventhough in drought stress. Meanwhile, *S. melongena x S. dasyphyllum* hybrid, *S. insanum, and S. melongena x S. insanum* hybrid were also showed tolerance to drought stress (figure 13). The less reduction of accumulation dry matter occured on those genotypes.

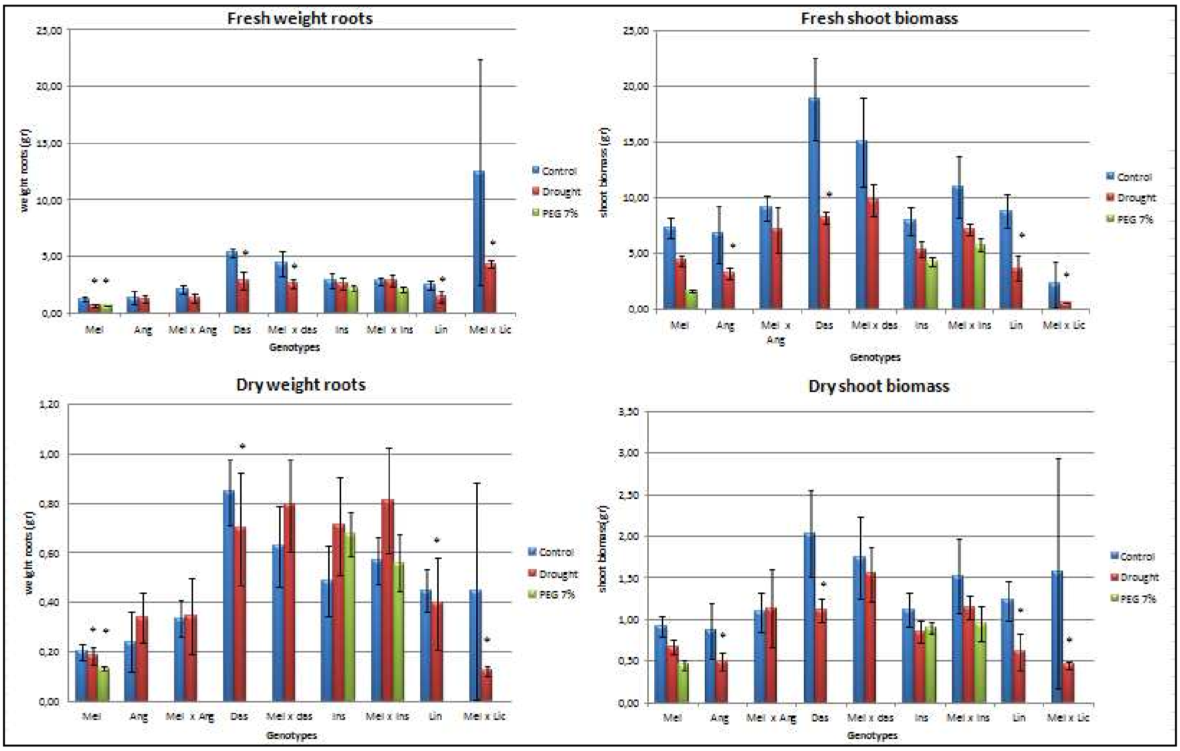

Figure 13. Bar diagram showing estimation of fresh weight roots, dry weight roots, fresh shoot biomass, and dry shoot biomass for every accession of *Solanum spp.* assayed under control and water deficit treatment. * indicate significant differences between treatment values within an accession at P-value < 0.05

Figure 14. Comparation between control and water-deficit of *Solanum melongena x Solanum anguivi* (taken from front of the pots). Deficit-watered plant experienced reduction at plant height and leaves size. However, they still growth well.

Figure 15. Comparation between control and water-deficit of *Solanum melongena x Solanum anguivi* (taken from above). It seems that the leaves of *Solanum melongena x Solanum anguivi* hybrid were could grow better in drought.

4.2.Physiological state analysis

Drought stress can impair plant growth rate by influence their physiological and biochemical process, such as photosynthesis, transpiration, ion uptake, etc. (Farooq *et al*., 2009[b]). Plants under drought stress tend to reduce their photosynthesis. In severe drought, plants can close their stomata, decrease their internal CO_2 concentration (Ci), reduce transpiration rate, and inhibit photosynthesis rate (Dulai, 2006; Rahbarian *et al*., 2011). However, despite the closure of stomata, the drought-tolerant plants usually are able to maintain their photosynthesis under drought stress.

The multifactorial analysis of variance showed that there were significant differences in the average values among treatments (table 10) for the photosynthetic rate (A), transpiration rate (E), stomatal conductance of H_2O (g_s) and the intercellular CO2 concentration (C_i). There were also significant differences in the averages among accessions, and there were no treatment x accession interaction.

Table 6. Multifactor ANOVA results table showing the effects of the *Solanum* accessions and treatments (water deficit and control) and their genotype over the photosynthetic rate (A), transpiration rate (E), stomatal conductance of H2O (g_s) and the intercellular CO_2 concentration (C_i) values.

		Mean squares			
	df[1]	A (μmol CO_2 m^{-2} s^{-1})	E (mmol H_2O m^{-2} s^{-1})	g_s (mol H_2O m^{-2} s^{-1})	C_i (μmol CO_2 mol^{-1})
Main effects					
Treatment (T)	1	17.68^{**}	2.99^{***}	0.013^{***}	5273^{*}
Accession (A)	8	13.92^{***}	0.83^{***}	0.01^{***}	18321^{***}
Interactions					
TxA	8	3.90^{ns}	0.22^{ns}	0.001^{ns}	1940^{ns}
Error	55	1.92	0.16	0.0006	1281

[1] Degrees of freedom; ^{ns} non-significant, *, **, *** significant at P-value < 0.05, 0.01, and 0.001 respectively

In general, the IRGA showed low values for photosynthetic variables (A, E, g_s and C_i) this means that all plants, regardless the treatment, were a little bit stressed (table 7) This could be due to the fact that the measures were done out of the growth chamber in a windy morning of March, therefore plants were under wind (which causes the closure of the stomata) and in a cold morning (which does not stimulate the metabolism). Nevertheless, ANOVA analysis showed there were significant differences among treatments indicating that there existed a drought stress.

Table 7. General averages by treatment of the 17 *Solanum* accessions assayed. Each value is the mean of at least 37 plants ± SE.

	A (μmol CO_2 m^{-2} s^{-1})	E (mmol H_2O m^{-2} s^{-1})	g_s (mol H_2O m^{-2} s^{-1})	C_i (μmol CO_2 mol^{-1})
Control	4.4 ± 0.3	1.29 ± 0.1	0.07 ± 0.01	263± 11
Water deficiency	3.7 ± 0.3	0.84 ± 0.06	0.05 ± 0.005	250± 8

Photosynthetic rate (A), transpiration rate (E), stomatal conductance of H_2O (g_s) and the intercellular CO_2 concentration (C_i) values

Despite the lack of interaction T x A, the individual analysis of the photosynthesis measurements showed that the stress effect of the water deficit condition was not equal in every accession (Figure 16). *Solanum melongena* (Mel) and *Solanum dasyphyllum* (Das 1) were very sensitive to drought. Interestingly, *Solanum melongena x Solanum dasyphyllum* hybrid *(*Mel x Das) were not affected by the water deficit. *Solanum Insanum* (Ins) showed significant reduction in stomatal conductance, intercellular CO_2 concentration and transpiration rate but it has higher photosynthetic rate. *Solanum melongena x Solanum lichtensteinii* hybrid (Mel x Lic) also showed great sensitivity to water deficit conditions.

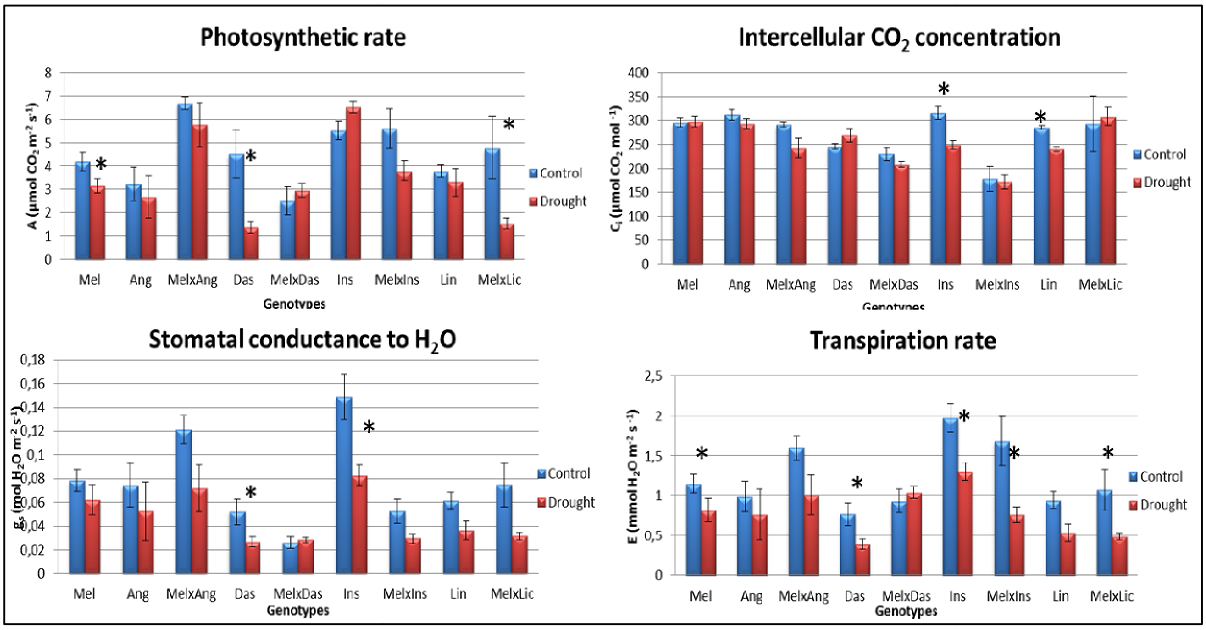

Figure 16. Bar diagram showing the average value for the photosynthetic measures for every accession assayed under control and water deficit conditions. * indicate significant differences between treatment values within an accession at P-value < 0.05.

Solanum melongena x Solanum insanum hybrid has significant reduction only in transpiration rate. Whereas *Solanum linneanum* only has significant reduction in intercellular CO_2 concentration.

4.3. Green-Color Level Analysis

Chlorosis usually occurs in the plants which experience water stresses. It happens as consequence of chlorophyll degradation in the leaves. Chlorophyll, which comprises of chlorophyll a and chlorophyll b, is responsible for green colour in the leaves. It has main role for catch the light in different spectrum for photosynthesis process in the leaves. However, the chlorophyll breakdown will occur along with leaf senescence as effect of drought (Hortensteiner and Krautler, 2011). As declining of chlorophyll, leaves won’t able to do the photosynthesis. Thus, it will cause the lack of nutrition in the plants which will lead to cell plant death. On the other hand, the drought-tolerance plants usually had higher chlorophyll content than sensitive one under water scarcity (Pastori and Trippi, 1992; Zaeifizade and

Goliov, 2009). They still can manufacture carbohydrates to survive in dry environment. The simple analysis on green-color level was done during this study using colorimeter. The CIE L, a and b scale were used for analyze the color in the eggplants leaves (Figure 17).

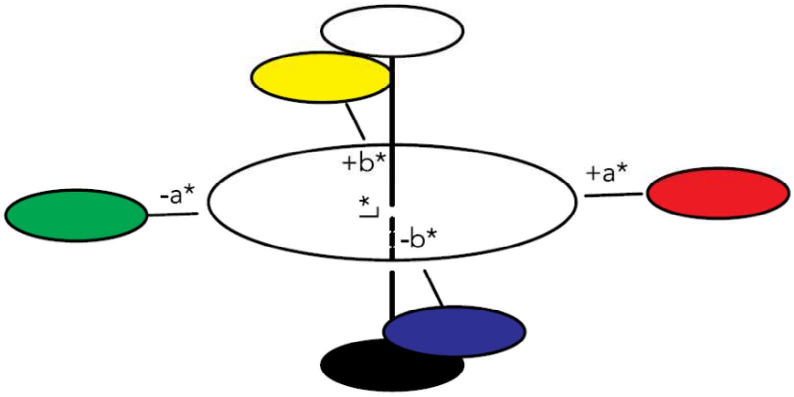

Figure 17. Diagram CIE L*, a* and b* scale. L means the lightness of color. The L axis runs from top to bottom. Maximum L = 100, defines as white. Minimum L =0, defines as black. The a and b axis have not specific limited. Positive a is red. Negative a is green. Positive b is yellow. Negative b is blue (courtesy : x-rite.com)

Water stress will cause chlorosis in the leaves. Green color in the leaves will turn into yellow color. In the mild case, the leaf tissue is pale green and the leaf veins remains green. In the moderate case, the tissue between leaf veins is bright yellow. In the advanced case, the leaf tissue will be pale white to pale yellow. The leaf marginal may develop brown, angular spot between tissue, wither and drop prematurely (The Morton Arboretum, 2016).

ANOVA analysis for color level in leaves of *Solanum* spp. (table 8) exhibited that the significant differences occured among genotypes. Drought treatment did not really influence the green/yellow color change level. However, each of genotypes possesed different changing green/yellow-color level under drought condition.

Table 8. Multifactor ANOVA results table showing the effects of the *Solanum* accessions and treatments (water deficit and control) and their interaction over green/yellow color-level in *Solanum* spp. leaves.

		Mean squares		
	df[1]	L	a	B
Main effects				
Treatment (T)	1	57,63*	15,82^{ns}	129,40**
Accession (A)	8	144,02***	44,64***	171,11***
Interactions				
TxA	8	8,76^{ns}	16,42***	40,89**
Error	176	12,14	4,65	16,08

[1] Degrees of freedom; ^{ns} non-significant, *, **, *** significant at P-value < 0.05, 0.01, and 0.001 respectively

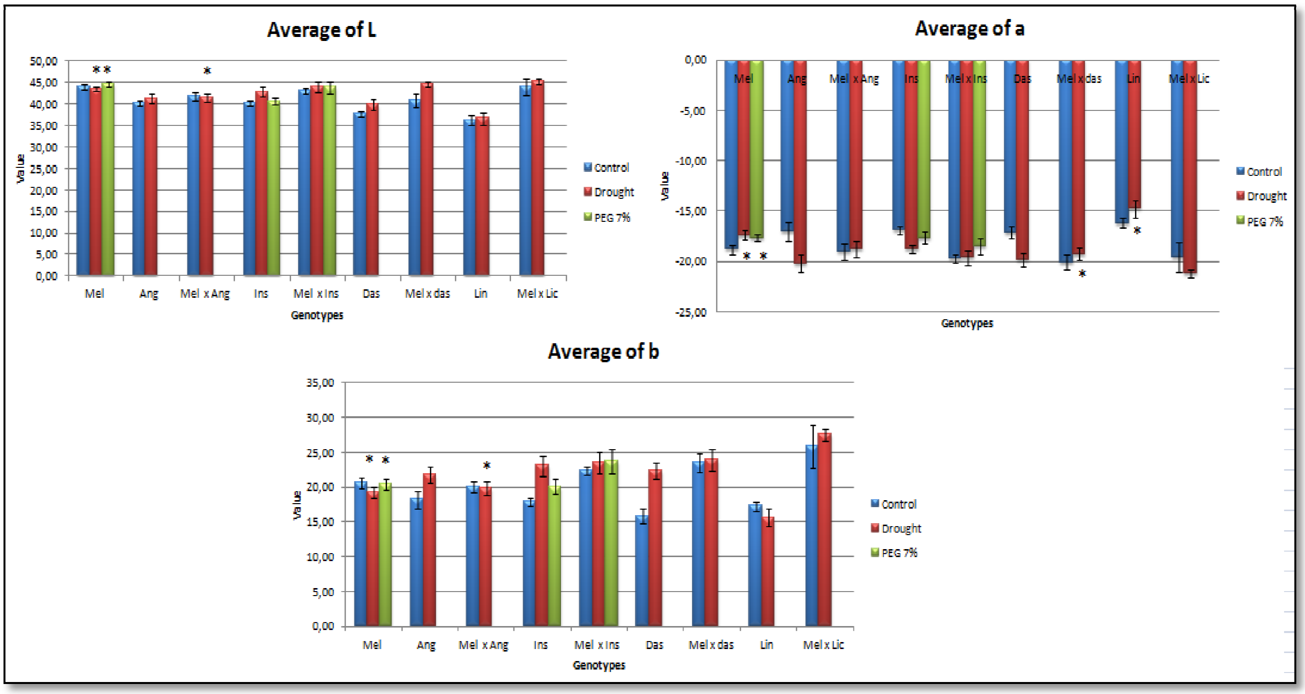

Figure 18. The average value of color in the eggplants leaves for every acession assayed under control, water-deficit and PEG 7%. . * indicate significant differences between treatment values within an accession at P-value < 0.05

The individual measurement of color on the leaves using colorimeter also showed that the drought do not really influence the alteration color of *Solanum* spp. leaves (figure 18). However, it showed that *S. melongena* was very affected by water-deficit treatment since it experienced a reduction on bright and green/yellow level. On the other hand, *Solanum anguivi*, *S. insanum* and their hybrid with *S. melongena* exhibited stability bright green-yellow color on their leaves. In addition, *S. melongena x S. dasyphyllum* was also showed tolerance to drought as well as their color consistency.

4.4. Water-Use Efficiency

Analysis on the water-use efficiency means measure the plant's efficiency on using water. WUE can be analyzed from different level and temporal scales, ranging from instantaneous measurement into integrative ones at the plant and crop level. Sometimes, instantaneous analysis was used as representative for explain WUE in the plant (Medrano *et al*., 2015). Nonetheless, it must be noted that analysis on WUE biomass and WUE yield were needed spatial and temporal scale which differ from Instantaneous WUE (Tambussi *et al*., 2007).

In this research, the measurement of WUE was only done to the leaf level (WUE intrinsic and WUE instantaneous) and biomass (WUE biomass). The individual analysis on accession of *Solanum* spp. exhibited that there is differences between WUE intrinsic/instantaneous and WUE biomass.

Multifactorial analysis on the WUE intrinsic (table 9) showed that the significant differences occured among genotype, but not among treatment and their interraction. Meanwhile, ANOVA analysis over WUE instantaneous (table 9) exhibited the significant differences among genotype, treatment and their interraction. It assumed that the drought do not influence the photosynthesis rate, but did affect to the transpiration rate of *Solanum spp* (figure 19).

Table 9. Multifactor ANOVA results table showing the effects of the *Solanum* accessions and treatments (water deficit and control) and their interaction over WUE intrinsic, WUE instantaneous, and system WUE

		Mean squares		
	df[1]	WUE intrinsic	WUE instantaneous	SystemWUE
Main effects				
Treatment (T)	1	1708,34[ns]	6,05[*]	3,54[ns]
Accession (A)	8	6299,68[***]	4,3[***]	1,76[ns]
Interactions				
TxA	8	840,79[ns]	3,23[**]	0,80[ns]
Error	55	493,05	1,08	0,92

[1] Degrees of freedom; [ns] non-significant, [*], [**], [***] significant at P-value < 0.05, 0.01, and 0.001 respectively

According to the result, it showed that *S. melongena, S. Dasyphyllum* and *S. melongena x S. lichtensteinii* hybrid were very sensitive to drought condition. Their WUE are lower than others.

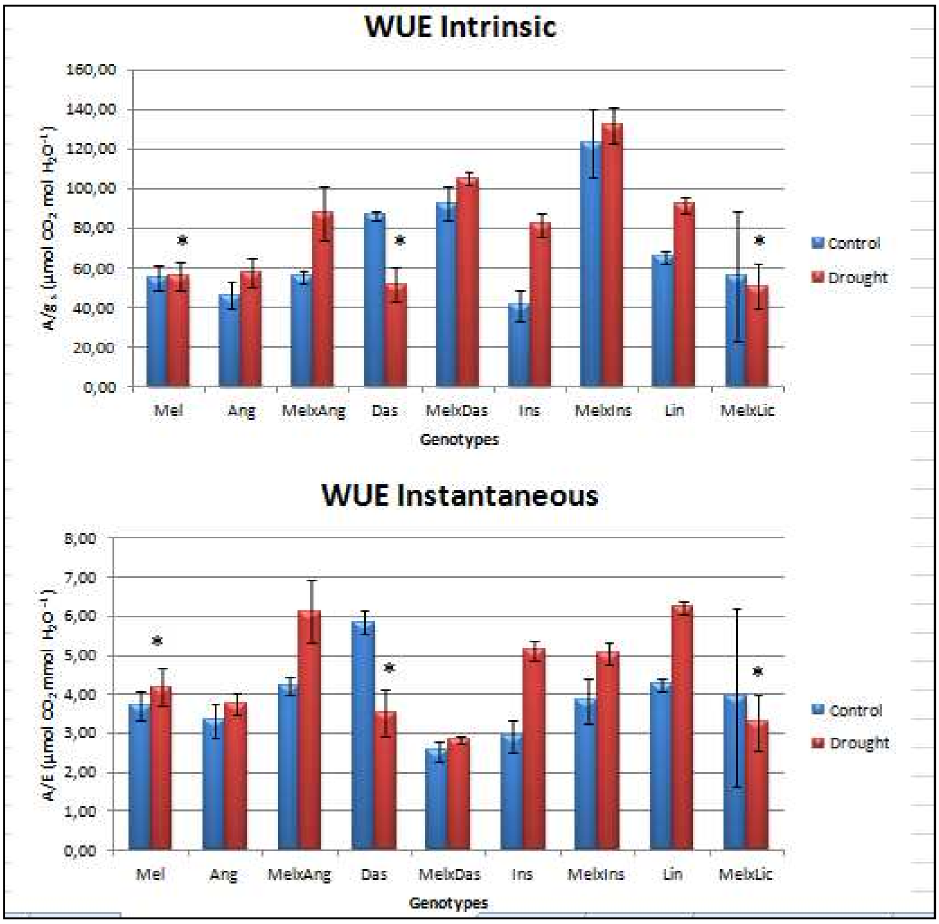

Figure 19. Bar diagram showing the average value of WUE intrinsic and WUE instantaneous for every acession of *Solanum spp*. assayed under control and water-deficit treatment. * indicate significant differences between treatment values within an accession at P-value < 0.05.

S. anguivi, *S. insanum* and their hybrid with *S. melongena* have higher WUE than others. In addition, *S. linneanum* also showed their ability to maintain their WUE intrinsic and instantaneous. Despite *S. dasyphyllum* has not good response to drought, their hybrid with *S. melongena* exhibited a good maintenance in their photosynthesis rate and their transpiration rate.

On the other hand, the system WUE biomass (whole plant WUE) has difference result with the WUE in the single leaf. Multifactorial analysis over system WUE biomass (table 9) showed there were not any significant differences among genotype, treatment and their interraction.

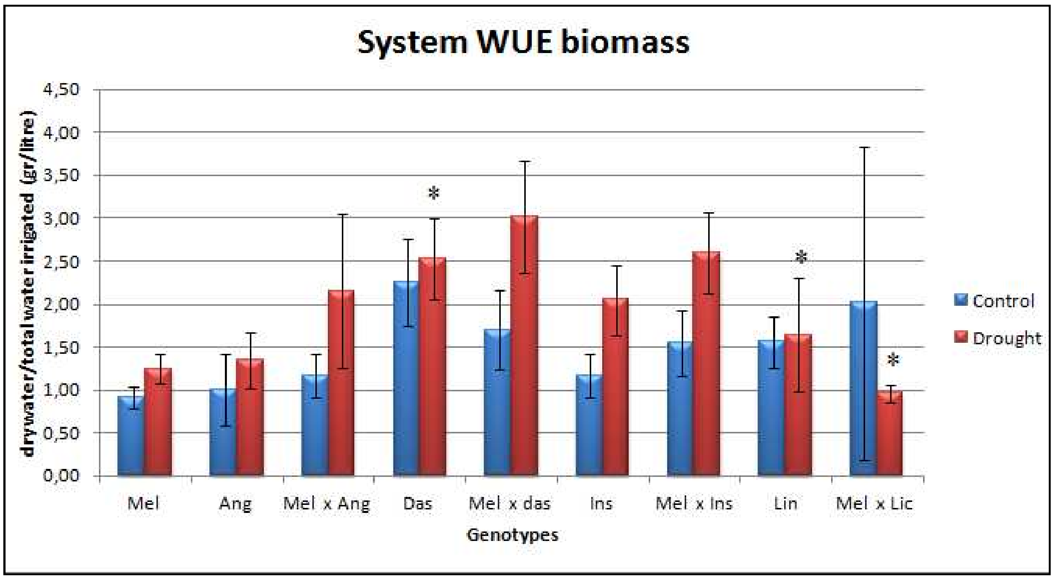

Figure 20. Bar diagram showing the average of system WUE biomass for every acession of *Solanum spp.* assayed under control and water-deficit treatment. * indicate significant differences between treatment values within an accession at P-value < 0.05.

The ratio of accumulation dry matter per total amount of irrigated water for every acession under water-deficit treatment showed that most genotype of plants under water-deficit have higher of WUE than control one (figure 20). *Solanum anguivi* (Ang), *S. insanum* (Ins) and their hybrid with *S. melongena* have higher WUE biomass. In addition, *Solanum melongena x Solanum dasyphyllum* (Mel x das) possesed the best of WUE biomass from any of its parents.

The comparison between WUE intrinsic and WUE whole plant exhibited difference result. The difference on the WUE biomass and WUE intrinsic likely due to their method approach. It should be noted that the accumulation of assimilated dry matter in plant needed longer time. Moreover, the measurement on dry weight for WUE biomass also comprises of dry weight in leaf, shoot, and roots, whereas WUE intrinsic only measured one single leaf. Therefore, the higher WUE should not be equated with yield or biomass since we have to consider other factors which influence the drought-tolerance traits.

4.5.Final Remarks

Eggplants, their wild relatives and their hybrid have ability to survive in drought condition. The variation level of drought-tolerance on eggplant have detected in the previous research. A simple analysis was conducted for comparative study on nine genotypes of *Solanum spp*. and their hybrid. The pot experiment under control, water-deficit treatment and PEG 7% treatment irrigation on those 9 genotypes assumed that water scarcity certainly influence plant growth rate (leaf area, and plant height), biomass, green level color, water-use efficiency, photosynthesis rate, stomatal conductance and transpiration rate in the *Solanum spp.*

Drought stress often reduce plant growth development, decrease chlorophyll content, decline photosynthetic rate, close stomata and reduce dry weight of plant. According to these experiments, drought treatment induced an average reduction of 17% in plant height, 30% in leaf area, and 32% in dry shoot biomass. Nevertheless, drought-tolerance plant has ability to minimize drought impact by maintain their photosynthesis rate and evapotranspiration rate to produce great yield. Likewise, *S. anguivi, S. insanum*, and their hybrid with *S. melongena* (*Solanum melongena x Solanum insanum* hybrid, *Solanum melongena x Solanum anguivi* hybrid) emerge as genotypes that tolerant to drought condition. In addition, *Solanum melongena x Solanum dasyphyllum* hybrid also seems has ability for survive in drought stress. Those genotypes can minimize drought impact to themselves since they only experience slight reduction on their plant height and their size area. Their roots dry weight in water-deficit treatment have an average weight increment of 32% than control one which means their roots grew longer to find the moisture soil. They also could maintain their photosynthetic rate and minimize their transpiration. Furthermore, they have higher water-use efficiency comparing with other genotypes.

By contrast, *Solanum melongena*, *Solanum dasyphyllum*, *Solanum linnaenum* and *Solanum melongena x solanum lichtensteinii* exhibited sensitivity in drought condition. Their growth plant rate, dry weight roots, plant height and size area seems experience significant decrease. They also seems couldn’t manage their water-use efficiency. Based on the result, it can be assumed that the hybrid eggplants possess drought-tolerance traits. It probably due to the introgression of drought-tolerance traits from the wild relatives to eggplant was successful. However, it seems that the ability of drought-tolerance also formed when eggplant and wild genotypes were united.

The eggplant hybrids tend have more tolerant to drought condition, such as *Solanum melongena x Solanum insanum* hybrid, *Solanum melongena x Solanum anguivi* hybrid and *S. melongena x S. dasyphyllum* hybrid. Nevertheless, it depends on the wild relatives, whether it has tolerant to drought stress or not. Likewise, the previous research (Zamkova, 2015) about roots of eggplants, wild relatives and their hybrid in vitro compelling evidence as pot experiment. It was explained that *Solanum melongena x Solanum insanum* hybrid and *Solanum melongena x Solanum anguivi* hybrid have longer deep growing roots with many lateral roots. In the other words, those genotypes have more tolerant to dry environment.

Having said that, *Solanum melongena x Solanum lichtensteinii* hybrid seems less tolerant than other genotypes.

Low and uneven germination of wild relatives and hybrids were the main problems of this research. Most of eggplants, wild relatives and their hybrid seeds did not germinated in petridish which only filled by water, except *Solanum melongena*, *Solanum melongena x Solanum dasyphyllum*, *Solanum melongena x Solanum insanum*, *Solanum insanum* (data not shown). Most of them germinated and grown better in pot which filled by soil. Nonetheless, *Solanum tomentosum* (Tom), *Solanum incanum* (Inc), *Solanum lichtensteinii* (Lic), *Solanum tomentosum x Solanum melongena* (Tom x Mel) did not germinate both in petridish nor in pot. Likewise, previous in vitro experiments (Zamkova, 2015) conviced the similar evidence as pot experiment. It was explained that wild eggplants and their hybrids showed difficulties in germination. Therefore, several protocol (soaking, adding KNO3, place plate in the light, and bleaching) were made to solve this germination problems in the previous research.

The limited number of growing *Solanum* spp. become first obstacle to gain information from PEG 7% treatment. PEG 7% treatment has function as water-deficit treatment which leads to drought in plants. Since it is similar to water-deficit treatment, so PEG 7% treatment only used for comparison with water-deficit treatment.

Maintaining eggplants, wild relatives and hybrids growth and development assayed under control, water deficit treatment and PEG 7% treatment were an important thing to have significant result. Since each genotypes has different ability to absorb water, so differ amount of water has applied according to their treatments (see material and method). It has function for keep and maintain soil moisture in eggplants. An amount of fertilizer has also provided to when they seems emergence.

In spite of having lack of information, the tentative result showed that there were a few of candidate wild relatives and their hybrid which more tolerant to drought stress as mentioned before. However, an advanced research are needed in the future for obtain new information.

5. CONCLUSION

The simple analysis on eggplants, wild relatives and their hybrids (*Solanum spp.*) assumed that there were variation level of drought-tolerance. The analysis were focused on plant growth rate (plant height and leaf area index), biomass, green-color level, photosynthesis rate, transpiration rate, stomatal conductance, intercellular CO_2 concentration and water-used efficiency. The comparison between control and water-deficit treatment exhibited that drought induced an average reduction of 17% in plant height, 30% in leaf area, and 32% in dry shoot biomass. Accordingly, it concluded that *S. anguivi, S. insanum, S. melongena x S. anguivi* hybrid, *Solanum melongena x Solanum insanum* hybrid, and *Solanum melongena x Solanum dasyphyllum* hybrid were more tolerant to drought condition.

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