EFFECTS OF SOIL WATER DEFICIT ON THE GROWTH AND PHYSIOLOGY OF SELECTED AFRICAN NIGHTSHADES *(Solanum scabrum* Mill. AND *Solanum villosum* Mill.*)*

BY

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ABSTRACT

Environmental stresses, such as water stress have a major impact on plant growth and survival. Lack of water causes reductions in growth rate and physiological processes affecting bioproductivity, which in turn lower agricultural production thereby contributing to food insecurity. This research was designed to investigate the effects of soil water deficit on growth and physiology of two African nightshades, which are widely consumed due to their high nutritional value. The aim of the study was to determine the effects of water deficit on their growth, chlorophyll fluorescence and gas exchange parameters, and on their chlorophyll and nitrogen content. The experiment was carried out at Maseno University, Botanic Garden glasshouse at Maseno University. The experiment was laid out as a completely randomized design (CRD) two factorial, consisting of four treatments and three replications. The treatments were: T1-watering daily (control), T2-watering after every three days (the 3rd and 6th day), T3-watering the 9th day and T4-watering the 12th day. African nightshades (*S. scabrum* Mill. and *S. villosum* Mill. subsp. *miniatum*, seeds were grown in 20 litre plastic pots in loam moist soils having a pH of around 4.6 to 5.4. Growth parameters measured included; plant height and the stem diameter using a meter rule and a veneer caliper respectively, and by counting the number of leaves. The root to shoot ratio was determined at the end of the experiment. Stomatal conductance and leaf temperature were determined by use of a steady-state porometer. Chlorophyll fluorescence was determined by use of a portable fluorescence monitoring system. Soil moisture content was determined gravimetrically. Chlorophyll content was determined through extraction and absorbance of chlorophyll solution read spectrophotometrically. Foliar chlorophyll content was determined using a chlorophyll meter. Statistical analysis involved analysis of variance (ANOVA) using MSTAT-C statistical computer package to determine the differences between the two species and the four treatments on the parameters using the Least Significant Difference test at 5% level. Results showed that the two species of African nightshades were significantly ($p \leq 0.05$) affected by water deficit. Growth parameters slightly increased with increase in water deficit and reduced significantly ($p \leq 0.05$) with further increase in water deficit. Water deficit caused a decrease in stomatal conductance and an increase in leaf temperature. The root to shoot ratio increased with increase in water deficit. Foliar chlorophyll content decreased with increasing water deficit among the treatments but also increased throughout the experimental period in all the treatments. Chlorophylls *a*, *b* and total chlorophyll also showed a general decrease with increasing water deficit. From the results obtained, it can be concluded that among the two species *S. scabrum* Mill. was more tolerant to water deficit and therefore it is recommended to be grown in water deficient regions as compared to *S. villosum* Mill. The results of this study can be used to recommend better management plant strategies to drought, as it considers effects of water deficit on the growth and physiology of the two African nightshade species.

CHAPTER ONE

1.1 Background information

Food and nutrition is a basic need for life yet agricultural production has been declining in Africa since 1980 and Kenya is no exception (Oniango, 2001). For instance, over 89% of Kenyans are food insecure and are malnourished especially in rural areas (Oniango, (2001). Kenya's agricultural production remains inadequate and has not made any progress on the food security front (Nyoro *et al.,* (2007), as a consequence Kenyans remain food insecure and are increasingly relying on emergence food supplies and commercial food imports moreso for survival (MOA, 2010). In Africa, African nightshades *S. scabrum* Mill. and *S. villosum* Mill. are probably the second most important group of indigenous leaf vegetables after Amarathus (Schippers,2002). In some places they even surpass exotic vegetables such as cabbages and kales. In Kenya more than 80% of its landmass is either arid or semi-arid (Luvaha *et al.*, 2008), it is characterized by a high population of poor households whose entire livehood depends on farming as an economic activity, and drought as worsened the poverty status, but Africa nightshades can perform well in areas with limited rainfall Schippers (2002).

Plants are often subjected to periods of soil water deficit during their life cycle due to erratic nature of rainfall (Silva *et al.,* 2007). Water deficit is one of the main limiting factors affecting plant distribution and impose selective pressure in the evolution of plant morphology and physiology (Bohnert *et al.,* 1995; Boyer, 1982; Stebbins, 1995). Any shortage in water supply in relation to the requirement of plants results in water deficit hence plants become stressed. Water is needed for numerous processes during plant growth and development, for it constitutes 50-90% of the weight of all plant tissues (Kramer, 1983). Water deficit occurs when water potential in the rhizosphere is sufficiently negative to reduce water deficit to suboptimal levels for plant growth and development (Zhongjin and Tamar,

2003). Water can limit crop growth and productivity either because of unexpected dry periods or abnormally low rainfall that makes regular irrigation necessary (Salisbury and Ross, 1992). Water deficit is often associated with regions receiving insufficient rainfall, however under adequate rainfall or irrigation plants may experience transient stress during noon hours of hot days. This transient stress has detrimental effects that persist long after water deficit removal (Mckersie and Ya'acov, 1994). The most frequent cause of water stress in plants is a sub optimal soil moisture supply, salinity and rate of transpiration in excess of the rate of absorption of water by roots (Bohnert *et al.*, 2004). As a general rule, severe water deficit begins to be evident in most plant species when the water potential drops to about -0.14 to -0.15 MPa (Levitt, 1980). At this level most physiological processes (for example, cell enlargement, growth and net photosynthesis and respiration) reach very low levels or cease altogether (Noggle and Fritz, 1977). Most plant physiological processes are influenced directly by plant water deficit and indirectly by atmospheric water deficit (Levitt, 1980).

The consequences of water deficit to the plants if unrelieved may develop into permanent wilting, dehydration and finally death (Fitter and Hay, 1987). Therefore, leafy vegetable growers might rely heavily on irrigation to meet production goals. However, water for irrigation is a limited resource and its effective management is critical not only in reducing wasteful usage but also in reducing production costs and sustaining productivity. Sustainable production of African nightshades can be attained through technology that makes effective and efficient use of the erratic rainfall resource (Ogindo, 2003), because they are highly recommended due to their high nutritional quality (Modi, *et al.*, 2006). Identification of drought tolerant African nightshade species is thus crucial in sustaining production in areas where water supply is limited (Fageria, 2007). Whereas African nightshades leaves are harvested and used for human consumption, Muthomi and Musyimi (2009) reported a significant reduction in leaf numbers for *S. scabrum* Mill. seedlings exposed to water deficit,

but it is not clear whether *S. scabrum* Mill and *S. villosum* Mill. have a similar response in growth and physiology. Thus this study focused on the effects of soil water deficit on the growth and physiology of two African nightshade species *S. scabrum* Mill and *S. villosum* Mill. which are very important leafy vegetables in Kenya. Not much has been done on the effects of soil water deficit on the growth and physiology of leafy vegetables (Thobile *et al.*, 2010). A study on *Brassica napus* L. showed a reduction in plant yield in response to water stress imposed at different plant growth stages, but limited their research to plant growth and morphology (Dixon, 2007). Plant responses to water deficit include changes in growth of shoots and roots, and acceleration of the plant development (Lannucci *et al.*, 2000, Ma *et al.*, 2006). Water deficit is one of the most important factors limiting plant growth, metabolism, yield and evapotranspiration (Mustafa *et al.*, 2011). Therefore, soil water deficit will reduce the number of leaves, leaf area, shoot height and chlorophyll content, which will consequently reduce biomass production and increase root to shoot ratio with increase in water deficit (Luvaha *et al.*, 2008). The reduction in photosynthates supply is, at least in part, counteracted by the mobilization of storage compounds. Water availability and quality affects growth and physiological processes of all plants since water is the primary component of actively growing plants ranging from 70-90 % of plant fresh mass (Gardner *et al.*, 1984). Water deficit affects adversely plant growth and development throughout the world (Boyer, 1982). Biomass production is proportional to water use, thus water use is constant for a species in a given environment, however the water lost by transpiration and biomass production in terms of dry matter or CO_2 fixed are linked through water use efficiency (Levitt, 1980). Luvaha *et al.* (2008), investigated the effect of water deficit in Mango (*Mangifera indica)* and observed that water deficit affects growth, development, yield and quality of plants in the greenhouse and field conditions. Water deficit is known to greatly affect growth and yield of vegetables (Thobile *et al.*, 2010). The importance of leafy vegetables in the developing countries has

been recognized only recently due to their nutritional (dietary) and medicinal values (Prasad *et al.*, 2008). They are more nutritious than cabbages especially in iron and calcium content (Onyango, 1993). With the onset of the market economy and modernisation of agriculture in Africa, attention has been given to crops that offer potential for export. As a result, exotic vegetables have become more prestigious than indigenous vegetables and conventional agronomy has, to a large extent, concentrated on conserving the genetic resources of exotic rather than indigenous vegetables (Schippers, 2002). Therefore there is need to investigate the two African nightshades species, which are threatened with extinction as they have to compete for attention with the much more popular exotic vegetables (Maundu *et al.*, 1999a). Another reason for a decline in indigenous vegetable usage is that the indigenous knowledge on their production methods, preservation, use and nutritive value is not anymore systematically transmitted from one generation to another (Anonymous, 1998). Furthermore, urbanisation has also led many growers to prefer exotic vegetable crops.

African leafy vegetables possess several agronomic advantages. For instance, unlike the exotic vegetables they can produce seeds under tropical conditions, they have a short growth period and can withstand both abiotic and biotic stresses (Onyango *et al.*, 2005; Mwai *et al.*, 2007). The nutritive value of African nightshade depends on many factors, including season of growth, soil fertility, plant age and the part utilized. Leaves harvested during the vegetative stage have higher protein content than those harvested after the onset of flowering. Imbamba (1973) studied the effect of plant age on protein content of African nightshade and observed that the highest levels of proteins were present 53 days after planting, and declined after flowering. The ability of these vegetables to grow fast and be harvested within a short period makes them useful in sustaining nutrition intervention programmes. They offer variety of vegetable to be used and can contribute in broadening the food base. The African nightshade can play an important role in food security, especially during periods of scarcity. This is due

to the fact that they can be stored through preservation. However, such a prolonged storage leads to valuable food components being lost through damage by insects, rodents and other vermin. When the storage is under humid conditions then they are destroyed by bacteria and fungi (Onyango, 2001).

African nightshades have the potential to contribute to poverty alleviation and nutritional security because they are easy to grow, require minimum production inputs, are rich in vitamins and minerals, have phytochemicals and anti-oxidant properties and can earn income to growers, yet their growth and physiology has not been fully investigated. Currently over 10 million people in Kenya suffer from chronic food insecurity and poor nutrition, and between two to four million people require emergency food assistance at any given time on the other hand, nearly 30% of Kenya's children are classified as undernourished, and micronutrient deficiencies are widespread (Ombalo, 2010). Several medicinal values were recognised e.g. in Kenya, where unripe fruits of *S. villosum* are applied to aching teeth, leaves are used for stomach-ache, an extract from pounded leaves and fruits used to treat tonsillitis, and even roots are boiled in milk and given to children as tonic (Maundu *et al.*, 1999a). Unfortunately, only few important crops get more attention through the development of appropriate varieties and agronomic research, while there is a tendency for the rarely cultivated crops to disappear (Schippers, 2002).

1.2 Statement of the problem

Most parts of Kenya are affected by drought, which in turn reduces water availability to plants (Luvaha *et al.*, 2008). Limitations to water lower agricultural production thus contributing to food insecurity and malnutrition problems. If drought stricken areas in Kenya are exploited by the production of water deficit tolerant African nightshade species, Kenya's food security would be improved. The two species have the potential to alleviate poverty, malnutrition and contribute to food security. *Solanum scabrum* Mill., is widely used as a cooked vegetable, a

medicinal plant, fodder for cattle and goats, and both the leaves and purple to black fruits contain anthocyanin pigments used as a dye or as a kind of ink while *S. villosum* Mill. subsp. *miniatum* (Bernh. ex Willd.) Edmonds is among the widely consumed African nightshade species in Kenya and many parts of sub-Saharan Africa (Abukutsa-Onyango, 2007; Smith and Eyzaguirre, 2007). There is paucity of knowledge on the effects of varying soil water deficits on the growth and physiology of the nightshade species for drought tolerance, and on which species between *S. scabrum* and *S. villosum* could be more resistant to drought. The current study, considered different soil water levels to better understand nightshade response to soil water deficit which has been observed to cause reductions in lateral branching, leaf production, shoot height and rates of leaf and shoot expansion and yield in both herbaceous and woody plants (Osorio *et al.*, 1998; Ngugi *et al.*, 2003; Sikuku *et al.*, 2012). Therefore understanding how the two species respond in their growth and physiology to water deficit is imperative for optimal production in the future.

1.3 Objectives

The main objective of this study was to investigate the effects of water deficit on growth and physiology of two African nightshades (*Solanum scabrum* Mill. and *Solanum villosum* Mill. subsp. *miniatum* (Bernh. ex Willd.) Edmonds.

Specific objectives

The specific objectives were:-

1. To determine the effects of water deficit on growth of African nightshades *Solanum scabrum* and *Solanum villosum*.
2. To determine the effects of water deficit on photosynthetic capacity, and leaf temperature of *Solanum scabrum* and *Solanum villosum*.

3. To determine the effects of water deficit on chlorophyll content and foliar chlorophyll content of *Solanum scabrum* and *Solanum villosum*.

1.4 Hypotheses

It is hypothesized that:-

1. Soil water deficit significantly reduces the growth of *Solanum scabrum* and *Solanum villosum*.
2. Soil water deficit significantly reduces the photosynthetic capacity and leaf temperature of *Solanum scabrum* and *Solanum villosum*.
3. Soil water deficit significantly reduces chlorophyll content and foliar chlorophyll content of *Solanum scabrum* and *Solanum villosum*.

1.5 Justification

African nightshades have been documented to be rich in nutritional value with high contents of vitamins A and C, minerals and supplemental proteins (Schippers, 2000). Despite these advantages, these vegetables have been neglected for many years by policy makers, agriculturists, educationists and even research funding agencies. They are normally considered and referred to variously as minor, poor man's crop or just weeds and therefore their potentials have not been fully exploited (Onyango, 2002).

African nightshades play an important role in income generation and subsistence. Recent surveys carried out in Western Kenya markets provided evidence that they offer a significant opportunity for poor people to earn a living as producers and traders without requiring large capital investments (Schippers, 2000). They are a source of employment for those outside the formal sector in urban areas in many African cities because of their short, less labour intensive production systems, low levels of purchase inputs and high yields

(Schippers, 2000). Research on water use has concentrated mainly on food crops such as maize, sorghum, wheat and rice while ignoring African indigenous vegetables such as the African nightshade vegetables which are equally greatly affected in their growth and physiology by water deficit. Being the most important leafy vegetable like any other crop, African nightshades are prone to water deficit (Schippers, 2002). However, there is scarce documented literature on growth, chlorophyll fluorescence, leaf temperature and gas exchange parameters, and chlorophyll content and foliar chlorophyll content of the two species to water deficit. According to Muthomi and Musyimi (2009), the growing of African nightshade *S. scabrum* Mill. seedling in water deficit conditions, proved to cause reductions in growth, however these investigations were limited to the growth responses of the seedlings, this aspects have not been fully studied, yet they might be contributing to the low productivity of the vegetables.

Therefore studying the effects of water deficit on their growth and physiology provided a better understanding of the African nightshades response to soil water deficit, and the conditions, under which they can be grown for higher yields to help alleviate poverty, enhance their value, generate income to farmers and improve human health.

CHAPTER TWO

2.1 African nightshades

African nightshades are known by various names by different ethnic communities in Kenya; Mnavu (Kiswahili), Osuga (Luo), Lisutsa (Luhya), Rinagu (Kisii), Managu (Kikuyu), Kitulu (Kamba), Momoi (Maasai) and Isoiyot (Kipsigis) (Chweya, 1997). African nightshades are widespread indigenous vegetables that are presently semi-cultivated. They are marketed countrywide just like *Amarathus spp* (Chweya, 1997).

African nightshades belong to the genus *Solanum* in the family *Solanaceae.* This family is made up of approximately 90 genera and 3000 species that are well distributed throughout the tropical and temperate regions of the world (Edmonds and Chweya, 1997). Maundu *et al.* (1999b) identified nine species of African nightshades while only five of these species are common in Kenya. They include:- *Solanum nigrum* L., *Solanum villosum* Miller., *Solanum americanum* Miller., *Solanum scabrum* Miller and *Solanum physalifolium.* They are commonly found as weeds in cultivated fields and in pruned tea fields. The three forms easily recognized in Kenya are *Solanum scabrum, Solanum americanum* and *Solanum villosum,* all being used as leafy vegetables (Maundu, 1997). *Solanum scabrum* is very common in both the highland and lowland regions of West and East Africa and its origin is likely to be in the warm humid forest belt of West and Central Africa (Fontem and Schippers, 2004). In contrast, *S. villosum* is purported to have its origin in Eurasia, while it is sometimes speculated to have originated from southern Europe (Manoko and Van Der Weerden, 2004).

2.2 Botanical description of *S. scabrum* Mill. and *S. villosum* Mill.

2.2.1 *Solanum scabrum* Mill.

Plants are erect, glabrescent to subglabrous, lateral branches sparse, usually spreading horizontally. Plant can reach 1.00m in height and become 1.20 m wide when undisturbed. Stems are prominently ribbed, ridged with distinct teeth. Leaves are large 10-12 cm long and 6-8 cm wide, with entire to sinuate margins. Apices are acute to obtuse. Inflorescence is simple or forked, often extended cymes, 6-14 flowered. Calyces 1.9-3.5 (4.5) mm: Sepals usually reflexed away from the berry.

Flowers are stellate, white (or with purplish tinge). Basal star, yellow green, 7-9 mm. Anthers are brown or purplish brown, 2.5-3.3 mm. Styles are 2.9-4.5 mm long, not exerted beyond anthers.

Berries are broadly ovoid, dark purple, 15-17 mm broad, remaining on the plant and adhering to erect pedicels at maturity. Cytology: is hexaploid when 2n=72 (an autoallopolyploid) (Schippers, 2002).

2.2.2 *Solanum villosum* Mill.

Plant are subglabrous to hairy, up to 50 cm high with glandular hairs. Leaves are 2-7 cm by 1.5-4 cm, rhombic to ovate- lanceolate, margins entite to sinuate dentate. Inflorescence simple, umbellate or small cymes with 3-5 flowers. Peduncles short, erect but long pendicels deflexed in front. Calyces 1.2-2.2 mm, deflexed or adhering to mature berry. Corollas white 4-8 mm, 3-5 times as long as the calyx. Anthers are yellow, 1.5-2.5mm. Styles 2.9-5 mm long, rarely beyond anthers. Berries are elliptic or oval shaped, less commonly round 6-10 mm broad falling when ripe (Schippers, 2002).

2.3 Ecological conditions necessary for nightshades growth

African nightshades are found nearly in all parts of Kenya at altitudes ranging from 620-2200m above sea level (Kemei *et al.*, 1997). Nightshades require large amounts of nitrogen and therefore do well in soils that are rich in organic matter. They also grow well on land covered with ash from recently burned vegetation. African nightshades are commonly found as weeds e.g. in cultivated fields, under trees and in shaded areas near buildings (Maundu *et al.*, 1999a). This is especially true for *S. scabrum* and *S. villosum*, which prefer fertile soils with high nitrogen content and rich in organic matter (Fontem and Schippers, 2004; Manoko and Van Der Weerden, 2004). Plant growth was significantly increased by nitrogen application with 5g/plant (19g CAN/plant) being optimum (Murage,1990). Beta carotene, crude protein, nitrates and phenolic leaf content significantly increase while ascorbic acid, crude fibre and oxalate leaf contents are significantly reduced by nitrogen application. Therefore although nitrogen application improves leaf yield and the nutritive quality of African nightshades, it leads to increased phenolic compounds and accumulation of nitrates in the leaves (Murage,1990).

2.4 Growth and morphology of *S. scabrum* Mill. and *S. villosum* Mill.

2.4.1 *Solanum scabrum* Mill.

Solanum scabrum Mill. can be recognized with relative ease by its strong green or purple stems with more or less serrated wings. Plants are usually about 60 cm high but could grow to 1.20m or more. There are both small and large leaved cultivars with different leaf shapes. Leaves have entire to sinuate margins and apices that are acute to obtuse. It is the only species whose berries remain on the plant at maturity. The dark purple fruits have a distinct bloom when young and become glossy when they get older (Schippers, 2002).

2.4.2 *Solanum villosum* Mill.

Solanum villosum Mill. subsp. *miniatum* (Bernh. Ex Willd.) Edmonds plants grow up to 1 m high, spreading or erect, short branches to 3rd level. Stems are green with node colour ranging from green to purplish green to purple with greenish purple. Leaves are lanceolate to ovate with entire, sinuate, sinuate-dentate or dentate margins that may have clearly defined lobes or none, leaf apex is acuminate to acute, light green to green lamina with light green or green veins. Leaves are ovate with finely lobed dentate margins and acute apex greenish purple with light green veins (Schippers, 2002).

2.5 Propagation of African nightshades

Propagation of African nightshades is mainly through seed. Vegetative propagation can also be used as a possible option when seed availability is limited (Schippers, 2002). According to Mwafusi (1992) vegetatively propagated plants branched, spread and yielded significantly less than those raised from seed. Furthermore, plants that were vegetatively propagated gave leaves which had significantly more glycoalkaloids than those raised from seeds. (Mwafusi, 1992) established that de-flowering increased leaf yield by 40% with a yield of 2154Kg / ha of leaf.

2.6 Nutritional value of African nightshades

African leafy vegetables occupy an important place among food crops as they provide adequate amounts of crude fiber, carotene, a precursor of vitamin A, vitamin C, riboflavin, folic acid and mineral salts like calcium, iron, phosphorous, among others (Schippers, 2000). They form cheap and best source of food (Prasad *et al.*, 2008). In spite of these merits, limited information is available on the effect of water deficit on growth and physiology of African nightshades (*Solanum scabrum* Mill. and *Solanum villosum* Mill. subsp. *miniatum* (Bernh. ex

Willd.) Edmonds which may help enhance their production and sustainable utilization among farming communities.

2.7 Water deficit and plant growth

Due to water deficits, the physiology of crops is disturbed which causes a large number of changes in morphology and anatomy of plants (Babein *et al.*, 2011). Plant growth is defined as a permanent change in volume of plant tissues accompanied by a change in form and this process could be significantly retarded by soil water deficit (Donatelli *et al.*, 1992; Ghobadi *et al.*, 2006). Plant growth solely depends on cell division, enlargement and differentiation and all can be delayed by water deficits (Kramer, 1983). Water deficit affects both elongation and expansion growth (Anjum *et al.*, 2003; Bhatt and Srinivasa, 2005). Shoot growth, particularly growth of leaves is generally more sensitive to soil water deficit than root growth (Hopkins and Huner, 2004). According to Imana *et al.* (2010), in tomato, water stress results in significant decrease in chlorophyll content, leaf relative water content and vegetative growth. Furthermore, severe water stress (40% of pot capacity) reduced the plant height by 24%, the stem diameter by 18% and chlorophyll content by 32% compared to the control. Krieg and Sung (1986), observed a reduction in the plant leaf area by decreasing the initiation of new leaves, with no significant changes in leaf size as a result of water stress. According to Sikuku *et al.* (2010) plant height decreased with increasing water deficit in three rice varieties of *O. sativa*.

When water is scarce in the soil or when there is excess water in the soil, the balance between uptake and transpiration is disturbed and plants become stressed (Fitter and Hay, 1987). Physiological modifications are the first responses of the plants to water deficit. The plant response begins with stress recognition at the cellular level *via* activation of signal transduction pathways. The root signal to leaves through messengers, promoting stomatal

closure, leaf rolling and leaf abscission (Rodrigues *et al.*, 2011). These results further showed that the expression of genes encoding calcium binding protein, protein kinase and phosphatises were altered under water stress.

Crop water stress is the result of interactions between factors in the rhizosphere in relation to the amount of moisture available to plants (Onyango, 1996). Water deficit during vegetative stage reduces plant height and plant leaf area. However, the effects of water deficit during this stage vary with the severity of stress and age of the crop. Long duration plant varieties suffer less yield damage than short duration varieties as long vegetative period could help the plant to recover when stress is relieved (Jones and Flowers, 1989). Specht *et al.* (2001) while working on soyabeans observed a decrease in stem length under water deficit conditions and Wu *et al.* (2008) reported a 25% reduction in plant height in water stressed citrus seedlings. Studies in leafy vegetables by Thobile *et al.* (2010) revealed that the critical growth stage in leafy vegetables is the vegetative stage, hence need for sufficient soil water to meet plant demand for vegetative growth. Leaf expansion during this vegetative stage is very sensitive to water deficit (Thobile *et al.*, 2010). Cell enlargement requires turgor to extend the cell wall and a gradient in water potential to bring water into the enlarging cell. Water deficit decreases leaf area, which reduces the intercepted solar radiation (Salisbury and Ross, 1992). Water deficit reduces the uptake of nutrients since most of the elements are absorbed via the roots through active diffusion. Water deficit reduces the rate of dark respiration and translocation of assimilates and sometimes it changes the pattern of partitioning of photosynthates at the expense of quality and quantity of economic yields (Boyer, 1982). Occurrence of early stages of water deficit leads to poor crop establishment and increased seedling mortality in the rice (Jose *et al.*, 2004). Leaf water potential has been recognized as an indicator of plant water status, while osmotic adjustment is an adaptive process, which assists in the maintance of turgor under water limiting conditions (Jongdee *et al.*, 1998).

2.8 Water deficit on total biomass production

Long periods of severe soil water deficit conditions, particularly at water sensitive growth stages causes reduced assimilation of carbon and decreased biomass production (Demir *et al.*, 2006). Plant productivity under drought stress is strongly related to the process of dry matter partitioning and temporal biomass distribution (Kage *et al.*, 2004). Tahir and Mehid (2001) noted diminished biomass due to water stress in almost all genotypes of sunflower. Studies have shown that more dry matter is partitioned to the root as compared to the shoot in plants facing drought (Arora and Mohan, 2001). This may be as a result of a plant changing the pattern of partitioning of assimilates in favour of the roots for root extension so as to extract soil moisture at deep layers of soil and prevent dehydration (Chatterjee and Maiti, 1988). In rice, drought tolerant rice varieties have a more extensive root system which grow to greater depth and have a higher root to shoot ratio than the susceptible varieties so as to extract soil moisture at deep layers of soil (Chatterjee and Maiti, 1988). Greater plant fresh and dry weights under water deficit conditions are desirable characters. A common adverse effect of water deficit on crop plants is the reduction in fresh and dry biomass production (Farooq *et al.*, 2009).

Sikuku *et al.* (2010) confirmed a reduction in whole plant dry weight with an increase in water deficit in rice. Similar results were observed by Pattanagul and Thitisaksakul, (2011) where water stress caused a significant reduction in shoot growth of rice. Cengiz *et al* (2006) observed that water deficit reduces the total plant dry weight, but affects shoots more than roots causing a larger root : shoot ratio while Quisenberry and Mc Michael (1991) observed a decreased shoot to root ratio of plants grown under conditions of severe water deficit. African nightshades (*Solanum scabrum* Mill.) seedlings, exposed to water deficit had a total dry matter reduced by 27-43% (Muthomi and Musyimi, 2009), however there was need therefore to determine the effects of soil water deficit on the total dry matter for *S. scabrum* Mill. and *S.*

villosum Mill. The reduction in leaf area was ascribed to avoidance mechanism aimed at reducing plant water consumption hence conserving water during periods of drought. Masinde *et al.* (2005) related this reduction in leaf area to decrease in interception of solar radiation and consequently decreasing biomass production for most crops.

An increase of root to shoot ratio under drought conditions was related to ABA content of roots and shoots (Sharp and LeNoble, 2002). Shoot and root dry matter ratios increases under drought stress not because of an increase in root mass but due to a relatively greater decrease in shoot mass (Blum, 2005). Root mass rarely increases under stress, however, root length and depth may increase in a drying soil even at a reduced total root mass.

2.9 Water deficit and photosynthesis

Photosynthesis like any other physiological process is affected by the conditions of the environment in which it occurs (Devlin and Witham, 1986). The effect of water deficit on photosynthesis depends on the plants adaptations to water deficit and the intensity and duration to which the plant is exposed to water deficit (Levitt, 1980; Chaves *et al.*, 2002; Martim *et al.*, 2009). According to Kaiser (1987), the intensity of this effect influences the capacity of different species to cope with the drought which also depends on the plant genetic background. Water deficit in plant tissue develops under drought conditions and the ability to maintain photosynthetic machinery functional under water deficit is a major importance for drought tolerance (Zlatev and Yordanov, 2004). Pieters and El-souki, (2005), reported photosythesis as one of the main metabolic processes that determine crop production and is directly affected by drought stress. According to Kramer (1983), photosynthesis could be retarded through reduction in plant leaf area, closure of stomata and decreasing the carbon fixation efficiency process. In sunflower (Hopkins and Huner, 2004), stomatal closure had minor effect on photosynthesis because the direct effects on the photosynthesis activity of

chloroplast decrease the demand for CO_2 and the level of CO_2 inside the leaf remains relatively high.

According to Colom and Vazzana (2003) water stress causes large reduction in leaf chlorophyll and carotenoid content, which directly affects photosynthesis rates, while Shaw and Laing (1966) observed a decrease in photosynthesis, when water content of the leaves was reduced by 5% to 15% below the maximum leaf saturation and photosynthesis stopped when leaves lost 50% of their maximum water content. Lawlor and Cornic, (2002) observed a decrease in photosynthetic rates of leaves as a result of decrease in leaf relative water content and water potential.

Water and CO_2 follow the same diffusion pathways but inverse direction, hence transpiration is beneficial to photosynthesis and any resistance in the diffusion pathway of CO_2 from the atmosphere to the sites of carboxylation within the mesophyll cells may increase with water stress (Mustafa *et al.*, 2011). Loss of turgor pressure in the guard cells leads to closure of the stomata whilst increased ABA levels under water stress triggers stomatal closure. Rise in the level of ABA in plants has often been associated with water stress (Luvaha *et al.*, 2008). An increase in ABA at the start of water stress leads to a decrease in transpiration and leaf expansion in drought tolerant plant (Milborrow, 1987). Mustafa *et al.* (2011) worked on drip irrigated cotton and observed that water deficit affected the water use, seed cotton yield, dry matter and some yield components such as plant height and number of bolls per plant of cotton. Water deficit further decreased leaf expansion, photosynthesis, rate of leaf production, rate of transpiration, leaf senescence, nutritional quality and total yield in general.

Warren *et al.* (2011) studied the responses to water stress of gas exchange and metabolites in *Eucalyptus* and *Acacia* spp and observed reductions in photosynthesis caused by water stress which was attributed to a reduced concentration of CO_2 at the sites of

carboxylation and/or impairments of mesophyll metabolism. The concentration of CO_2 at the sites of carboxylation were less than the atmospheric CO_2 concentration owing to a series of gas-phase (air) and liquid-phase (mesophyll cell) resistances, at least some of which were affected by water stress.

Under water deficit, cells lose their turgidity causing stomatal closure. This limits the rate of CO_2 diffusion through the stomata causing a decline in the photosynthetic rate. Luvaha *et al.* (2008), observed that internal CO_2 content seemed not to be affected by water deficit. While low CO_2 assimilation under water deficit, without a corresponding decline in internal CO_2 could have been due to non-stomatal effects on the photosynthetic process, possibly due to an increase in the mesophyll resistance as was suggested by Cornic *et al.* (1989). Damages in the primary photochemical and biochemical process may occur simultaneously (Lawlor, 2002). CO_2 maximal assimilation reflects the results of the mesophyllic impairment (Zlater and Yordanov, 2004). In many experiments it has been shown that photosynthesis decreases when stomatal conductance decreases (e.g Tenhunen *et al* 1985; Nielsen and Orcutt, 1996 and Mafakheri *et al.*, 2010). Stomatal closure and the resulting CO_2 deficit in the chloroplast is the main cause of decreased photosynthesis under water deficit (Flexas and Medrano, 2002), whereas others argue that low ATP content caused by a reduction in ATP sythase is the likely explanation for decreased photosynthesis under water deficit (Lawlor, 2002 and Tang *et al.*, 2002).

Previous studies of water deficit on leafy vegetables are scarce. For instance Thobile *et al.* (2010) investigated the response of local wild mustard (*Brassica* species) landraces to water stress and found out significant reductions in their morphology as a result of water stress, yet there is no information on their physiological response. Muthomi and Musyimi (2009) investigated the growth response of African nightshade (*S. scabrum*) seedlings to water deficit, and observed reductions in growth as a result of water deficit, however their

research was limited to plant growth and biochemistry. The general scarcity of information on physiological response to water deficit partly contributed to the basis for the current research.

2.10 Water deficit and transpiration rate

A large portion of the absorbed water is translocated to the leaves and is lost to the surrounding atmosphere due to the anatomical features of the plant (Devlin and Witham, 1986). According to (Salisbury and Ross, 1992), high rates of transpiration can lead to decreased photosythesis, and that the driving force for transpiration is the gradient in water vapour density from mesophyll layer of the leaf beyond the boundary layer to the atmosphere. The availability of soil water to the roots of a plant and the efficiency of its absorption has a profound influence on the rate of transpiration (Devlin and Witham, 1983; Zinolabedin *et al.*, 2008). High transpiration rates are favoured by large root : shoot ratio which ensures the transpiring plant is supplied with adequate water. Absorption of water by the plant may lag behind the release of water via transpiration without noticeably affecting the plant for a short period, if the condition is prolonged, water deficit develops and the plant will wilt. Some plants avoid water deficit by water conservation and have adaptations that limit the rate of water loss thus prevent the development of detrimental plant water deficits by conserving soil water for an extended period thus maintaining soil and plant water potential suitably high over sufficient period for growth (Jones, 1996).

Shahenshah, *et al.* (2010) observed a decrease in transpiration in cotton and peanut with low water deficit and this decrease continued with further increase in water deficit. Similar results were observed in rice where a general decline was observed in transpiration rate as water deficit increased (Sikuku *et al.*, 2010). According to Imana *et al.* (2010), in tomato, a decrease in plant growth as a result of water stress was attributed to a reduction in the transpiration rate. Onyango, (1996) worked on rainfed rice (*Oryza sativa* L.) and observed

instances of decreased water potential when exposed to water stress. Moaveni *et al.* (2011), showed that a converse relationship exists between transpiration rates and water deficits in wheat. It was further observed by Moaveni *et al.* (2011), that stomatal conductance and cuticular conductance reduced with increased water stress but turgor pressure was maintained.

2.11 Water deficit and Stomatal Conductance

According to Li *et al* (2004) and Inamullah and Isoda (2005), water stress decreased stomatal aperture, stomatal conductance and transpiration rate. The reduction in the soil moisture may lead to lower water content in the leaves, causing guard cells to lose turgor and hence the stomatal pores to reduce. In addition, an increase in stomatal resistance may lead to reduced water transport into the leaves, resulting in a decrease in stomatal conductance which in turn decreases transpiration and also limits photosynthesis (Pereira *et al.*, 2000). Decreased leaf water potential leads to stomatal closure and ultimately results in low transpiration, which in turn increases leaf temperature (Fukai *et al.*, 1999).

Stomatal closure could be triggered by the accumulation of abscisic acid (ABA), which is a drought tolerant mechanism (Devlin and Witham, 1986). Even though closure of stomates improves water use efficiency under water stress conditions, this decreases carbon assimilation due to reduction in physical transfer of CO_2 molecules. It also leads to increased leaf temperature, which interferes with the biochemical processes (Forbes and Watson, 1994). Mafakheri *et al.* (2010) reported that transpiration and stomatal conductance decreased in chicken pea cultivars exposed to drought stress as one of the first response of plants to drought is stomatal closure restricting gas exchange between the atmosphere and the inside of the leaf.

2.12 Effect of water deficit on chlorophyll content

High chlorophyll content is a desirable characteristic because it indicates a low degree of photoinhibition of photosynthetic apparatus, therefore reducing carbohydrate losses for grain growth (Moaveni, *et al.*, 2011). According to (Moaveni, *et al.*, 2011) water stress conditions caused reductions in chlorophyll content in wheat varieties. Similar observations were also made by Alireza, *et al.* (2011) in *Matricaria chamomilla* L. a medicinal plant. Studies by Randall *et al.* (1977) on the consequence of drought stress on the organization of chlorophyll into photosynthetic units and on the chlorophyll-protein composition of mesophyll and bundle sheath chloroplast of *Zea mays* found that most of the chlorophyll lost in response to water deficit occurs in the mesophyll cells with a lesser amount being lost from the bundle sheath cells. All of the chlorophyll loss can be accounted for by reduction on the lamellar content of the light harvesting chlorophyll a/b protein (Randall *et al.*, 1977). Studies by Kura- Hotta *et al.* (1987) on rice seedlings showed that the chlorophyll content of leaves decreases during senescence suggesting that the loss of chlorophyll is a main cause of inactivation of photosynthesis. Potato leaves have also showed a significant decline in chlorophyll content with increasing water deficit (Nadler and Bruvia, 1998). Furthermore, water deficit induced reduction in chlorophyll content has been ascribed to loss of chloroplast membrane, excessive swelling, distortion of the lamellae vesiculation and the appearance of lipid droplets (Kaiser *et al.*, 1981). According to Levitt (1980), chlorophyll content in plants often decreases with increased mesophyll resistance commonly observed in water deficient regions, [illegible] changes in the ratio of chlorophylls a and b (Anjum *et al.*, 2003). Manivannan *et al.* (2007) reported a large decline in the chlorophylls *a*, *b* and total chlorophyll content in [illegible] chlorophylls *a* and *b* are prone to soil water deficit (Farooq *et al.*, 2009). Drought stress different sunflower varieties caused by water deficit and Shamshi (2010) while working on wheat cultivars reported that drought stress reduces concentration of chlorophyll *b* more than

chlorophyll *a*. Cengiz *et al.*, (2006) and Pastori and Trippi (1992) reported that resistant genotypes of wheat and maize had higher chlorophyll content than sensitive genotypes under water stress. Chlorophyll degradation is one of the consequences of drought stress that may result from sustained photoinhition and photobleaching (Long *et al.*, 1994; Kiani *et al.*, 2008). The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo oxidation and chlorophyll degradation. According to Chaves *et al.*, (2002), water deficit inhibits chlorophyll synthesis by inhibiting light dependent conversion of protochlorophyllide into chlorophyllide and also inhibits synthesis of chlorophylls *a* and *b* along with their inclusion into developing pigment protein complexes of the photosynthetic apparatus. Water deficit also causes a reduction of minerals nutrients essential for chlorophyll synthesis like magnesium since most of the elements are absorbed via roots through passive diffusion hence reduction in chlorophyll content (Reddy *et al.*, 2004).

Nitrogen is a component of the photosynthetic apparatus within the chlorophyll content (Sugiharto *et al.*, 1990; Egli and Suchmid, 1999; Shangguan *et al.*, 2000; Da matta *et al.*, 2003; Makuto and Koike, 2007). According to De Groot *et al.* (2003) nitrogen limitation in plants affected chlorophyll concentration and thus absorbance and light harvesting. Similarly, high nitrogen concentration brings about an increase in cell wall rigidity and osmotic water adjustment (Fife and Nambiar, 1997; Saneoka *et al.*, 2004). Furthermore Yao *et al.* (2008) showed that high N increased Fv/Fm and the effective quantum yield of photochemical energy conservation in PSII (change Fv/Fm). This N supply in turn might alleviate photoinhibition and photodamage caused by water stress (Wang and Kellomaki, 1997).

2.13 Water deficit and leaf temperature

Leaf temperatures differ between stressed and non-stressed plants. Generally, well watered plants have low leaf temperatures as compared to stressed plants. In maize (*Zea mays* L.) plants an increase in temperature of sun-lit leaves in severely stressed plants was 4.6 °C above the air temperature while the temperature difference in the well watered plants was found to be zero or negative (Gardner *et al.*, 1981). This was in agreement with Björkman and Demming-Adams, (1994); Chow (1994) and Carpentier (1996). Carpentier (1996) observed that under conditions of water scarcity, plants are often subjected to a high temperature which increases their vulnerability to light stress and consequently the photoinhibition. High air temperatures increase with the rate of transpiration to enhance cooling of leaves by evaporation (Burke *et al.*, 1990) while low leaf temperatures and water deficit had been identified as being powerful inhibitors for plant growth (Rapacz *et al.*, 2001; Ercoli *et al.*, 2004; Çakir, 2004; Rodríguez *et al.*, 2005; Xia *et al.*, 2009).

2.14 Water deficit and chlorophyll fluorescence

Measuring chlorophyll fluorescence has become a very important method of obtaining rapid, semi-quantitative information on photosynthesis, both in the field and the laboratory (Netondo, 1999). The photochemical efficiency of PSII is determined by the Fv/Fm ratio, which is reduced during periods of drought stress. The Fv/Fm ratio represents the maximum quantum yields of the primary photochemical reaction of PSII. Environmental stresses that affect PSII efficiency leads to a characteristic decrease in the Fv/Fm ratio (Krause and Weis, 1991; Mamnouie *et al,;* 2006). The Fv/Fm ratio is an indicator of plant stress resulting from damage to photosystem II (Demming and Björkman, 1987). Chlorophyll fluorescence is a useful tool for quantification of the effect of abiotic stress on photosynthesis (Krause and

Weis 1991; Tezara *et al.*, 2005). An inverse relationship occurs between chlorophyll content and chlorophyll fluorescence under water stressed conditions (Pereira *et al,* 2000).

Under drought stress, disturbances of photosynthesis at the molecular level are connected with the low electron transport, through photosystem II and or with structural injuries of PSII and the light-harvesting complexes (Van Rensburg and Krüger, 1993; Hura *et al.*, 2007). According to Zanella *et al.* (2004) low Fv/Fm ratio is the main consequence of photoinhibitory damage and may be attributed to the down regulation of photosystem II activity and impairment of photochemical activity. This may be due to reduction of photosynthesis directly as a result of water deficit hence dehydrating the protoplasm thereby lowering its photosynthetic capacity (Vurayai *et al.*, 2011).

Studies have proved that changes in photosystem II fluorescence may result from damage in the reaction center or from regulatory processes external to the reaction centre including non radiative dissipation or increased excitation transfer to PSII (Demming-Adams, 1990). Bjorkman and Powles (1984), showed that in *Nerium oleander* L. water stress caused photo inhibitory damage in the photosynthetic system and that water stress predisposes the leaves to photoinhibition. The amount of functional PSII reaction centers in a given leaf is the result of the rates of damage and degradation and of repair of PSII (Antelmo *et al.*, 2010). At the molecular level the light dependent inactivation and repair of PSII under water deficit is accompanied by damage and degradation of D1 protein and replacement of its protein in thylakoid membrane by one newly synthesized (Adir *et al.*, 1990; Barber and Anderson, 1992).

Studies by Sikuku (2007) on NERICA rice varieties showed no significant effect in maximum photochemical efficiency of water stressed and non water stressed plants while studies conducted by Antelmo *et al.* (2010) observed a decrease in maximum photochemical efficiency in rice varieties. Efforts to determine differences in chlorophyll fluorescence as a

result of water deficit in indigenous vegetables proved to be very limited and this formed the basis for the current research.

CHAPTER THREE

3.1 Experimental Site

The experiment was set up in a polythene shade at Maseno University Botanic Garden Western Kenya. The area is approximately 1500m above sea level with a latitude of 0° 1'N-0°2'S and longitude 34° 25'E-34° 47. The mean annual day temperature is 20°C with the average maximum daily temperature not exceeding 31°C and the average minimum night temperature not dropping below 15°C (Otieno *et al.*, 1993; GOK, 2002). The minimum and maximum temperatures inside the polythene shade were 27 ± 8 °C and 37 ± 8 °C respectively with a relative humidity of 39 ± 5%, and photosynthetic flux density (PPFD) from 400 - 600 µmol photons $m^{-2}s^{-1}$. The soils at Maseno are classified as acrisol being well-drained, deep reddish brown clay with pH ranging between 4.6 and 5.4 (Mwai, 2001).

3.2 Experimental Layout and Treatments

The experiment was set up between January and July 2012. Seeds of African nightshade *Solanum scabrum* Mill and *Solanum villosum* Mill. subsp. *miniatum* (Bernh. ex Willd.) Edmonds were obtained from the University Botanic Garden, Maseno. Soil was dug from a portion of the garden and then solarised after which it was filled into 20 litre Polyvinyl (PVC) pots up to three-quarters full (15Kgs) with perforated bottoms to allow for proper drainage of water then sowing was done.

The experimental design was Completely Randomized Design (CRD) with two factorial consisting of four treatments and three replications. A total of (24) pots were used. The four treatments were:

I Watering daily (control)- (T1) with water deficit level of -45.84 litres.

II Watering every 3rd and 6th day- (T2) with water deficit level of 5.79 litres.

III Watering every 9th day- (T3) with water deficit level of 45.11 litres.

IV Watering every 12th day- (T4) with water deficit level of 91.19 litres.

Plants were irrigated with normal tap water using a hand sprinkler for twelve days in order to improve root development (Imana *et al.*, 2010). Treatments were then initiated when the plants were 24 days old. Subsequently 1.5 litres of water was applied to each pot.

3.3 Measurement of Parameters

3.3.1 Soil moisture content

The soil moisture content was determined gravimetrically, whereby samples were scooped from the topsoil, 10 cm from the top using an auger between 10.00a.m and 11.00 a.m. During soil extraction care was taken to minimize root destruction. The scooped samples were immediately placed in polythene tubes (non-perforated) to avoid any moisture loss. The fresh weights (W_1) were taken using an electronic weighing balance (Denver instrument, Model XL-31000, Germany). Samples were then dried in an oven for 48 hours at 72^0C and the dry weight (W_2) obtained. The measurements were done at every 13th day after initiation of treatments and the average values obtained. The percentage water content (W) was calculated as demonstrated by Nguyen *et al.* (2013).

$$W = \frac{W1 - W2}{W1} \times 100 \quad \text{eqn 1.}$$

Where;

W1 = fresh weight

W2 = dry weight

W = percentage soil moisture content

The determination of field capacity was also done gravimetrically. The upper limit of field capacity was determined by watering soil thoroughly to drainage and then allowed to drain for 24 - 48 hours then soil samples were collected at 10 cm. The scooped samples were immediately placed in polythene tubes (non-perforated) to avoid any moisture loss. The fresh weights (W_1) were taken using an electronic weighing balance (Denver instrument, Model XL-31000, Germany). Samples were then dried in an oven for 48 hours at 72°C and the dry weight (W1) obtained, and the percentage water content (W) was calculated as shown in equation (1) above. The lower limit for plant water extraction (permanent wilting point) was determined by growing plants to flowering without limiting water intake, after which water intake was limited until permanent wilting was achieved. The percentage water content by mass was calculated at the permanent wilting point. The levels of moisture deficit imposition for each treatment in terms of percentage was calculated as demonstrated by Nguyen *et al.* (2013).

$$AWC = FC - WP \quad \text{.....eqn 2.}$$

$$\text{Water deficit} = \frac{FC - T1}{AWC} \times 100 \quad \text{.....eqn 3}$$

Where;

AWC = available water content

WP = wilting point

FC = field capacity

T1 = treatments

3.4 Plant Growth Parameters

3.4.1 Shoot height

Shoot height was measured using a meter rule, from the stem base up to the shoot apex once after every twelve days. This began on the first day after initiating treatments and was done for a period of 84 days.

3.4.2 Number of leaves

The number of fully expanded fresh leaves per plant on the main stem and branches were counted and recorded once every twelve days. Counting began on the first day after initiating treatments and was done once after every twelve days for a period of 84 days.

3.4.3 Stem diameter

The diameter of each seedling was measured by use of a vernier calliper at a height of 10 cm from the stem base. Measurements began from the day treatments were initiated and were done after every twelve days for a period of 84 days.

3.4.4 Root to shoot ratio determination

These measurements were done at the end of the experiment (97^{th} DAS). The plants were carefully uprooted after loosening the soil and rinsed under tap water. The root masses that were embedded in the soil were carefully removed by soaking the rooting media in water and sieving out all the root segments. The plants were then separated into shoot and root, dried in an oven at 70 °C for 48 hrs and then weighed using an electronic weighing balance (Denver Instrument Model XL-31000, Germany). (Sikuku *et al.*, 2010). The ratio of root : shoot biomass was computed as a percentage according to Sikuku, *et al.* (2010).

$$\text{Root: shoot ratio} = \frac{\text{Root dry weight}}{\text{Shoot dry weight}} \times 100 \quad \text{.....eqn 4.}$$

3.5 Physiological measurements

3.5.1 Leaf stomatal conductance

Leaf stomatal conductance measurements were carried out using a leaf porometer (L1-1600, LICOR, Nebraska, USA). The measurements were conducted between 0900 and 1200 hours on fully sun exposed top leaf from an area of 0.7 cm^2. Measurements began from the day treatments were initiated and were done after every 12 days. The following were the adjustments or specifications during measurements; in the polythene shade, the aperture was set at 0.7 cm^2, cuvette air temperature varied between 26^{o} to 37^{o} C, relative humidity varied between 43% to 63% (Sikuku *et al.*, 2010).

3.5.2 Leaf chlorophyll fluorescence and temperature

Chlorophyll fluorescence measurements were carried out using a portable fluorescence monitoring system, (Model FMS 2, Hansatech Instruments, Germany) as shown in *plate 1*. Measurements began from the day treatments were initiated and were done after every twelve days. Three plants per treatment were sampled and measurements were done on the fourth fully expanded leaf. The leaves used for the measurements were dark adapted for 30 minutes using the dark adaptation clips and then illuminated for 6 seconds with actinic light to induce fluorescence. The initial fluorescence (Fo) and the maximum fluorescence (Fm) was measured and the variable fluorescence (Fv=Fm-Fo) and the Fv/Fo ratio was calculated (Sikuku *et al.*, 2010). The leaves used for the measurement of ETR were light adapted for 30

minutes and then illuminated for 6 seconds with actinic light to induce fluorescence. The PAR during measurements ranged from 400 - 600 µmol photons $m^{-2}s^{-1}$. Leaf temperature was also determined using a portable fluorescence monitoring system, (Model FMS 2, Hansatech Instruments, Germany) as shown in *plate 1*, and as demonstrated by (Sikuku *et al.*, 2010).

Plate 3.1 Measuring chlorophyll fluorescence and leaf temperature using a portable fluorescence monitoring system.

3.6 Biochemical measurements

Two methods were used to determine leaf chlorophyll content i.e destructive and non destructive measurements

3.6.1 Chlorophyll concentration determination

This was determined through the destructive method. Chlorophyll content was determined using the procedure of Arnon (1949) and Coombs *et al.* (1987) as described by Netondo (1999). The 4th youngest fully expanded compound leaf was randomly sampled from each of

the treatments. In the laboratory 0.5g of each fresh leaf tissue, minus the mid-rib, was weighed, cut into very small pieces and put in a specimen bottle. 10ml of 80% acetone solution was added to the bottle and care taken to ensure that the leaf tissue was fully immersed in the acetone. The set up was then kept in the dark for 7 days for chlorophyll to be extracted by the acetone. 1ml of solution was filtered from each of the bottles. The filtrate was then diluted with 20 ml 80% acetone solution. Using a spectrophotometer (Model Nova spec II JAPAN), the absorbance of the chlorophyll solution was read at 645nm and 663 nm. 80% acetone solution was used as the blank, and the final volume of the extract was 21 millilitres.

The respective chlorophyll concentration in milligrams (mg) of chlorophyll per gram (g) of leaf tissue extracted was calculated using the formula of Arnon (1949) as follows.

mg *chl a* / g leaf tissue =12.7 (D663)-2.67 (D645) x V /1000xW.....eqn. 5

mg *Chl b* / g leaf tissue =22.9 (D645)-4.68 (D663) x V / 1000xW.....eqn. 6

mg *tChl* / g leaf tissue =20.2 (D645)+8.02 (D663) x V / 1000xW.....eqn. 7

Where; D= optical density (absorbance) at the given wavelengths (645nm or 663nm).

V= volume of the extract in millilitres (ml).

W= fresh weight in grams (g) of leaf tissue from which the extract was made

3.6.2 Foliar chlorophyll content determination

This was determined through the non-destructive method. These measurements were carried out by use of a Minolta SPAD 502, JAPAN chlorophyll meter. The SPAD 502 chlorophyll meter instantly measured the amount of chlorophyll content by clamping the meter on the abaxial surface of the leafy tissue and an indexed foliar chlorophyll content reading (0-99.9) was received in less than 2 seconds. The optical device determined the interaction of the thylakoid chlorophyll with incident light (Jifon *et al.*, 2005). The measurements were carried out between 0930 and 1300 hrs, and begun when the plants were twelve days old and were

carried out after every twelve days for a period of 84 days. Four leaves per plant with 3 SPAD values per leaf were determined, plants were sampled and measurements were done from an area of 2.5cm^2 of the fully expanded leaves as demonstrated by (Silva *et al.*, 2007; Markwell *et al.*, 1995; Marquard and Tipton, 1987).

3.7 Statistical analysis of data

Data were subjected to Analysis of variance (ANOVA) using MSTAT-C statistical computer package (Michigan State University, MI). Mean separation was done using the Least Significant Difference (LSD) test at 5% level.

CHAPTER FOUR

4.1 Soil moisture content

There was a significant difference in soil moisture content at ($p \leq 0.05$) between all treatments in all days. T1 had the highest moisture content followed by T2, T3 and T4 respectively as shown in Fig 4.1.

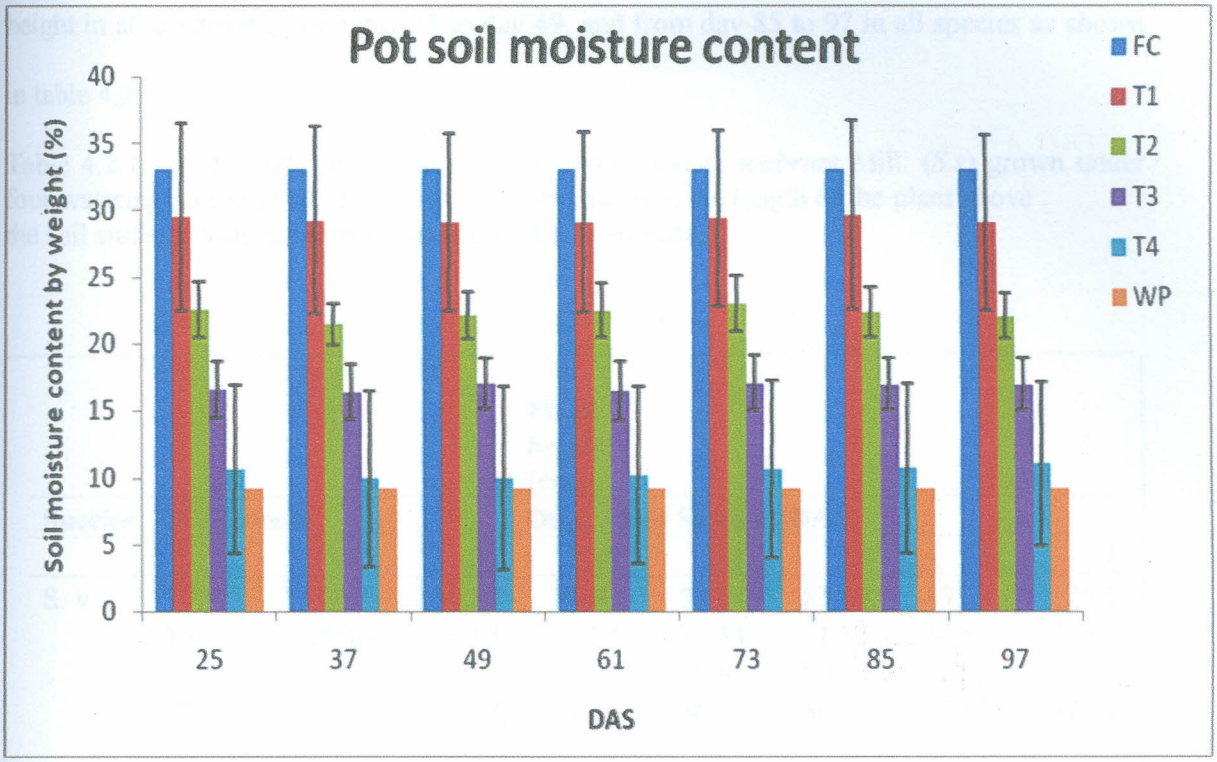

Fig.4.1 The upper limit (FC) and lower limit (WP) levels for soil moisture content and the four treatments (T1, T2, T3 and T4) for *S. villosum* Mill. and *S. scabrum* Mill. grown under different watering regimes. (means of three replicates ± SE). LSD (0.05) T= 0.2506, 0.2282, 0.2326, 0.2889, 0.2125, 0.1419, 0.1931, for DAS 25, 37, 49, 61, 73, 85, and 97 respectively.

4.2 Plant growth parameters

4.2.1 Plant height

There was no significant difference in plant height at ($p \geq 0.05$) between the two species for all days. There was a significant difference at ($p \leq 0.05$) between treatments in all days. There was a significant difference at ($p \leq 0.05$) between the species and the treatments in days 49, 61, 73, 85, and 97, while days 25 and 37 they were non-significant. The highest increase in height was in T1 followed by T2, T3 and T4 respectively. There was a general increase in plant height in all treatments from day 25 to day 49, and from day 85 to 97 in all species as shown in table 4.2.1.

Table 4.2.1: Plant height of *S. villosum* Mill. (*S.v*) and *S. scabrum* Mill. (*S.s*) grown under four watering regimes (T1, T2, T3 and T4), measured at the length of the plant above the soil surface. Values represent means of three replicates.

		Plant height (cm)						
Species	**Treatments**	**Days After Sowing (DAS)**						
		25	37	49	61	73	85	97
S. v	T1	32.83	38.83	46.33	53.83	53.83	69.67	76.5
	T2	29.83	36.17	43.5	51.33	51.33	65.83	73.83
	T3	33.67	35.17	43.17	50.83	50.83	66	72
	T4	33.5	37	40.83	46.17	46.17	55.67	57.33
S. s	T1	31.83	41	49.5	57	57	71.83	80.5
	T2	28.83	37.5	45.33	52.5	52.5	67	74.67
	T3	29.67	39.33	45.83	53.5	53.5	72.17	77.83
	T4	32.33	37.33	42	45.67	45.67	58	60.5
LSD Species		**0.6**	**0.78**	**0.67**	**0.84**	**1.27**	**1.32**	**1.11**
LSDTreatments		**0.85**	**1.1**	**0.94**	**1.19**	**1.8**	**1.86**	**1.57**

LSD (Least Significance Difference at 5% level of significance), T1-watering daily (control), T2-watering after every three days (the 3rd and 6th day), T3-watering the 9th day and T4-watering the 12th day.

4.2.2 Stem diameter

There was no significant difference at ($p \geq 0.05$) in stem diameter between the two species. There was a significant difference at ($p \leq 0.05$) in stem diameter on all days except days 73 and 85. There was a significant difference in treatments at ($p \leq 0.05$) between species and treatments on all days except days 25 and 97. There was a steady increase in stem diameter. The highest increase in diameter was in T1 followed by T2, T3 and T4 respectively as shown in table 4.2.2.

Table 4.2.2: Stem diameter of *S. villosum* Mill. (*S.v*) and *S. scabrum* Mill. (*S.s*) measured 10 cm above the soil surface, grown under four watering regimes (T1, T2, T3 and T4). Values represent means of three replicates.

		Stem diameter (cm)						
Species	**Treatments**	**Days After Sowing (DAS)**						
		25	37	49	61	73	85	97
S.v	T1	2.5	2.17	2.5	2.58	2.92	3	3.5
	T2	2.17	2.417	2.5	2.75	2.83	3	3
	T3	2	2.33	2.53	2.58	2.83	2.92	3
	T4	2.5	2.5	2.75	2.75	2.92	2.67	1.92
S.s	T1	2	2.67	2.83	2.83	2.92	2.92	3
	T2	2.5	2.58	2.67	2.92	3	3	3
	T3	2.25	2.5	2.67	2.75	2.92	3	3.5
	T4	2.33	2.58	2.67	2.83	3	2.83	2.25
LSD species		**0.03**	**0.06**	**0.04**	**0.03**	**0.04**	**0.08**	**0.03**
LSD treatments		**0.05**	**0.08**	**0.06**	**0.05**	**0.05**	**0.11**	**0.24**

LSD (Least Significance Difference at 5% level of significance), T1-watering daily (control), T2-watering after every three days (the 3rd and 6th day), T3-watering the 9th day and T4-watering the 12th day.

4.2.3 Number of leaves

There was no significant difference at ($p \geq 0.05$) in number of leaves between the species in all days. There was a significant difference at ($p \leq 0.05$) in all treatments. There was a significant difference at ($p \leq 0.05$) between the species and the treatments in all days. The highest increase in number of leaves was in T1 followed by T2, T3 and T4 respectively. There was a decline in number of leaves for both species in T4 from day 73 to day 97 as shown in table 4.2.3.

Table 4.2.3: The number of leaves of *S. villosum* Mill. (*S.v*) and *S. scabrum* Mill. (*S.s*) grown under four watering regimes (T1, T2, T3 and T4). Values represent means of three replicates.

		Number of leaves						
Species	**Treatments**	**Days After Sowing (DAS)**						
		25	37	49	61	73	85	97
S.v	T1	19.33	28.33	37.67	44.67	53	61.67	62
	T2	16	20	23.67	26.67	31.33	35	38.33
	T3	11.33	14	16	18.33	20.33	22.67	26
	T4	12	13.33	15	15.67	15.67	13.67	12.67
S.s	T1	18.33	22.67	26	29.67	33.67	37.33	41
	T2	13	15.33	17.33	20	22.33	25.67	29
	T3	13	15.33	17.33	20	22.33	25.67	29
	T4	13	15	16.33	17	16.67	16	14.67
LSD Species		**0.28**	**0.35**	**0.4**	**0.53**	**0.75**	**0.88**	**1.65**
LSD treatments		**0.39**	**0.49**	**0.57**	**0.75**	**1.05**	**1.24**	**2.34**

LSD (Least Significance Difference at 5% level of significance), T1-watering daily (control), T2-watering after every three days (the 3rd and 6th day), T3-watering the 9th day and T4-watering the 12th day.

4.2.4 Root to shoot ratio

There was a significant difference in root to shoot ratio at ($p \leq 0.05$) between the treatments. There was a significant difference in root to shoot ratio at ($p \leq 0.05$) between the species and the treatments. The root to shoot ratio increased with increase in water deficit from T1, T2, T3 and T4 respectively, with *S. villosum* having the highest root to shoot ratio followed by *S. scabrum* as shown in Fig 4.2.4.

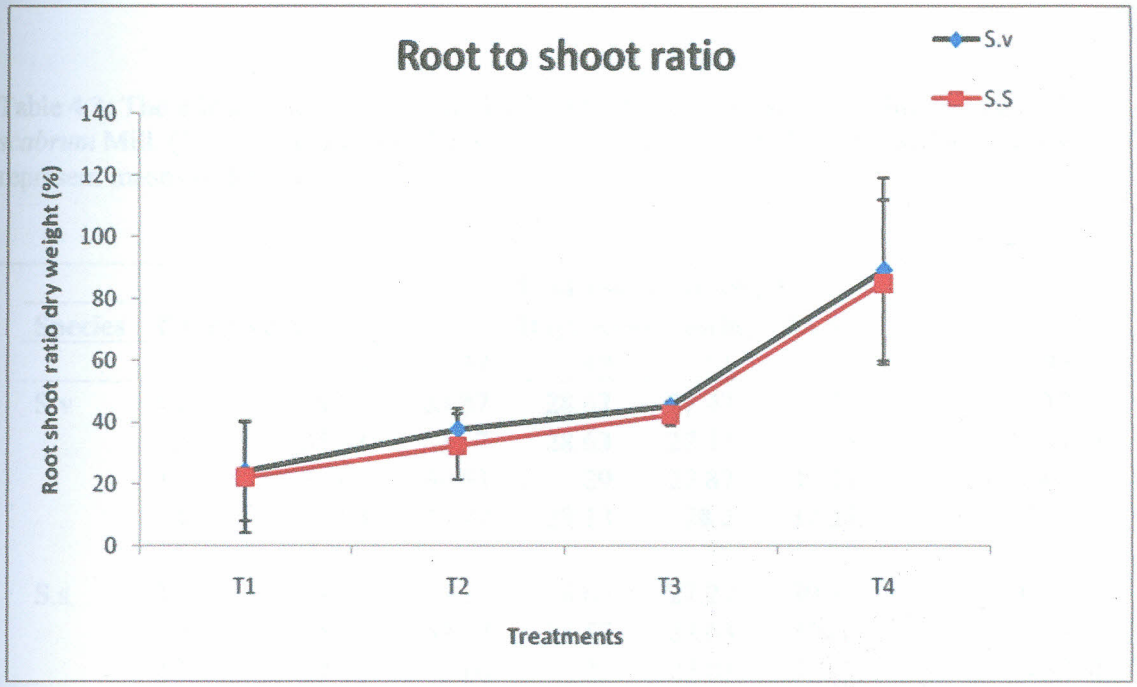

Fig 4.2.4. The root: shoot ratio of *S. villosum* Mill. (S.v) and *S. scabrum* Mill. (S.s) grown under four watering regimes (T1, T2, T3 and T4), (means of three replicates ± SE). LSD (0.05) for Species = 0.6805. LSD (0.005) for Treatments = 0.9624

4.3 Leaf temperature

There was a significant difference in leaf temperature at ($p \leq 0.05$) between the two species on days 25 and 37 while there was no significant difference at ($p \geq 0.05$) on days 49, 61, 73 and 85. All treatments were significant at ($p \leq 0.05$) on all days. There was a significant difference at ($p \leq 0.05$) between the species and the treatments in all days except on day 25. The highest temperatures were on T4 followed by T3, T2 and T1 respectively as shown in table 4.3.

Table 4.3: The effect of water deficit on leaf temperature of *S. villosum* Mill. (*S.v*) and *S. scabrum* Mill. (*S.s*) grown under different watering regimes (T1, T2, T3 and T4). Values represent means of three replicates.

		Leaf temperature (°C)					
Species	**Treatments**	**Days After Sowing (DAS)**					
		25	37	49	61	73	85
S.v	T1	33.2	33.87	28.67	27.47	31	31.17
	T2	35.13	31.7	28.63	27.77	31.6	32.97
	T3	34.63	30.53	29	27.87	32.23	34.6
	T4	34.3	29.77	29.13	28.2	32.27	34.87
S.s	T1	31.63	34.17	28.63	27.27	30.47	30.63
	T2	35.07	32.07	28.57	27.63	31.63	32.73
	T3	34.67	30.6	29	27.83	32.07	34.33
	T4	34.43	29.5	29.07	27.83	32.2	34.9
LSD species		**0.31**	**0.15**	**0.05**	**0.06**	**0.11**	**0.17**
LSD Treatment		**0.44**	**0.21**	**0.08**	**0.08**	**0.16**	**0.24**

LSD (Least Significance Difference at 5% level of significance), T1-watering daily (control), T2-watering after every three days (the 3rd and 6th day), T3-watering the 9th day and T4-watering the 12th day.

4.4 Gas exchange parameters

4.4.1 Stomatal conductance

There was no significant difference at ($p \geq 0.05$) in stomatal conductance between the two species. There was a significant difference in stomatal conductance at ($p \leq 0.05$) between the treatments in all days except day 25 and day 73. There was no significant difference at ($p \geq 0.05$) between the species and the treatments in all days. The highest conductance was observed in T1, followed by T2, T3 and T4 respectively as shown in table 4.4.1.

Table 4.4.1: Leaf stomatal conductance of *S. villosum* Mill. *(S.v)* and *S. scabrum* Mill. *(S.s)* grown under four watering regimes (T1, T2, T3 and T4). Values represent means of three replicates.

		Stomatal conductance ($mmol\ m^{-2}\ s^{-2}$)					
Species	**Treatments**	**Days After Sowing (DAS)**					
		25	37	49	61	73	85
S.v	T1	20.27	20.6	15.34	32.8	16.93	22.83
	T2	15.23	14.53	13.81	20.14	16.73	15.9
	T3	6.47	12.23	10.59	19.03	9.13	12.47
	T4	3.5	10.27	9.24	5.83	5.9	10.43
S.s	T1	17.16	13.23	18.15	22.2	24.33	17.93
	T2	16.8	9.71	12.1	20.63	18.7	15.33
	T3	14.79	8.61	12.07	7.18	10.33	15.17
	T4	14.53	7.75	11.73	4.91	8.1	8.27
LSD Species		**3.02**	**1.52**	**1.79**	**3.19**	**3.87**	**2.79**
LSD Treatments		**4.27**	**2.15**	**2.53**	**4.51**	**5.48**	**3.94**

LSD (Least Significance Difference at 5% level of significance), T1-watering daily (control), T2-watering after every three days (the 3rd and 6th day), T3-watering the 9th day and T4-watering the 12th day.

4.4.3 Chlorophyll fluorescence

4.4.3.1 Fv/Fm

There was no significant difference in Fv/Fm at ($p \geq 0.05$) between the two species in all days except on day 25. There was no significant difference at ($p \geq 0.05$) between the treatments in all days except on day 25 and day 61. There was no significant difference at ($p \geq 0.05$) between the species and the treatments in all days. The highest Fv/Fm ratio was observed in T1, followed by T2, T3 and T4 respectively in both species as shown in table 4.4.2.

Table 4.4.2: The Fv/Fm ratio of *S. villosum* Mill. (*S.v*) and *S. scabrum* Mill. (*S.s*) grown under four watering regimes (T1, T2, T3 and T4). Values represent means of three replicates.

		Fv/Fm ratio			
Species	**Treatments**	**Days After Sowing (DAS)**			
		25	37	49	61
S.v	T1	0.92	0.81	0.79	0.69
	T2	0.87	0.72	0.61	0.5
	T3	0.76	0.63	0.53	0.5
	T4	0.63	0.55	0.44	0.35
S.s	T1	0.98	0.89	0.79	0.69
	T2	0.87	0.79	0.67	0.52
	T3	0.7	0.64	0.53	0.43
	T4	0.61	0.54	0.48	0.31
LSD Species		**0.03**	**0.01**	**0.02**	**0.01**
LSD Treatments		**0.04**	**0.02**	**0.03**	**0.01**

LSD (Least Significance Difference at 5% level of significance), T1-watering daily (control), T2-watering after every three days (the 3rd and 6th day), T3-watering the 9th day and T4-watering the 12th day.

4.4.3.2 ETR

There was no significant difference ($p \geq 0.05$) in ETR between the two species in all days. The treatments were significant ($p \leq 0.05$) on days 25 and day 61 only. There was no significant difference at ($p \geq 0.05$) between the treatments and the species. There was no clear-cut trend in the ETR between the four treatments in both species. However, higher ETR was observed in the control (T1) on the day 25 followed by T2, T3 and T4 respectively in both species as shown in table 4.4.3.

Table 4.4.3: The ETR of *S. villosum* Mill. (*S.v*) and *S. scabrum* Mill. (*S.s*) grown under four watering regimes (T1, T2, T3 and T4). Values represent means of three replicates.

		ETR (μmol electrons m^{-1} s^{-1})			
Species	**Treatments**	**Days After Sowing (DAS)**			
		24	36	48	60
S.v	T1	307.28	81.38	38.38	21.43
	T2	163.85	66.91	31.66	21.48
	T3	230.02	52.58	32.79	18.78
	T4	135.81	61.61	45.46	31.79
S.s	T1	275.06	64.84	39.49	21.38
	T2	200.33	52.51	127.03	24.87
	T3	195.31	58.49	65.72	36.21
	T4	210.7	75.53	50.31	18.08
LSD Species		**21.61**	**6.34**	**15.9**	**2.44**
LSD Treatments		**30.56**	**8.96**	**22.48**	**3.46**

LSD (Least Significance Difference at 5% level of significance), T1-watering daily (control), T2 watering after every three days (the 3rd and 6th day), T3-watering the 9th day and T4 watering the 12th day.

4.5 Biochemical measurements

4.5.1 Chlorophyll content

4.5.1.1 Chlorophyll *a*

Chlorophyll *a* was not significant at ($p \geq 0.05$) between the species. The treatments had a significant difference at ($p \leq 0.05$) on day 25 and day 49. The species and the treatments had no significant difference at ($p \geq 0.05$) on all days. As water deficit increased chlorophyll *a*, decreased, hence chlorophyll *a* was highest in T1, followed by T2, T3 and T4 respectively for the two species as shown in table 4.5.1.

Table 4.5.1: The effect of water deficit on chlorophyll *a* extracted from fresh leaves of *S. villosum* Mill. (*S.v*) and *S. scabrum* Mill (*S.s*) grown under four watering regimes (T1, T2, T3 and T4).

		Chlorophyll *a* ($mg\ g^{-1}$)			
Species	**Treatments**	**Days After Sowing (DAS)**			
		25	37	49	61
S.v	T1	4.98	4.37	5.045	7.52
	T2	3.98	3.34	4.64	5.7
	T3	3.64	4.01	4.41	6.71
	T4	4.11	2.55	2.64	7.13
S.s	T1	4.76	5.05	5.34	6.8
	T2	4.15	5.31	4.79	7.19
	T3	4.58	4.46	3.31	6.19
	T4	4.98	3.85	4.84	5.67
LSD Species		**0.24**	**0.49**	**0.39**	**0.39**
LSD Treatments		**0.34**	**0.69**	**0.55**	**0.55**

LSD (Least Significance Difference at 5% level of significance), T1-watering daily (control), T2-watering after every three days (the 3rd and 6th day), T3-watering the 9th day and T4-watering the 12th day.

4.5.1.2 Chlorophyll *b*

Chlorophyll *b*, was not significant at ($p \geq 0.05$) in the species in all the days. Water deficit treatments were not significant at ($p \leq 0.05$) on days 25, 37 and 73. The species and treatments were not significant at ($p \geq 0.05$) in all days. Chlorophyll *b* was highest in T1, followed by T2, T3 and T4 respectively for the two species as shown in table 4.5.2.

Table 4.5.2: The effect of water deficit on chlorophyll *b* extracted from fresh leaves of *S. villosum* Mill. (*S.v*) and *S. scabrum* Mill (*S.s*) grown under four watering regimes (T1, T2, T3 and T4). Values represent means of three replicates.

		Chlorophyll *b* ($mg\ g^{-1}$)			
Species	**Treatments**	**Days After Sowing (DAS)**			
		25	37	49	61
S.v	T1	1.49	1.49	0.78	2.9
	T2	1.12	1.12	1.19	2.11
	T3	0.97	0.97	1.35	2.1
	T4	1.5	1.5	0.59	2.22
S.s	T1	1.55	1.55	1.88	2.28
	T2	1.01	1.01	1.15	2.76
	T3	1.69	1.69	0.89	2.32
	T4	1.45	1.45	0.98	2.2
LSD Species		**0.16**	**0.16**	**0.15**	**0.16**
LSD Treatment		**0.23**	**0.23**	**0.21**	**0.23**

LSD (Least Significance Difference at 5% level of significance), T1-watering daily (control), T2-watering after every three days (the 3rd and 6th day), T3-watering the 9th day and T4-watering the 12th day

4.5.1.3 Total chlorophyll content

Total chlorophyll content was not significant at ($p \leq 0.05$) between the species in all days. There was no significant difference at ($p \geq 0.05$) between the treatments except on day 25. There was no significant difference at ($p \geq 0.05$) between the species and the treatments. Total chlorophyll was highest in T1, followed by T2, T3 and T4 respectively for both species. There was a limited increase in total chlorophyll content with increase in water deficit as shown in table 4.5.3.

Table 4.5.3: The effect of water deficit on total chlorophyll extracted from fresh leaves of *S. villosum* Mill. grown under four watering regimes (T1, T2, T3 and T4). Values represent means of three replicates.

		Total chlorophyll (mg g^{-1})			
Species	**Treatments**	**Days After Sowing (DAS)**			
		25	37	49	61
S.v	T1	6.47	6.465	5.155	10.414
	T2	5.11	5.11	5.82	7.47
	T3	4.61	4.61	5.76	8.82
	T4	5.27	5.27	3.23	9.34
S.s	T1	6.31	6.31	7.22	9.05
	T2	5.15	5.15	5.94	9.95
	T3	6.27	6.27	4.21	8.51
	T4	6.43	6.43	5.16	7.87
LSD Species		**0.36**	**0.53**	**0.49**	**0.47**
LSD Treatment		**0.5**	**0.76**	**0.71**	**0.67**

LSD (Least Significance Difference at 5% level of significance), T1-watering daily (control), T2-watering after every three days (the 3rd and 6th day), T3-watering the 9th day and T4-watering the 12th day.

4.5.1.4 Foliar chlorophyll content

There was a no significant difference between the species at ($p \geq 0.05$) in all days except day 25. Treatments were significant at ($p \leq 0.05$) in all days except days 75 and 97. Species and treatments were significant at ($p \leq 0.05$) in all days except in day 75. There was a limited increase in foliar chlorophyll content with increase in water deficit as shown in table 4.5.4.

Table 4.5.4: The effect of water deficit on foliar chlorophyll content of *S. villosum* Mill. (*S.v*) and *S. scabrum* Mill (*S.s*) grown under four watering regimes (T1, T2, T3 and T4). Values represent means of three replicates .

		Foliar chlorophyll content (SPAD values)						
		Days After Sowing (DAS)						
Species	**Treatments**							
		25	37	49	61	73	85	97
S.v	T1	49.27	49.8	50.1	51.33	52.3	52.93	50.33
	T2	44.23	45.57	46.33	46.83	47.27	47.77	47.9
	T3	34	34.83	36.13	36.6	37.5	38.8	39.43
	T4	25.7	26.47	27.07	28.07	28.63	29.77	30.4
S.s	T1	48.73	49.23	50.43	51.23	52.33	50.97	47.93
	T2	42.33	43.73	43.87	48.67	46.2	46.2	48.43
	T3	31	33.2	34	35.23	35.9	39.67	38.07
	T4	24.67	25.47	26.67	27.77	29.1	29.6	29.97
LSD Species		**1.17**	**0.23**	**0.16**	**0.59**	**0.2**	**0.66**	**0.59**
LSD Treatments		**0.23**	**0.32**	**0.22**	**0.83**	**0.29**	**0.93**	**0.83**

LSD (Least Significance Difference at 5% level of significance), T1-watering daily (control), T2-watering after every three days (the 3rd and 6th day), T3-watering the 9th day and T4-watering the 12th day.

CHAPTER FIVE

5.1 Effect of soil moisture content on *S. scabrum* Mill. and *S. villosum* Mill.

Soil water content decreased with decreasing frequency of irrigation (Fig 4.2). This was in agreement with results of Martim *et al.* (2009), on grapevine and Siddique *et al.* (2000), on wheat plants. According to (Thobile *et al.*, 2010) moisture requirements for plants differ with the species, stage of development and plant age. Losses of moisture from the soil may be attributed to surface evaporation, transpiration through the leaves and water absorbed by the roots (Luvaha *et al.*, 2008). In well watered plants the soil moisture content was more or less similar in both species, implying that when water is not limiting, the two species have got the same rate of water absorption, utilization and water loss.

5.2 Effect of water deficit on plant growth

Plants require water and nutrients for growth and survival, but increased water deficit will limit nutrient availability hence can be detrimental to plant growth and development (Wu *et al.*, 2011). Although there was no significant difference in plant height between the two species, the depression of plant height in T3 and T4, unlike T1 and T2 (Table 4.2.1), could have resulted from a reduction in plant photosynthetic efficiency as reported by Castonguay and Markhart (1992). During the initial stages of development (vegetative stage) the plants were adjusting to water deficit and therefore did not require a lot of water unlike during their last stages of development. Reduction in plant height under water deficit could be due to leaf number reduction which is a result of physiological changes that occur under water stress. A general decline in plant height with increasing water deficit showed that growth allocation was diverted to the stems. A similar study on rice (*Oryza sativa*) genotypes showed reduction in plant

on cell division, enlargement and differentiation and all can be delayed by water deficit (Thobile *et al.*, 2010). Shoot growth, particularly growth of leaves is generally more sensitive to soil water deficit than root growth. Extreme water deficit (T4) had the lowest plant height for both species probably due to reduced cell turgor affecting cell division and expansion, and as noted by Salisbury and Ross (1992), cell enlargement requires turgor to extend the cell wall and a gradient in water potential to bring water in the enlarging cell, but water deficit suppresses cell expansion and cell growth due to low turgor pressure. Under mild water deficit in T1 plant height increased the highest, while the contrary was with T4 where plant height was limited probably due to internodal elongation, leaf initiation and expansion by inducing epinasty of leaf and petiole, leaf senescence, leaf chlorosis, and leaf abscission.

At the start of water deficit, changes in the leaf number were more visible, where T1 had the highest increase in number of leaves, whereas T4 had the least number of leaves (Table 2.4.3). Decrease in leaf number, could be attributed to reduction in cell expansion which was common in T4, possibly due to carbohydrate depletion due to mobilisation and export. Among the two species *S. scabrum* Mill. had the highest number of leaves as compared to *S. villosum* Mill. thus *S. scabrum* Mill. might have had higher photosynthetic rates, and subsequently increased photosynthate allocations to other plant organs. This is in agreement with Sah and Zamora, (2005) where maize plants subjected to water deficit had significantly reduced leaf area and number. Reduced leaf number in plants under water stress reduces light interception by a plant and eventually reduces biomass production (Masinde *et al.*, 2005). Shoot growth, particularly growth of leaves is generally sensitive to soil water deficit than root growth (Hopkins and Huner, 2004).

Reduction in stem diameter with increase in water deficit (Table 4.2.2), may have been as a result of reduced cell size and cell number as a result of lower rates of cell division and cell enlargement respectively. In well watered treatments (T1 and T2), the stem base often

swelled at the initial stages of growth. This results were in agreement with those obtained in tomato (*Lycopersicon esculentum* Mill.) where the smallest stem diameter of plants was observed in those that received the least amount of water (Imana *et al.*, 2010). However, according to Bimpong *et al.* (2011), the stem growth of the two species of the African nightshades may have been inhibited at low soil moisture content despite complete maintenance of turgor in the growing regions as a result of osmotic adjustment. This suggests that the growth inhibition may be metabolically regulated possibly serving an adaptive role by restricting the development of transpiring leaf in water stressed plants (Sharp, 1996).

5.3 Effect of soil water deficit on chlorophyll content and foliar chlorophyll content

Water deficit caused a general reduction in chlorophyll *a*, in T4, DAS 37 for both species, chlorophyll *b*, in T4 DAS 49 for both species and in estimated total chlorophyll in T4 DAS 49 (Tables 4.5.1, 4.5.2 and 4.5.3). Foliar chlorophyll content generally reduced with increasing water deficit. (Table 4.5.4). Similar results were observed in maize (Anjum *et al.*, 2011) and in barley (Kuroda *et al.*, 1990). Nikolaev *et al.* (2010) observed a decline in chlorophyll content from 15% to 13% in water stressed wheat as compared with well watered plants in three varieties of wheat. Chlorophyll content is one of the indices of photosynthetic activity (Bojovic and Stojanovic, 2005), and according to Montagu and Woo (1999), water deficit can destroy chlorophyll and inhibit its synthesis. Extreme water deficit leads to dehydration of the plant tissue resulting in an increase in oxidative stress, causing deterioration in chloroplast structure and an associated loss of chlorophyll hence a decrease in the photosynthetic activity (Jafar *et al.*, 2004). The losses in chloroplast activity, possibly due to leaves dehydration include decreases in the electron transport rate and photophosphorylation and this might be associated with changes in conformation of the thylakoids and of coupling factor (ATP-synthetas- a sub unit of the thylakoids) and decreased

substrate binding by coupling factor (O'Neli *et al.*, 2006). Dehydration of leaves could be as a result of chlorophyll pigments not being resistant to stress, hence chlorophyll *a* and chlorophyll *b* were almost constant in T1 and T2 possibly due to the inhibition of biosynthesis of precursors of chlorophyll under moisture deficit as reported by Moaveni *et al.* (2011). While the contrary was with chlorophyll *b* which slightly increased under higher water deficit (T3 and T4), probably due to increased protein synthesis, and increased nitrogen metabolism (Sing *et al.*, 2008). The significant decrease in total chlorophyll content under water deficit, might be attributed to the increased degradation of chlorophyll pigments due to stress induced metabolic imbalance (Steinke and Stier, 2003). According to Colom and Vazzana, (2003) water deficit causes large reductions in chlorophyll and carotenoid content, which directly affects photosynthesis due to poor light absorption and conversion into useful energy. Kirnak *et al.* (2001) found that water deficit resulted in significant decrease in chlorophyll content, among other parameters for plant growth under high water stress, which resulted in less fruit yield and quality. Steinberg *et al.* (1990) reported a reduction of chlorophyll concentration in peach trees subjected to different levels of water stress, and was in agreement with the results of this study, that showed water deficit in the pot grown indigenous vegetables produced a reduction in total chlorophyll content subjected to different levels of water stress. The reduction in chlorophyll content in this study, might have been exacerbated by excess light which caused greater degradation, whereas a reduction in light harvesting, chlorophyll proteins (LHCPs) content was an adaptive defence mechanism of the chloroplast (Sing *et al.*, 2008). On the other hand, reduced stomatal conductance leading to a decrease in carbon assimilation might have contributed to decreased photosynthetic rate, as a result of the inhibitory effect of decreased water content on leaf development (Medrano *et al.*, 2002; Fariduddin *et al.*, 2009).

5.4 Effect of soil water deficit on root to shoot ratio

The observed increase in root : shoot ratio with increase in water deficit (Fig 4.2), in the current study may be attributed to increased allocation of biomass from shoot to root , which is in agreement with previous results obtained in *M. indica* rootstock by Luvaha *et al.* (2008). Masinde *et al.* (2005), reported similar results in *Cleome gynandra*. These observations were ascribed to differential sensitivity of the root and shoot biomass production to soil water deficit, in amaranthus by (Liu and Stutzel, 2004) and in wheat by Liu *et al.* (2004). Under low soil water content, the roots grow deeper in search for water. Roots therefore become the second line of defense after leaf area reduction. Water deficit usually changes the source-sink relationship thus altering assimilate partitioning. Under water stress, the roots become the stronger sink. Sharp and Davis (1979), observed significant accumulation of solutes in the root tips of un-watered plants which resulted in the maintenance of root turgor for the duration of water deficit treatment. Higher root length at lower depth provides the ability of crop to survive under drought by acquiring more water. Many plants have developed mechanisms to cope with a restricted water supply. Plants can avoid drought stress by maximizing water uptake e.g. tapping ground water by deep roots or minimizing loss e.g. stomatal closure (Jie *et al.*, 2010). Generally plants increase root : shoot ratio under water deficit conditions (Westgate and Boyer, 1985). Water deficit cause a decline in the growing zones. Increased root surface area allows more water to be absorbed from the soil and could be an adaptive response to *S. scabrum* Mill. and *S. villosum* Mill. to water deficit. This implies that increased root : shoot ratio during soil moisture deficit might have continued at very low water potentials which in turn inhibited the shoot growth, and a reduction in shoot growth coupled with continued root growth would result in an improved plant water status under extreme water deficit conditions (Sharp and Davies, 1985).

5.5 Effect of water deficit on chlorophyll fluorescence

The patterns of changes in fluorescence parameters observed in this study (Tables 4.4.2 and 4.4.3) are consistence with those reported under water deficit conditions in barley (Mamnouie *et al.*, 2006) and Bambara groundnuts (Vurayai *et al.*, 2011). Estimates of ETR describe the ability of photosytems to use incident light thereby giving an indication of the overall photosynthetic capacity of the plant (Uku and Bjork, 2005), while the flow of electrons through photosystem II is indicative under many conditions of the overall rate of photosynthesis (Pereira *et al.*, 2004; Flexas *et al.*, 2004). Although there was no significant difference in ETR between the two species *S. villosum* had a higher ETR rates with increase in water deficit, implying that it was more tolerant to water deficit since low ETR under water deficit suggests low tolerance to water deficit (Santos *et al.*, 2009). In severe water deficit Fv/Fm ratio decreased indicating a reduction in efficiency of PSII centers, or possibly due their damage, because according to Zanella *et al.* (2004) low Fv/Fm ratio is the main consequence of photoinhibitory damage and may be attributed to the down regulation of photosystem II activity and impairment of photochemical activity. This is because water deficit reduces photosynthesis directly hence dehydrated protoplasm has a lowered photosynthetic capacity (Vurayai *et al.*, 2011). The decrease in Fv/Fm indicates, to some extent, the occurrence of photoinhibition due to photoinactivation of PSII centers, possibly attributed to DI protein damage (Bjorkman and Powles, 1984). The constant Fv/Fm values for *S. villosum* in DAS 61 for T1 and T2 is an indication that there is no loss in the yield of PSII photochemistry and confirmed the resistance of the photosynthetic machinery to water deficit stress, as earlier found by Chaves *et al.* (2002) and Cornic and Fresneau, (2002), while high Fv/Fm values in T1 for the two species in all days, may have resulted in an increase in dry matter production because of the return to normal photosynthetic rates. The higher ETR and

Fv/Fm ratio in well-watered plants observed in this study agree with those of Maricle *et al.* (2007). The standard Fv/Fm ratio is 0.83 but typically ranges from 0.75 to 0.85 for normal healthy plants (Demming and Björkman, 1987). In the present study, Fv/Fm ratio of the two species ranged from 0.980 to 0.787 for T1 in DAS 25, 37 and 49, these values were slightly high possibly due to higher temperatures that increased enzymatic activity (Viera and Necchi, 2006). Similar results were obtained in beans as indicated by Miyashita *et al.* (2005) and in NERICA rice varieties as reported by Sikuku *et al.* (2012). While the decrease in electron transport along with photosystem II may also be due to the inhibition of energy transfer from carotenoids to chlorophyll or according to (Sikuku *et al.*, 2012) in rice among the NERICA varieties the decrease in ETR may be associated with increases in excitation energy quenching in the PSII antennae which are generally considered indicative of down regulation of electron transport. ETR described the ability of photosystems to use incident light thereby giving an indication of the overall photosynthetic capacity of the plant which is exhibited by the flow of electrons through PSII under many conditions of the overall rate of photosynthesis.

5.6 Effect of soil water deficit on leaf temperature and gas exchange parameters

The significant difference in gas exchange between the two species at the initial stages of growth and the later stages of growth may have been due to less water being acquired for growth and low transpiration rates hence high temperatures. On the other hand during the flowering stage demand for water was high hence high transpiration rates consequently lowering leaf temperatures. The highest temperatures were reported in T4 and decreased with a decrease in water deficit (Table 4.3). Leaf temperatures increased with increasing water deficit. Generally, well watered plants had low leaf temperatures as compared to stressed plants.

Stomatal conductance in water stressed plants was generally lower as compared to the well-watered plants (Table 4.4.1). A decline in stomatal conductance with increase in water deficit might have helped plants to avoid dessication because severe water deficit could also have increased ABA concentrations that regulate the opening and closing of the stomata. The reduction in stomatal conductance and transpiration in stressed plants might have been due to the reduction in leaf number as observed in T4 that reduced number of stomata, thereby decreasing the rate of water flow into the plant. The reduced stomatal conductance might have decreased the intercellular CO_2 concentration in turn reducing the CO_2 assimilation rate (Zhao *et al.*, 2010).

The two species of African nightshades had a reduction in stomatal conductance as a result of increasing water deficit in the leaves. These results are in agreement with those of Upretty and Bhatia (1989), who reported that stomatal conductance in the leaves of mungbean decreased with increase in water deficit. Reduction in stomatal conductance decreases transpiration and limits CO_2 assimilation rate (Tezera *et al.*, 2002). The contrary was in sunflower where stomatal closure had a minor effect on photosynthesis because the direct effects on the photosynthetic activity of chloroplast decreased the demand for carbon dioxide and the level of carbon dioxide inside the leaf remained relatively high (Hopkins and Huner, 2004). Nonstomatal limitations such as reduction in photosynthetic pigment concentration and reduction in photosytem II activity may partly account for the decreased rates of photosynthesis (Pierce *et al.*, 2007). According to Cornic and Fresneau (2002), stomatal closing is the main reason reducing photosynthesis rates as a result of water deficit because the maximum value of photosynthesis can be recovered by supplying sufficient amount of CO_2 to the leaves. Therefore the causes of low photosynthesis under water deficit depend not only on the stress and plant variety but also on the complex interaction between the age of the plant and the leaves as well as the light intensity (Flexas *et al.*, 2004). Stomatal conductance

in T1 and T2 was slightly higher as compared to T3 and T4 this might have resulted in increased CO_2 diffusion into the leaves to attain higher photosynthetic rates which favoured higher biomass in T1 and T2 (Siddique *et al.*, 2000).

CHAPTER SIX

6.1 Summary

The research provides results on the effects of soil water deficit on the growth and physiology of *S. scabrum* Mill. and *S. villosum* Mill. From the data presented, it is evident that water deficit affected all the physiological, biochemical and agronomic parameters of *S. scabrum* Mill and *S. villosum* Mill.

The growth of *S. scabrum* Mill. was higher compared to *S. villosum* Mill. The reduction in plant height was attributed to reduction in plant cell turgor which affected cell division and elongation. Generally stem and leaf growth were inhibited at low water levels despite complete maintenance of turgor in the growing regions as a result of osmotic adjustment. Extremely stressed plants showed adaptive features to survive and this included:- shedding of leaves. The root : shoot ratio increased with increase in water deficit. *S. villosum* Mill. had a higher root : shoot ratio compared to *S. scabrum* Mill. This indicates that *S. scabrum* is more tolerant and well adapted to water deficient regions unlike *S. villosum*.

Stomatal conductance was higher in well-watered plants compared to extremely stressed plants. Generally, leaf temperature increased with increase in water deficit.

Water deficit caused a general reduction in Fv/Fm ratio, and this was attributed to down regulation of photosystem II activity and occurrence of photoinhibition due to photoinactivation of PSII centers, possibly due to the resistance of the photosynthetic machinery to water deficit stress.

Generally chlorophyll *a* decreased with increase in water deficit while chlorophyll *b* slightly increased under higher water deficit and generally decreased, possibly due to increased protein synthesis, and increased nitrogen metabolism. There was no significant change in

chlorophyll between the two species. Foliar chlorophyll content was almost the same in the two species but increased with increase in water deficit, as stomatal conductance decreased with increase in water deficit.

6.2 Conclusions

- Based on the results of this study, it can be concluded that plant height decreased with increase in water deficit and *S. scabrum* Mill. had the highest plant height as compared to *S. villosum* Mill. The stem diameter also decreased with increase in water deficit and *S. scabrum* Mill. had a higher stem diameter as compared to *S. villosum* Mill. The number of leaves decreased with increasing water deficit. Although *Solanum villosum* Mill. had the highest number of leaves in T1 as compared to *Solanum scabrum* Mill., *S. scabrum* Mill. had the highest number of leaves in both treatments. *S. villosum* Mill. had a higher root : shoot ratio as compared to *S. scabrum* Mill.
- There was a reduction in leaf temperature, Fv/Fm and ETR and this may be attributed to down regulation of photosystem II activity and occurrence of photoinhibition due to photoinactivation of PSII centers.
- There was a general reduction in chlorophyll and foliar chlorophyll content with increase in water deficit, this may be due to the inhibition of biosynthesis of precursor's chlorophyll and increased degradation of chlorophyll pigments as a result of stress induced metabolic imbalance.
- Based on the results obtained from this study, it can also be concluded that for optimum growth of the two species, soil water should be maintained at T1 of the field capacity.
- The results indicate that *S. scabrum* Mill. is well adapted in terms of growth and physiology to water deficit compared to *S. villosum* Mill. therefore it can grow well in drought affected areas.

6.3 Recommendations

From the results obtained it can be recommended that:-

1. Soil water deficit significantly affected the growth of *S. scabrum* Mill. and *S. villosum* Mill. Therefore I recommend growth measurements as suitable parameters for determining the effects of water deficit on the different indigenous vegetables, and that *S. scabrum* Mill is more tolerant and well adapted to water deficient regions.
2. Soil water deficit significantly reduced chlorophyll fluorescence and gas exchange parameters of *S. scabrum* Mill. and *S. villosum* Mill. The significant differences among the plant species suggest that the two parameters are suitable in determining physiological differences to water deficit. I therefore recommend investigations on other species of African nightshades.
3. Chlorophyll and foliar chlorophyll contents of *S. scabrum* Mill. and *S. villosum* Mill. decreased with increase in soil water deficit this suggests that the two parameters are also suitable in determining the effects of water deficit on indigenous vegetables and therefore they should be adopted for future physiological studies.

6.3 Suggestions for further studies

1. Future studies should evaluate other indigenous vegetables in different climatic and soil water deficit conditions in order to predict their growth and physiology for future sustainable production.
2. Determination of leaf water potential as an indicator of plant water status should be studied in future to help understand better the water potentials in plants in relation to soil water deficit.
3. Determination of tissue mineral nutrient content as an indicator of plant growth should also be studied in future to help understand better the mineral nutrient uptake in plants in relation to soil water deficit.

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