GROWTH AND PHYSIOLOGICAL RESPONSE OF SEVEN AMARANTH SPECIES TO SOIL WATER DEFICIT



BY

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ABSTRACT

Soil water deficit is a principal abiotic factor that limit plant growth and development in dry areas. Insufficient moisture contribute to soil water deficit and some negative effects on plants such as reduced growth, yield, altered biomass allocation, reduced photosynthesis and decreased plant survival. Differences in soil water deficit responses of plants may be a consequence of different physiological and morphological adaptations. In arid and semi-arid areas, water shortage is becoming an increasing problem because of the unreliable and limited rainfall and it significantly contributes to food shortage especially in Kenya. Amaranth species are among the most popular and widely consumed micronutrient rich African indigenous leafy vegetables in Kenya; however, published information is limited concerning growth and physiological response of amaranth species to soil water deficit. This research was therefore designed to evaluate the response of seven widely cultivated amaranth species in Kenya:- Amaranthus blitum (L), Amaranthus retroflexus (L), Amaranthus spinosus (L), Amaranthus albus (L), Amaranthus cruentus (L), Amaranthus hypochondriacus (L) and Amaranthus tricolor (L). to soil water deficit in relation to their growth and physiology. The experiment was carried out at Kenya Agricultural and Livestock Research Organisation, Kisii Centre. The experiment was laid out as completely randomized design, consisting of four treatments, seven species and three replications. The treatments were: watering daily (T1), watering every 3rd and 6th day (T2), watering every 9th day (T3) and watering every 12th day (T4). Seeds of the seven amaranth species were grown in 20 litre plastic pots in loam moist soils having a pH of around 4.6 to 5.4 in a glasshouse condition. Data collections commenced on the twelfth day before initiating treatments and were collected after every twelve days. Growth parameters measured included; shoot height, stem diameter and root to shoot ratio. Gas exchange parameters were determined from one leaf per plant per treatment per replication and this included, stomatal conductance, net carbon (iv) oxide assimilation rate intercellular carbon (iv) oxide concentration and transpiration rate by use of a portable infrared gas analyzer. Leaf water potential, chlorophyll fluorescence, chlorophyll a, b and total chlorophyll concentrations were determined. Data was subjected to analysis of variance and separation of means using the Least Significant Difference at 5% level. The seven species of amaranth were significantly (p≤0.05) affected by soil water deficit. Growth parameters decreased with increase in water deficit and reduced significantly (p≤0.05) with further increase in soil water deficit. Root to shoot ratio increased with increase in soil water deficit. CO₂ assimilation rate decreased significantly with increase in water deficit while intercellular CO2 decreased with increasing soil water deficit. Water deficit caused a significant decrease in stomatal conductance. Leaf transpiration decreased significantly with increase in water deficit, while leaf water potential increased with an increase in water deficit. The relative leaf water content showed a significant reduction with increase in water deficit. Chlorophylls a, b and total chlorophyll decreased with increasing soil water deficit. There was a significant interaction between soil water deficit treatments and amaranth species. From the results obtained, it can be concluded that among the seven species of amaranth, Amaranthus albus, and A. hypochondriacus are more adaptive to soil water deficit and therefore can be grown in water deficient regions.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Globally, water is the major environmental factor limiting agricultural production. Plant water deficit is also the major problem in low rainfall areas of Kenya and by extension it is a worldwide problem affecting plant growth, distribution and yield in many ways and to varying degrees (Oniang'o, 2001). Whereas global climatic change has made the situation dire for agricultural production (Pan et al., 1996), the use of the commonly cultivated drought tolerant Amaranth vegetable crops such as Amaranthus blitum (L), Amaranthus retroflexus (L), Amaranthus spinosus (L), Amaranthus albus (L), Amaranthus cruentus (L), Amaranthus hypochondriacus (L) and Amaranthus tricolor (L), might be a solution to this global problem (Vorster et al., 2005). According to Schippers, (2002) indigenous crops may be having a strong tendency to tolerate drought, thereby forming the basis for this research.

Plants often undergo periods of soil water deficit during their life cycle due to the erratic nature of rainfall (Silva *et al.*, 2007). Water deficit occurs when water potential in the rhizosphere is sufficiently negative to reduce water availability to sub optimal levels for plant growth and development (Zhongjin and Tamar, 2003). The most frequent cause of water deficit in plants is a sub optimal soil moisture supply, salinity and a 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 soil water potential drops to about -0.14 to -0.15 MPa (Sikuku *et al.*, 2012). At this level most physiological processes (for example cell enlargement, growth, and net photosynthesis) reach very low levels or cease altogether (Noggle and Fritz, 1977). Amaranth according to Neluheni *et al.* (2007), has been regarded as a relatively drought tolerant plant, thus suggesting that a

reasonable growth and physiology can be realized at a given water deficit level, which this research sought to establish.

Presently, amaranth is the major African Indigenous vegetable in the African continent and with special reference to Kenya, most Kenyans living in the rural areas consume large quantities of Amaranth species. A previous survey conducted revealed that they form an important diet for urban dwellers (MOA, 2010). Amaranth species have enjoyed a revived interest as an agronomic crop by several people from various parts of Kenya (Neluheni et al. 2007). They are important for food and nutritional security during periods of drought and poor harvests as well as for income generation because they have high protein content (16-18%) on the average which is highly digestible (Vorster et al., 2002). It also contains high quantities of lysine and sulphur containing amino acids such as methionine, (Gudu and Gupta, 1988). Despite their large consumption and agronomic potential, drought threatens their growth and yields yet they have the potential role in improving the nutritional and economic status of marginalized and poor rural, urban and peri-urban populations (GoK, 2002). Increasing the production of amaranth vegetables will improve food security situation in Kenya especially during periods of drought, because the demand of African Leafy Vegetables (ALVs) such as Amaranth surpasses their supply, which is highly dependent on factors such as poverty, urbanization, accessibility of fresh produce markets and seasonality of production (Vorster et al., 2002). Amaranth has the potential to broaden man's food base in Africa, probably due to its ability to adapt to new environments and extremely diverse climates especially those with limited water availability (Vorster et al., 2002).

Plant water deficit develops when the evaporative demand of the atmosphere upon the leaves exceeds the capacity of the roots to extract water from the soil (Jomo *et al.*, 2014b). Jomo, (2013) further noted that the strain of drought is developed when crop demand for water is not met by the supply and plant water status is reduced. Water deficit is defined as

the insufficient moisture necessary for a plant to grow normally and complete its life cycle (Cabuslay *et al.*, 2002). Water deficit is a major problem in low rainfall areas and has contributed to high food insecurity and poor malnutrition in rural areas, and it has been described as the single physiological and ecological factor upon which plant growth and development depends more heavily than other factors (Nogues and Baker, 2000). Kenya for instance, according to Jomo, (2013), is considered as water scarce country because her 80% of agricultural production is dictated by water for irrigation as a result of low rainfall and uneven distribution (AVRDC, 2003). On the other hand, meteorological scientists have always predicted increased evapotranspiration and lower rainfall amounts, which limit the normal growth, yields and physiological tolerance of amaranth which have the potential to be used in enhancing food security (Bhagirath *et al.*, 2013).

Although the amaranth species selected were probably the most important group of indigenous leaf vegetables followed by African nightshades in Africa (Schippers, 2002), literature on their growth, physiological and biochemical response to water deficit is still scarce. For instance, Liu and Stutzel (2004), focused on biomass partitioning, specific leaf area and water use efficiency of amaranth (*Amaranthus* spp.) in response to drought stress, and their results indicated significant reductions as a result of limited water. However, according to Sullivan and Ross (1979), and Mitra (2001) drought stress tolerance is a complex characteristic and it is difficult to assess species that are resistant to drought stress, since their expressions depend on the action and interaction of not only morphological but also physiological and biochemical characteristics of the plant. Therefore data on CO₂ assimilation rate, intercellular CO₂, transpiration rate, stomatal conductance, chlorophyll contents and chlorophyll fluorescence, leaf water potential and relative leaf water contents is conspicuously lacking yet it could help in understanding their response to soil water deficit. There is need therefore to evaluate the promising amaranth species to soil water deficit in a

bid to ensuring constant food supply and proper use of water, which in comparison with food crops, have been identified to occupy an important place 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). According to Larncom, (1991); Alleman *et al.* (1996) and Palada and Chang (2003) a 100g portion of amaranth provides the same amount of vitamins as 600g Swiss chard or 280g of cabbage, thus forms cheap and best source of food and nutrition.

Abiotic stress such as water deficit stress commonly limits growth, yield and physiology of major crop species and this must be true for amaranth. Besides its nutritional value, it is perceived to be a prospective crop for marginal lands and semi-arid regions due to its tolerance towards low soil water contents, these properties qualify amaranth to be described as a drought tolerant crop (Steckel *et al.* 2004). In this regard, a study by Myers (1974) while comparing eight different crops with respect to drought tolerance, including their physiological responses, did indicate that amaranth plants have an astonishing capacity to recover after a period of severe drought stress. Oyedele *et al.* (2002), argued that drought tolerance in amaranth might be due to the ability of the crop to shut down transpiration through wilting while recovering easily when moisture is made available.

To cope with water deficit stress generally, plants have developed mechanisms that include both avoidance and tolerance (Tucker, 1986; Paland and Chang, 2003). Water deficit avoidance may result from specific morphological characteristics such as a decrease in leaf number and shape, reduced stem diameter and reduced plant height. Tolerance, on the other hand, results from altered physiological processes such as reduced CO₂ assimilation rates, intercellular CO₂, transpiration rate and stomatal conductance, leaf temperature and biochemical contents of the plant. In many cases, water deficit stress is detected by reduced

soil moisture contents, leaf water potentials and relative leaf water contents, which results in reversible damage to cellular and sub cellular structures and functions (Kigel *et al.*,1977). This research sought to evaluate the growth and physiological response of the commonly known and cultivated seven amaranth species in East Africa, because they have not only been described as water deficit-tolerant crop probably due to their capability of repairing damaged tissues and resuming normal metabolic functions faster than other leaf vegetables (Tucker, 1986; Paland and Chang, 2003), but also because of their reported competitive advantage of resuming normal cellular functions, such as photosynthesis, sooner after water deficit than other plants (Gou and Al-Khatib, 2003).

According to Sullivan and Ross (1979), stomatal closure is completely responsible for decreasing photosynthetic rate as soil water deficit increases because of the impeded CO₂ supply. Whereas Hopkins and Huner (2004) further, stated that stomatal closure in sunflower proved to have a minor effect on photosynthesis because of the direct effect on the photosynthetic activity of the chloroplasts decreasing the demand for CO₂ and the level of CO₂ inside the leaf remaining relatively high. It is not clear the extent of stomatal closure at low water deficit level that is able to decrease photosynthesis completely due to decrease in the demand for CO₂ assimilation rate or whether stomatal closure could have been as a result of changes in transpiration rates as recently been suggested by Jomo *et al.* (2014c).

Zlatev *et al.* (2004) worked on beans under water deficit and observed a decrease in stem length. On the other hand, Wu *et al.* (2008) reported a 25% reduction in shoot height in water stressed citrus seedlings, but it is not clear if the seven amaranth species in this study would have a similar or different response when subjected to water deficit. Likewise, the extent of shoot, stem and number of leaves reduction might differ among the amaranth species, hence the desire to undertake this investigation.



Reports by Bogale *et al.* (2011) in wheat plants have showed that chlorophyll *a, b* and total chlorophyll contents reduced in some species while remaining unchanged in others during water deficit stress. It is not known whether such behavior occurs in amaranth and if it occurs, its implication on growth is not clear because an increase in water deficit might not merely lead to plant tissue dehydration but also to an increase in oxidative stress and subsequent deterioration in chloroplast structure and an associated loss of chlorophyll an argument partially supported by Jafar *et al.* (2004).

Results on African nightshades revealed that transpiration could be favored by large root: shoot ratio which ensured that the transpiring plant was well supplied with adequate water (Jomo, 2013). However, a similar study is yet to be done on amaranth. Therefore, the response on transpiration as a result of water deficit, and the strategy of water conservation by amaranth is not known, and this could help compare the tolerance of the seven species by evaluating their leaf numbers which might be reduced or even delayed as a result of increased water deficit in a bid to help in minimizing water loss through transpiration.

According to Jomo, *et al.* (2014b) soil moisture content requirements among two African nightshade species varied with the species, stage of development and the plants age. Any further losses as a result of soil water deficit could be attributed to transpiration and evapotranspiration which must be true for the seven amaranth species, however, the extent of variation has not been established and could have better ranked their absorption and utilization in terms of their water use efficiency with regard to varying water deficit levels.

The comparison between leaf water potential and relative leaf water content as indicators of plant water status had been done in Tomato (*Lycopersicon esculentum* Mill.) by Imana *et al.* (2010) a similar C₄ plant just like amaranth but did not conclusively ascertain the most sensitive integrator of plant water balance with increasing water deficit. Hopkins and Huner (2004) found out leaf growth for cereals to be completely inhibited at -1.00 MPa

whereas root growth still continued until water potential of the root tissues reached -0.45 MPa, this proved to be a higher stress hence the need to investigate it among the seven amaranth species. There was no imposition of such levels of water stress in the soil and tissues of the Amaranth species.

According to Ma *et al.* (2006), CO₂ assimilation rate seemed to reduce with increasing water deficit, while intercellular CO₂ (Ci) increased with increasing water deficit. However there was paucity of knowledge on the amaranth response in terms of CO₂ assimilation and intercellular CO₂ to increasing water deficit and the consequences of both stomatal and non-stomatal effects in the photosynthetic processes due to increase in mesophyll resistance.

Studies on rice varieties by Sikuku *et al.* (2012), have emphasized that changes in PSII fluorescence might have resulted from damage in the reaction centers or from regulatory processes external to the reaction centers including non radiative dissipation or increased excitation transfer to PSII. It is not known whether such behavior occurs among the selected amaranth species under different levels of water deficit imposition.

1.2: Statement of the research problem

Water deficit is an important principal ecological factor that affects the growth and physiology of crops in general. Most parts of Kenya being arid and semi-arid are affected by drought, which in turn reduces water availability to plants (Luvaha *et al.*, 2008), thereby causing significant reductions in plant height, stem diameter and number of leaves of both herbaceous and woody plants (Osorio *et al.*, 1998; Ngugi *et al.*, 2003; Sikuku *et al.*, 2012). This further lowers agricultural production thus contributing to food insecurity and malnutrition problems (GoK, 2002). Statistics have indicated that over 89% of Kenyans are food poor and are malnourished, especially in the rural drought stricken areas (Oniang'o, 2001), yet these drought stricken areas in Kenya are unexploited and can be used for the

production of drought tolerant amaranth species. Amaranthus, as ALV have the potential to alleviate poverty, malnutrition and contribute to food security in Kenya. They are highly recommended due to their high nutritional quality (Modi, 2006), and rich sources of vitamins, mineral trace elements, dietary fibre and proteins (Humphrey et al., 1983; Fafunso and Bassir, 1976). In spite of all these obvious advantages, there is scarce information on their response to drought. The ability of shutting down transpiration through wilting, while reducing stomatal conductance to help avoid desiccation, thereby decreasing intercellular CO₂ concentration and subsequently reducing CO₂ assimilation rate, might be different among the seven amaranth species. The extent to which Electron Transport Rate and the ratio of variable florescence to maximum fluorescence values decrease with increasing water deficit may not necessarily be attributed to photoinhibitory damage due to down regulation of photosystem II, because the losses in chloroplast activity can also be associated with changes in conformation of the thylakoids and of the coupling factor (ATP sythetase, a sub-unit of the thylakoids). Investigations on chlorophyll concentrations, as a result of damage of the chloroplasts membrane among the species might vary with different water deficit levels, and this might not be merely as a result of increased protein synthesis or increased nitrogen metabolism, since any loss in chlorophyll content will lead to an increase in oxidative stress and a subsequent deterioration in the chloroplast structure.

Leaf water potential and relative leaf water content being indicators of plant water status have been noted to reduce significantly with increasing water deficit however the lowest leaf water potential and relative leaf water content that could predispose the leaves to photo inhibition further inhibiting photosynthetic activity among amaranth could help understand their response to soil water deficit.

1.3: Justification of the study

The genus Amaranthus having about 70 species is characterized with a high degree of morphological diversity and a wide spectrum of adaptability to different ecological conditions (Gudu and Gupta 1988). Amaranth is a promising C₄ crop for semi-arid regions possibly due to its ability to adapt to diverse environments and its high nutritive value, hence its used as an animal feed whereas its leaves and seeds are suitable for human consumption (Drinic *et al.*, 2012). This will contribute to the nutritional well being and help in improving food security and provide additional information, particularly on the mechanism employed by the species on soil water deficit conditions. This will further help in deciding on the selection of tolerant species for specific agro ecological zones.

Amaranthus (*spp*) play an important role in income generation and subsistence. A previous survey 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). Further to this, it is seen by many as a new dicotyledonous pseudo-cereal and vegetable crop of high nutritional value and its development as an alternative crop has and still is yet to attract the attention of several researchers over the past and next decades (Aufhammer *et al.*, 1998; Coastea and Damason, 2001; Leon *et al.*, 2004). They are a source of employment for those outside the formal sector in peri-urban areas in many African cities because of their generally short, less labour intensive production systems, low levels of purchase of inputs and high yields (Schippers, 2000). Therefore, important in this study is to understand the response of the seven commonly cultivated amaranth species to soil water deficit for higher yields in order to help alleviate poverty, enhance their value, generate income to farmers and improve human health. This will in turn ensure reduced reliance on exotic vegetables through dissemination of

results and recommending to farmers the superior tolerant amaranth species which may be used in breeding for drought tolerance.

1.4: Objectives

1.4.1: General objective

To evaluate growth and physiological response of seven amaranth species *Amaranthus blitum* (L), *Amaranthus retroflexus* (L), *Amaranthus spinosus* (L), *Amaranthus albus* (L), *Amaranthus cruentus* (L), *Amaranthus hypochondriacus* (L) and *Amaranthus tricolor* (L), to soil water deficit.

1.4.2: Specific objectives

- 1. To determine the effect of soil water deficit on growth of the seven *Amaranthus* species.
- 2. To determine the effect of soil water deficit on gas exchange and chlorophyll fluorescence of the seven *Amaranthus* species.
- 3. To determine the effect of soil water deficit on chlorophyll content of the seven Amaranthus species.
- 4. To determine the effect of soil water deficit on leaf water potential and the relative leaf water content of the seven *Amaranthus* species.

1.5: Hypotheses

- 1. Water deficit significantly reduces the growth of the seven *Amaranthus* species.
- 2. Water deficit significantly reduces the gas exchange and chlorophyll fluorescence parameters of the seven *Amaranthus* species.
- 3. Water deficit significantly reduces chlorophyll content of the seven *Amaranthus* species.
- 4. Water deficit significantly reduces the leaf water potential and the relative leaf water content of the seven *Amaranthus* species.

CHAPTER TWO

LITERATURE REVIEW

2.1: African Leafy Vegetables

Amaranth is known by various names by different ethnic communities in Kenya; Mchicha (Kiswahili), Ododo (Luo), Tsimboka (Luhya), and Emboga (Kisii), (Chweya, 1997). Africa is richly endowed with many well-adapted indigenous food crops that have long been grown on the continent. African leafy vegetables (ALVs) are plant species of which the leafy parts, which may include young, succulent stems, flowers and very young fruit, are used as a vegetable (Thobile, 2010). African leafy vegetables embraces indigenous and indigenized plant species but the focus of this research was on amaranth which are indigenous species that are either genuinely native to a particular region, or which were introduced to that region long enough to have evolved through natural processes or farmer selection.

For most Amaranth species the young growth points and tender leaves are the plant parts that are used in the preparation of vegetable dishes. Petioles and in some cases young tender stems are also included, but old, hard stems are discarded (Vorster *et al.*, 2002). The leaves and other selected plant parts are prepared as potherbs or as relishes, primarily to accompany maize porridge and sorghum.

These crops play an important role in the food security of many resource poor farming families, and have a potential value as a genetic resource for the global community. However, researchers, policy-makers and farmers are yet to exploit the potential of these amaranth species in reducing food insecurity and poverty. The growing interest in these vegetables in both research and policy circles contrasts sharply with the negative image these plants have come to carry among important potential groups of consumers in the African society, particularly the youth and the urbanized, who tend to associate their consumption with poverty and the past (Vorster *et al.*, 2002; Hart and Vorster, 2006). Even in some rural areas,

a decline in the consumption of these species, particularly those that are harvested from the wild or as weeds, in favour of exotic vegetables has been observed (Schippers, 2002), an indication of the indigenous vegetables unexploited potential.

Leafy vegetables, including several AIVs such as Amaranth, are highly valued in the typical African diet as an accompaniment to carbohydrate-based staples. However, they are looked down upon by the urban dwellers (Modi, 2003), yet they are important as sources of essential vitamins, trace elements (iron and calcium) and other nutrients that are important for good health (Chweya and Eyzaguirre, 1996).

The role of leafy vegetables such as amaranth in the food consumption patterns of many households is highly variable and depends on factors such as poverty status, degree of urbanisation, distance to fresh produce markets and time of the year (Prasad *et al.*, 2008).

Quantitatively, the consumption of amaranth collected from the wild or as weeds tends to be inversely proportional to household income (Tshikalange and Van Averbekewv, 2006). Poor households tend to use these species more than their wealthier counterparts, because they lack the financial means to purchase exotic vegetables and to produce their own (Vorster et al., 2002). The use of amaranth species forms part of the safety net that rural people use to cope with poverty, disaster and livelihood stress (Rose and Guillarmod, 1974; Rubaihayo, 1997; Shackleton et al., 2000). During periods of drought, or when the breadwinner in the household becomes unemployed, affected rural households intensify their collection and consumption of amaranth (Shackleton et al., 1999; Dovie, et al., 2002; Shackleton, 2003). Social disturbances can also lead to increased use of amaranth. In poor rural communities consumption of amaranth is particularly important for women and children (Shackleton et al. 2002a). The use of amaranth species is also enhanced by remoteness because households in remote rural areas have limited access to fresh produce markets (Jansen van Rensburg and Vorster, 2005; Hart and Vorster, 2006). Urban households use

amaranth leafy vegetable collected from the wild less than rural households, because they lack access to sites where these vegetables grow naturally.

Concerns regarding agrobiodiversity use and conservation, coupled with poverty alleviation have greatly contributed to reawakened interest in amaranth and ALVs in general (Onyango et al., 2002). It is increasingly recognized that communities are, almost exclusively, the custodians of knowledge on how amaranth is grown and used, as well as their cultural value and genetic diversity (Aynehband, 2008). The best way to reduce the threat of loss of amaranth biodiversity is to improve their conservation through increased production and utilization, and improve their productivity in order to make them more competitive with exotics. Previous studies in East Africa by Mwai and Schippers, (2002) revealed increased use of ALVs such as amaranth and decreased use of exotics (cabbage, kale, spinach), mainly because ALVs require lower inputs to produce compared to exotics and consequently are more affordable for many rural households in the low-income bracket. ALVs especially the amaranth species are easily available and cheap in village markets, but expensive in under-supplied urban markets, indicating that they have the potential to become commercially important and increase their market share (Mwai and Schippers, 2004; Weinberger and Msuya, 2004). They are often cultivated in small kitchen gardens, and occasionally collected from the wild for domestic use and sale in markets (Maundu et al., 1999).

2.2: An overview of amaranthus spp

The family Amaranthaceae, and more specifically the genus *Amaranthus*, consist of about 70 species of which 40 are native to the Americas. Other species originated from Australia, Africa, Asia and Europe (Coastea and Demason, 2001). Amaranth has been grown as a crop in East Africa, Asia and Southern Mexico as long ago as 6700 BC (Akanbi and Togun, 2002). It is an erect, annual herb with average maturity height ranging between 60

and 120 cm and has been regarded as a weed (Muyonga et al., 2008). The plants dark-green leaves are oval with average length of two to four centimetres that often contain dark ring spots (Pedro et al. 1995). The abaxial leaf epidermis of young plants is also often purple-spotted, which makes the entire seedling to appear red in colour. Amaranthus species bear small flowers that are placed close to the stem. Leaves are consumed as a vegetable and the small grains of about 0.6 and 0.8 mg can be utilized as a cereal (Muyonga et al., 2008). Harvesting of leaves and tender shoots from cultivated plants starts about a month after sowing, or two to three weeks after the first rains, and stop as soon as the crop starts flowering Aynehband, (2008).

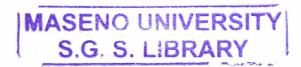
Dieleman *et al.* (1997) reported that harvesting amaranth leaves and tender shoot stimulates the crops vegetative growth making it an ideal alternative crop. Leaf and shoot harvesting from cultivated plants is done repeatedly at weekly intervals and are prepared and consumed in the same way as spinach. It can also be consumed together with sorghum, millet or maize meal porridge. Grain amaranth can be consumed as seeds or milled into flour to prepare food such as cookies, porridge, pancakes, bread muffins, crackers, pasta or other bakery foodstuffs (Muyonga *et al.*, 2008). Apart from its dietary importance, amaranth plants have a good history of medicinal uses. Fresh and dried leaf powder treats inflammation, gonorrhoea and haemorrhoids. Pounded roots of *A. cruentus* treat dysentery while leaf sap is used as eye wash to treat eye infections (Pedro *et al.*, 1995).

Amaranth is propagated through seeds that can be planted by direct sowing in the soil where it takes four to six days to emerge (Dieleman *et al.*, 1997). The amaranth seeds being too small to be sown alone, they may be mixed thoroughly with dry sand to obtain a homogenate mixture that can be broadcasted at the rate of one and half to two kg ha⁻¹. Amaranth seeds can alternatively be germinated in nursery trays and transplanted as seedlings

approximately four weeks after germination when the seedlings are about four to eight centimetre tall (Oyedele, 2002). Thinning may be done at about two weeks where needed. Once established, amaranth can effectively smother most grass weeds, and is remarkably drought-tolerant. Even though the crop is grown on a marginal land, amaranth leaf and grain yield increase with fertility of the soil Leon *et al.* (2004).

Although the crop is sensitive to frost, there are no reported major pest or disease problems associated with amaranth crop production. Pedro *et al.* (1995) reported that, although amaranth can withstand drier environments than most other vegetables, leaf production is boosted during occasional precipitation. Amaranth can be cultivated on marginal soils but will produce higher yields of better quality when planted in fertile well drained soils (Aynehband, 2008).

2.3 Ecological growth and morphology



2.3.1 Amaranth (Amaranthus spp.)

Amaranth is known as pigweed, cockscomb and hell's curse in English. It belongs to the Amaranthaceae family and is an extremely variable, erect to spreading herb. The height of mature plants varies between 0.3 m and 2 m, depending on the species, growth habit and environment. Some species have distinct markings on their leaves. Terminal and auxiliary inflorescences occur. The small seeds of the leafy amaranth are usually very shiny and dark brown to black, contrary to the grain types, which usually have seeds that are cream coloured. (Schippers, 2000; Van Wyk and Gericke, 2000; Vorster *et al.*, 2002; Hart and Vorster, 2006).

Amaranth is a C₄ plant that grows optimally under warm conditions (day temperatures above 25°C and night temperatures not lower than 15°C, bright light and adequate availability of plant nutrients (Van Den Heever and Coertze, 1996a; Maboko, 1999; Chigumira and Grubben 2004). The various amaranth species are tolerant to adverse climatic

conditions (Grubben, 2004; Maundu and Grubben, 2004), but prolonged dry spells induce flowering and decrease leaf yield (Chweya, 1997; Palada and Chang, 2003). Amaranth is photoperiod sensitive and starts to flower as soon as the day length shortens. Under cultivated conditions amaranth produces fresh leaf yields of up to 40 t ha⁻¹ (Van Den Heever and Coertze, 1996b; Maboko, 1999; Schippers, 2000; Mhlonthlo *et al.*, 2006). *Amaranthus blitum* (L), *Amaranthus retroflexus* (L), *Amaranthus Spinosus* (L), *Amaranthus albus* (L), *Amaranthus cruentus* (L), *Amaranthus hypochondriacus* (L), and *Amaranthus tricolor* (L) are among the most widely used amaranth species in Africa (Fox and Norwood Young, 1982; Schippers, 2000; Van Wyk and Gericke, 2000; Vorster *et al.*, 2002; Hart and Vorster, 2006), and this partly formed the basis for this research. The young leaves, growth points and whole seedlings of amaranth are harvested and cooked for use as a vegetable. Amaranth has also got other uses. The leaves and stems of *A. spinosus* are dried and ground for use as snuff (Hart and Vorster, 2006). In areas where in the past access to salt was limited, the whole dried plants of different amaranth species were burnt to produce ash, which was dissolved in water and the precipitate of the filtrate of the ash was used as salt (Fox and Norwood Young, 1982).

2.4 Effect of water deficit on plant growth

Water deficit according to Hsiao (1973), directly and physically reduces plant growth through reduction of cell turgor. Constable and Rawson, (1980) noted that the growth rate of cereal leaves is very sensitive to plant water status, since a small reduction in water potential of the root medium was able to limit the growth rate of maize and barley immediately. Amaranth spp investigated are however classified as pseudo cereals which might be limited in their growth even further considering the various water deficit levels. Water deficit during vegetative stage has been found to reduce plant height, and plant leaf area. However the effects during this stage vary with the severity of stress and age of the crop. Long duration

species suffer less yield damage than short duration species as long vegetative period could help the plant to recover when stress is relieved (Jones and Flowers, 1989). Thobile et al. (2010) while studying wild mustard leafy vegetables revealed that the critical growth stage is the vegetative stage, hence need for sufficient soil water to meet plant demand for vegetative growth but noted that leaf expansion during this vegetative stage is very sensitive to water deficit and that cell enlargement requires turgor to extend the cell wall and a gradient in water potential to bring water into the enlarging cell. According to Salisbury and Ross, (1992) water deficit decreases leaf area, which reduces the intercepted solar radiation, decreased leaf water potential leads to stomatal closure and ultimately results in low transpiration, which in turn increases leaf temperature (Fukai et al., 1999), however the response towards decreased water potential leading to stomatal closure in comparison with the leaf relative water content had not been established among the selected amaranth species. Stomatal closure could be due to the accumulation of Abscisic Acid, 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 CO₂ molecules. It also leads to increased leaf temperature, which reduces the biochemical processes (Forbes and Watson, 1994). Water deficit has also been found to reduce nutrient uptake, 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 moisture stress leads to poor crop establishment and increased seedling mortality (Jose et al., 2004). Leaf water potential has been recognized as the best 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). Kesari et al. (2005), while studying bentgrass clones suggested that

relative water content would better predict maintained growth under increasing water deficit than the simple measure of water potential. This research sought to determine whether leaf relative water content and leaf water potential decreased with increase in water deficit. Plants under water deficit have shown reductions in leaf area and number as a mechanism to reduce water loss through transpiration, and through the inhibition of leaf expansion. Whereas according to Muthomi and Musyimi, (2009) moderate water deficit reduces leaf area in African nightshades (*Solanum scabrum*, Mill) seedlings, and that leaf area reduction is a drought avoidance mechanism in plants subjected to water stress. Liu *et al.* (2004) reported that root length increased significantly in wheat (*Triticum aestivum*) cultivars in response to drought stress. The study in African nightshades (*Solanum scabrum*, Mill) seedlings a similar ALV by Muthomi and Musyimi, (2009) did not address the root to shoot ratio which is an important parameter for determination of plants under soil water deficit.

2.4.1 Effects of water deficit on yield components

Long periods of severe soil water deficit conditions, particularly at water sensitive growth stages causes reduced assimilation of carbon and decreased yield production (Demir et al., 2006). Plant productivity under drought stress is strongly related to the process of yield and dry matter partitioning and temporal biomass distribution (Kage et al., 2004). Mehid and Tahir, (2001) noted diminished yield due to water deficit in almost all genotypes of sunflower. Greater plant yields under water deficit conditions are desirable characters (Vurayai et al. 2011). A common adverse effect of water deficit on crop plants is the reduction in yield and dry biomass production (Farooq et al., 2009). This study was to evaluate the leaf numbers and root to shoot biomass as yield attributes among the selected amaranth species under soil water deficit treatments.

In leafy vegetables the critical growth stage is depended on the kind of crop grown and the purpose of growing such a crop. The vegetative stage is the critical stage and

according to Ma *et al.* (2006), water deficit occurring during the vegetative growth has been shown to have little effect on yield as compared to water stress occurring during the reproductive stage. This however is not the case with amaranth because it is considered as a pseudo cereal crop and the effects of water deficit might not be depended on its growth stage. The occurrence of water deficit at the vegetative stage will definitely reduce leaf area and dry matter as a result of reduced leaf expansion. These effects of water deficit at the vegetative stage will inhibit plant growth resulting in reduced leaf area, dry weight and leaf number.

Sikuku et al. (2010) showed a reduction in whole plant yield with an increase in water deficit in rice. Similar results were observed by Pattanagul and Thitisaksakul, (2011) where water deficit caused a significant reduction in yield of rice. Cengiz et al. (2006) observed that water deficit reduces yield and the total plant dry weight, but affects shoots more than roots causing a larger root: shoot ratio, however this information on amaranth species to soil water deficit conditions is conspicuously missing. The reduction in leaf area (yield) in African nightshades a similar leafy vegetable was ascribed to be an avoidance mechanism aimed at reducing plant water consumption thereby conserving water during periods of drought, however this is not known whether is the case with amaranth. Masinde et al. (2005) on the other hand related this reduction in leaf area (yield) to a decrease in interception of solar radiation and consequently decreasing biomass production for most crops.

2.5 Effects of water deficit on photosynthesis

Photosynthesis is a crucial process that supports growth and yield. It is known to be sensitive to water deficit in many higher plants species (Neluheni, 2004). Despite this there has been conflicting results, discussions and conclusions due to the plant species studied and the experimental procedures followed for investigation on photosynthesis (Gou and Al-Khatib, 2003). However water and CO₂ follow the same diffusion pathways but inverse direction hence transpiration is beneficial to photosynthesis and any resistance in the

diffusion pathway of CO₂ from the atmosphere to the sites of carboxylation within the mesophyll may increase with water stress. A rise in the level of ABA in plants has often been associated with water stress (Luvaha *et al.*, 2008) and is an initial response of the plant to water deficit. An increase in ABA at the start of water stress leads to a decrease in transpiration and leaf expansion in drought tolerant plants (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 boll 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.

Ackerson and Krieng (1977) observed a converse relationship between transpiration rates and water deficits in maize (*Zea mays*). On the other hand Premahandra *et al.* (1992) revealed osmotic adjustment under conditions of water stress on sorghum (*sorghum bicolor* L) and observed that stomatal conductance and cuticular conductance reduced with increased water stress but turgor pressure was maintained. Osmotic adjustment results from the accumulation of solutes within cells, which lowers the osmotic potential and helps maintain turgor of both shoots and roots as plants experience an increasing water deficit. This allows turgor-driven processes, such as stomatal opening and expansion growth, to continue, though at reduced rates, at lower water potentials (Turner and Jones, 1980; Blum *et al.*, 2005; Morgan, 1984). Osmotic adjustment positively affects growth and yield of food legumes under drought stress. Rodriguez *et al.* (2011) examined the osmotic adjustment capability of various pea cultivars and breeding lines under drought and found a linear relationship between yield and capacity of this mechanism.

According to Imana *et al.* (2010), in tomato water deficit stress resulted in significant decreases in the overall photosynthesis as a result of reduction in chlorophyll contents. 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. Further decrease in plant growth as a result of water stress was attributed to reduction in the transpiration rate. Onyango, (1996) worked on rain fed rice (*Oryza sativa* L.) and observed instances of decreased water potential when different varieties of *O. Sativa* were exposed to water stress, however among the amaranth species it is not clear how soil water deficit would affect leaf water potential and hence their photosynthetic capacity.

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 which was attributed to a reduced concentration of CO₂ at the sites of carboxylation and/or impairments of mesophyll metabolism. The concentration of CO₂ at the sites of carboxylation were less than the atmospheric CO₂ 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. A comparison by Shaw and Laing (1966) found that there was a direct correlation between the rate of photosynthesis per unit leaf area and water content of the leaf. Bhagirath (2013), further observed the maximum rate of photosynthesis when water content of the leaves was reduced by 5 to 15% below the maximum leaf saturation and photosynthesis stopped when the leaves lost 50% of their maximum water content. The decrease in relative water content resulting to reductions in photosynthesis among the amaranth species due to increasing soil water deficit is lacking and this partly formed the basis for this study.

Under water deficit, transpiration from the above ground parts has to be controlled to reduce the effects of soil water deficit through changes in the surface area of transpiring parts such as the leaves, physical changes in the transpiring surface and by regulating the stomatal opening and closing (Jomo *et al.*, 2014c). However, this might be common among succulent plants which according to Warren *et al.*, (2011), represent sensitive plants that control water

diffusion through the stomata causing a decline in the photosynthetic rate (Warren *et al.*, 2011). The reduction in transpiration rate may also be attributed to morphological changes such as increased cell wall lignifications (Netondo, 1999). Reduction in soil moisture may lead to lower water content in the leaves, causing guard cells to loose turgor thereby reducing the stomatal pores sizes. In addition, an increase in stomatal resistance may lead to reduced water transport in the leaves, resulting in a decrease in stomatal conductance which in turn decreases transpiration and also limits photosynthesis (Periera *et al.*, 2000). Luvaha *et al.* (2008), observed that internal CO₂ concentration seemed not to be affected by water deficit, therefore low CO₂ assimilation under water deficit, without a corresponding decline in internal CO₂ concentration which could be attributed 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). However it is not clear if such a response can occur in other plants such as amaranth.

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). 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). Bjorkman and Powles (1984), showed that in Nerium oleander L. water deficit caused

photoinhibitory damage in the photosynthetic system and that water stress predisposes the leaves to photoinhibition. The amount of functional PSII reaction centres in a given leaf is the result of the rates of damage and degradation repair of PSII (Antelmo *et al.*, 2010).

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. Recent studies on African nightshades by Jomo (2013), produced inconclusive results on the overall photosynthetic capacity of the plant which is exhibited by the flow of electrons through PSII. Their study was also limited to two African nightshades (*Solanum scabrum* Mill. and *Solanum villosum* Mill.), therefore this formed the basis for the current research because it was not known whether amaranth behave differently.

2.6 Effect of water deficit on chlorophyll content

Moaveni et al. (2011) showed water deficit conditions to cause 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 Fariduddin et al. (2009) 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 Brassica juncea 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 Sikuku et al. (2012) on rice seedlings showed that 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 which 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 Jomo *et al.* (2014a), chlorophyll content in plants often decreases with increased mesophyll resistance commonly observed in water deficient regions.

Chlorophylls *a* and *b* are prone to soil water deficit (Farooq *et al.*, 2009). Drought stress produces changes in the ratio of chlorophylls *a* and *b* (Anajum *et al.*, 2003). Manivannan *et al.* (2007) reported a large decline in the chlorophylls *a*, *b* and total chlorophyll content in different sunflower varieties caused by water deficit, Shamshi (2010) while working on wheat cultivars reported that drought stress reduced the concentration of chlorophyll *b* more than chlorophyll *a*, similar changes in chlorophyll *a* and *b* concentration as a result of increasing water deficit had not been established among the selected amaranth species, yet they are photosynthetic pigments which play a role in photochemical reactions of photosynthesis determining photosynthetic efficiency.

CHAPTER THREE

MATERIALS AND METHODS

3.1: Study site

The study site is at an altitude of between 1570 and 2015m a.s.l. Geographically, the region falls within the latitude range 0°, 30'S and 0°, 58 S and longitude 34°, 38' and 34° East. The soils are mainly loam soils classified as phaeozems, being well-drained, deep reddish brown clay with pH ranging between 4.6 and 5.4 (Otieno et al., 1993). 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. The area receives conventional type of rainfall of 1200-2000 mm due to its proximity to Lake Victoria and it is hilly in topography. MASENO UNIVERSIT S.G. S. LIBRARY

3.2 Experimental Layout and Treatments

The experiment was set up in an uncontrolled glasshouse conditions at Kenya Agricultural and Livestock Research Organisation (KALRO), Kisii Centre. The minimum and maximum temperatures inside the glasshouse ranged between 24 ± 8 °C and 32 ± 8 °C respectively with a relative humidity of $37 \pm 5^{\circ}/_{\circ}$, and photosynthetic flux density (PPFD) from $450 - 650 \, \mu \text{mol photons m}^{-2} \text{s}^{-1}$.

Seeds of Amaranth;-Amaranthus blitum (L), Amaranthus retroflexus (L), Amaranthus Spinosus (L), Amaranthus albus (L), Amaranthus cruentus (L), Amaranthus hypochondriacus (L) and Amaranthus tricolor (L), were obtained from Kenya Agricultural and Livestock Research Organisation, (KALRO) Kisii Centre, and sown in 20 litre PVC pots. The soil was dug from a portion of the garden and then solarised to remove weeds, plants and crop debris, and to break up large clods after which it was filled in pots with perforated bottoms to facilitate drainage. The pots had a 25 cm inner diameter and 40 cm depth each holding soil up to three-quarters full, and each pot weighed (15Kg) before sowing. The experiment was laid out as a Completely Randomized Design (CRD), consisting of four treatments, seven amaranth species and three replications. Four seeds were sown in each pot and after seven days thinning of immature plants was done remaining with two plants per pot (Imana *et al.*, 2010). Before initiating water deficit treatments plants were irrigated with tap water using a hand sprinkler to full saturation for ten days in order to improve root development (Vanassche and Laker 1989). After which 1 litre of water was applied to each pot and this was able to wet all the soil pots to full saturation, while the same 1 litre quantity of water was applied to subsequent 4 treatment regimes, comprising of T1 Watering daily (control), T2 Watering every 3rd and 6th day, T3 Watering every 9th day and T4 Watering every 12th day upto the end of the experiment. The pot soil field capacities of T1, T2, T3 and T4 were 34 %, 28 %, 22 % and 16 % respectively, and the soil moisture content for T1, T2, T3 and T4 were 29.7 %, 23.1 %, 18.1 % and 12.8 % respectively.

3.3 Measurements of Growth Parameters

3.3.1 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 begun the first day before initiating treatments. Measurements were done on one plant per pot in all replications.

3.3.2 Stem diameter

The diameter of one plant species per treatment per replication was measured by use of a vernier caliper with an accuracy of \pm 0.02, at a height of 10 cm from the stem base. This begun the first day after initiating treatments, and was done after every twelve days before treatments.

3.3.3 Number of leaves

The number of fully expanded fresh leaves of one plant species per treatment per replication on the main stem and branches were counted and recorded once after every twelve days.

Counting begun the first day after initiating treatments.

3.3.4 Root: Shoot ratio determination

These were calculated at the end of the experiment. 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 root 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 ratio was computed as a percentage according to Sikuku *et al.* (2010).

3.4 Measurements of Physiological parameters

3.4.1 Gas exchange

Measurements of gas exchange parameters involved one plant per species per treatment of each replication. Net carbon dioxide assimilation rate, transpiration rate, stomatal conductance and intercellular CO₂ concentration were determined by use of a portable infrared gas analyzer (IRGA) {Model: CIRAS 1-pp systems Ltd., Herts, U. K.}. Gas exchange was determined from an area of 2.5cm² of the fully expanded 4th leaf per species per treatment which had attained maturity. These measurements begun when the plants were twelve days old and were carried out after every twelve days before initiating treatments.

Measurements were done in the morning (0930-1130 HR) to avoid high afternoon temperatures (Zlatev *et al.*, 2004). The leaf cuvette temperature ranged between 28.5 °C and 32.6 °C.

3.4.2 Chlorophyll fluorescence

Chlorophyll fluorescence measurements were carried out using a portable fluorescence monitoring system, Hansatech model FMS 2 (Hansatech Instruments, Germany). The measurements begun when the plants were twelve days old and was carried out after every twelve days before watering. One plant species per treatment per replication were sampled and measurements were done on the fourth fully expanded leaf. The leaves to be used for the measurements were dark adapted for 30 minutes using the dark adaptation clips and then illuminated for 6 seconds to induce fluorescence. The initial fluorescence (Fo) and the maximum fluorescence (Fm) were measured and the variable fluorescence (Fv=Fm-Fo) and the Fv/Fm ratio was calculated (Jomo, 2013).

3.5 Measurements of Biochemical parameters

3.5.1 Chlorophyll content

Chlorophyll content was determined using methods of Arnon (1949) and Coombs *et al.* (1987) as described by Netondo (1999). The 4^{th} youngest fully expanded compound leaf was randomly sampled from all treatments. In the laboratory 0.5g of the fresh leaf tissue was measured and cut into small pieces into specimen bottle. 10ml of 80% acetone was added and the set up kept in the dark for 7 days for chlorophyll to be extracted by the acetone. 1ml of the filtered extract was diluted with 20ml of 80% acetone and absorbance of the chlorophyll solution measured using a spectrophotometer at 645 and 663 nm to determine the content of chlorophyll a and b and the total chlorophyll of the leaf tissue. This measurements were done after every twelve days before watering. The respective chlorophyll content in milligram of

chlorophyll per gram of leaf collected 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 2. mg *Chl b* / g leaf tissue =22.9 (D645)-4.68 (D663) x V / 1000xW......eqn 3. mg *tChl* / g leaf tissue =20.2 (D645)+8.02 (D663) x V / 1000xW......eqn 4. Where; D= absorbance measured at wavelengths 645nm and 663nm.

V= volume (ml) of the acetone extract.

W= fresh weight (g) of leaf tissue from which the extract was made. $mg \, chl \, a/g$ leaf tissue = Milligram chlorophyll a per gram leaf tissue $mg \, chl \, b/g$ leaf tissue = Milligram chlorophyll b per gram leaf tissue $mg \, tchl/g$ leaf tissue = Milligram total chlorophyll per gram leaf tissue

3.6 Leaf water potential

Leaf water potential was determined by use of scholander pressure bomb in the glass house pre-dawn to avoid transpiration of the excised leaf in the afternoon. The instrument allowed high pressure to be applied to the exterior of detached leaf while leaving the cut end of the leaf exposed to the air. The amaranthus leaf petiole was cut at the base using a razor blade. One fully expanded leaf exposed to sunlight of each species per treatment per replication were measured and immediately placed in the chamber with the cut end of the petiole protruding through the seal. The leaf was properly sealed in the pressure chamber using an appropriate slitted gasket. Pressure was then applied slowly until the water/sap appeared at the cut end of the petiole and this was observed using a magnifying glass and proper lighting. The gas supply was cut off immediately water/sap appeared. Therefore the pressure (MPa) required to produce the first wet appearances was recorded and assumed that it was equal but opposite to the negative tension which existed inside the twig before it was cut. The accumulated gas in the chamber was then released carefully and the leaf unmounted in

readiness for more measurements. Measurements were carried out on a clear sunny day after every twelve days before initiating treatments.

3.7 Relative leaf water content

Relative leaf water content was determined on the leaf of one plant species per treatment per replication. The leaves to be harvested were rinsed with distilled water to eliminate surface accumulation of dust two hours before harvesting. One gram of fresh leaf sample were cut using a cork borer and weighed immediately to get the fresh weight (Wf). The leaf disks were then floated in distilled water in a petri dish for three hours to get the turgid weight (Wt). The disks were then dried in an oven at 80°C until a constant weight was obtained to get the oven dry weight (Wd). Measurements begun from the day treatments were initiated and was done after every twelve days. The relative water content was calculated using the formula of Coombs *et al.* (1985) as follows;

Relative water content (R) = $(Wf - Wd) / (Wt-Wd) \times 100$eqn 5.

Where;

Wf = Fresh weight

Wd = Dry weight

Wt = Turgid weight

3.8 Statistical Analysis of Data

Data were analyzed using the statistical program (SAS, 2003). Differences between treatments as well as amaranth species were tested by a two-way analysis of variance (ANOVA). Treatment means were separated using Fisher's protected t-test least significant difference (LSD) test at 5% significance level (Snedecor and Cochran, 1980).

CHAPTER FOUR

RESULTS

4.1 Plant growth parameters

4.1.1 Shoot height

Soil water deficit generally reduced shoot height of all the amaranth species (Table 4.1.1.1). There were significant differences in shoot height among the amaranth species in response to soil water treatments (Table 4.1.1.1). The highest reduction in shoot height was in T4 treatments followed by T3, T2 and T1 respectively (Table 4.1.1.1). The highest height reduction in shoot height was observed in *Amaranthus tricolor*, followed by *A. spinosus*, *A. blitum*, *A. retroflexus*, *A. cruentus A. hypochondriacus* and *A. albus* respectively (Table 4.1.1.2). There was no significant interaction between soil water deficit treatments and amaranth species (P = 0.5702), appendix 3. There was a significant difference ($P \le 0.5702$) in shoot height reduction among the species in all days (Table 4.1.1.2).

Table 4.1.1.1: Shoot height for seven Amaranth species namely; S1 *A. albus*, S2 *A. hypochondriacus*, S3 *A. cruentus*, S4 *A. retroflexus*, S5 *A.blitum*, S6 *A. spinosus*, and S7 *A. tricolor*, grown under four watering regimes;- T1-Watering daily, T2-Watering every 3rd and 6th day, T3-Watering every 9th day and T4-Watering every 12th day. Values represent means of three replicates ± SE, in a 96 days period.

Amaranth species	Shoot height (cm) under four soil water treatments							
	T1 (Control)	T2	Т3	T4	Species mean	LSD values		
A. albus	62.17±5.01a	66.21±5.11b	62.75±5.25 c	59.25±5.35d	64.34±2.58d	0.6060		
A.hypochondriacus	67.54±5.04a	64.67±5.10b	61.25±5.24 c	57.75±5.34d	62.80±2.58f	0.6088		
A. cruentus	65.54±5.00a	62.67±5.06b	59.25±5.21c	56.04±5.31d	60.88±2.55e	0.5707		
A. retroflexus	64.00±5.04a 62.00±5.11a	61.17±5.10b 59.17±5.12b	57.79±5.24c 55.63±5.26 c	54.54±5.35d 52.63±5.43d	59.38±2.58b 57.35±2.60a	0.6229 0.6088		
A. blitum								
A. spinosus	60.63±5.06a	57.79±5.06b	54. 29±5.18 c	51.46±5.43d	56.04±2.58c	0.6173		
A. tricolor	58.6±5.00a	56.0±5.09b	52.9±5.23c	50.1±5.48d	54.42±2.58g	0.5707		
Treatments mean	63.9±1.89a	61.1±1.91b	57.7±1.96c	54.5±2.01d				
/ 1	cies mean (S) 0.2954 atments mean (T) 0.2 2							

Means with the same letter along the row are not significantly different at ($p \le 0.05$).

Table 4.1.1.2: Shoot height for the seven amaranth species namely; S1 *A. albus*, S2 *A. hypochondriacus*, S3 *A. cruentus*, S4 *A. retroflexus*, S5 *A.blitum*, S6 *A. spinosus*, S7 *A. tricolor*, in 12, 24, 36, 48, 60, 72, 84 and 96 DAT. Values represent means of three replicates \pm SE, in a 96 days period. LSD values; S1 = 0.8809, S2 = 0.873, S3 = 0.861, S4 = 0.859, S5 = 0.857, S6 = 0.807, S7 = 0.807.

		Shoo	t height (cm) un					
Species	12 DAT	24 DAT	36 DAT	48 DAT	60 DAT	72 DAT	84 DAT	96 DAT
S1	26.5±1.04h	36.1±1.47g	48.4±1.42f	62.2±1.15e	66.3±1.27d	81.1±1.68c	96.0±0.54b	98.3±0.70a
S2	25.3±0.95h	35.1±1.47g	46.4±1.42f	60.1±1.13e	65.3±1.27d	78.1±1.68c	95.0±0.55b	97.3±0.70a
S3	24.3±0.95h	33.1±1.47g	43.4±1.42f	58.6±0.94e	64.3±1.27d	75.2±1.64c	93.0±0.55b	95.3±0.70a
S4	22.3±0.94h	32.1±1.47g	42.4±1.42f	55.6±0.94e	62.2±1.24d	74.2±1.64c	92.0±0.54b	94.3±0.70a
S5	20.5±0.95h	31.1±1.45g	39.4±1.42f	51.5±0.98e	60.7±1.18d	72.2±1.64c	90.7±0.54b	92.9±0.69a
S6	19.4±0.95h	28.8±1.39g	40.4±1.42f	49.4±0.94e	59.2±1.32d	71.1±1.63c	88.9±0.48b	91.2±0.60a
S7	18.4±0.95h	27.8±1.39g	36.4±1.42f	48.4±0.94e	57.4±1.14d	69.3±1.40c	87.7±0.40b	89.9±0.50a

Means with the same letter along the row are not significantly different at ($p \le 0.05$).

4.1.2 Stem diameter

Soil water deficit generally reduced stem diameter of the amaranth species (Table 4.1.2.1). There were significant differences in stem diameter reduction among the amaranth species in response to soil water treatments (Table 4.1.2.1). The highest reduction in stem diameter was in T4 treatments followed by T3, T2 and T1 respectively (Table 4.1.2.1). The highest stem diameter reduction was observed in *Amaranthus tricolor*, followed by *A. spinosus*, *A. blitum*, *A. retroflexus*, *A. cruentus A. hypochondriacus* and *A. albus* respectively (Table 4.1.2.2). There was no significant interaction between soil water treatments and amaranth species (P = 0.1042), appendix 3. There was a significant difference at ($p \le 0.05$) in stem diameter among the species in all days (Table 4.1.2.2).

Table 4.1.2.1: Stem diameter for seven Amaranth species namely; S1 *A. albus*, S2 *A. hypochondriacus*, S3 *A. cruentus*, S4 *A. retroflexus*, S5 *A.blitum*, S6 *A. spinosus*, and S7 *A. tricolor*, grown under four watering regimes;- T1-Watering daily, T2-Watering every 3^{rd} and 6^{th} day, T3-Watering every 9^{th} day and T4-Watering every 12^{th} day. Values represent means of three replicates \pm SE, in a 96 days period. LSD values; S1 = 0.0154, S2 = 0.0112, S3 = 0.0122, S4 = 0.0103, S5 = 0.0112, S6 = 0.0111, S7 = 0.0141.

	Stem diameter (cm) under four soil water treatments								
T1 (Control)	T2	Т3	T4	Species mean	LSD values				
4.46±0.231 a	4.36±0.222 b	4.29±0.219 c	4.17±0.217 d	4.32±0.110 d	0.0154				
4.44±0.232 a	4.35±0.222 b	4.27±0.219 c	4.16±0.217 d	4.30±0.110 f	0.0112				
4.41±0.234 a	4.33±0.222 b	4.26±0.219 c	4.13±0.216 d	4.28±0.110 e	0.0122				
4.39±0.232a	4.31±0.222b	4.24±0.219c	4.12±0.217d	4.26±0.110 b	0.0103				
4.36±0.235 a	4.29±0.222 b	4.22±0.218 c	4.10±0.216 d	4.25±0.110 a	0.0112				
4.35±0.232 a	4.27±0.222 b	4.20±0.217 c	4.07±0.217 d	4.23±0.110 c	0.0111				
4.34±0.231 a	4.26±0.222 b	4.19±0.215 c	4.06±0.216 d	4.21±0.109 g	0.0141				
4.39±0.086a	4.31±0.082b	4.24±0.081c	4.116±0.080d						
ean (S) 0.0061				3					
s mean (T) 0.3092									
-	4.46±0.231 a 4.44±0.232 a 4.41±0.234 a 4.39±0.232a 4.36±0.235 a 4.35±0.232 a 4.34±0.231 a 4.39±0.086a ean (S) 0.0061 s mean (T) 0.3092	4.46±0.231 a 4.36±0.222 b 4.44±0.232 a 4.35±0.222 b 4.41±0.234 a 4.33±0.222 b 4.39±0.232a 4.31±0.222b 4.36±0.235 a 4.29±0.222 b 4.35±0.232 a 4.27±0.222 b 4.34±0.231 a 4.26±0.222 b 4.39±0.086a 4.31±0.082b ean (S) 0.0061 ss mean (T) 0.3092	4.46±0.231 a 4.36±0.222 b 4.29±0.219 c 4.44±0.232 a 4.35±0.222 b 4.27±0.219 c 4.41±0.234 a 4.33±0.222 b 4.26±0.219 c 4.39±0.232a 4.31±0.222b 4.24±0.219c 4.36±0.235 a 4.29±0.222 b 4.22±0.218 c 4.35±0.232 a 4.27±0.222 b 4.20±0.217 c 4.34±0.231 a 4.26±0.222 b 4.19±0.215 c 4.39±0.086a 4.31±0.082b 4.24±0.081c ean (S) 0.0061 s mean (T) 0.3092	4.46±0.231 a 4.36±0.222 b 4.29±0.219 c 4.17±0.217 d 4.44±0.232 a 4.35±0.222 b 4.27±0.219 c 4.16±0.217 d 4.41±0.234 a 4.33±0.222 b 4.26±0.219 c 4.13±0.216 d 4.39±0.232a 4.31±0.222b 4.24±0.219c 4.12±0.217d 4.36±0.235 a 4.29±0.222 b 4.22±0.218 c 4.10±0.216 d 4.35±0.232 a 4.27±0.222 b 4.20±0.217 c 4.07±0.217 d 4.34±0.231 a 4.26±0.222 b 4.19±0.215 c 4.06±0.216 d 4.39±0.086a 4.31±0.082b 4.24±0.081c 4.116±0.080d	4.46±0.231 a 4.36±0.222 b 4.29±0.219 c 4.17±0.217 d 4.32±0.110d 4.44±0.232 a 4.35±0.222 b 4.27±0.219 c 4.16±0.217 d 4.30±0.110f 4.41±0.234 a 4.33±0.222 b 4.26±0.219 c 4.13±0.216 d 4.28±0.110e 4.39±0.232a 4.31±0.222b 4.24±0.219c 4.12±0.217d 4.26±0.110b 4.36±0.235 a 4.29±0.222 b 4.22±0.218 c 4.10±0.216 d 4.25±0.110a 4.35±0.232 a 4.27±0.222 b 4.20±0.217 c 4.07±0.217 d 4.23±0.110c 4.34±0.231 a 4.26±0.222 b 4.19±0.215 c 4.06±0.216 d 4.21±0.109g 4.39±0.086a 4.31±0.082b 4.24±0.081c 4.116±0.080d team (S) 0.0061 team (T) 0.3092				

Table 4.1.2.2: Stem diameter for the seven amaranth species namely; S1 *A. albus*, S2 *A. hypochondriacus*, S3 *A. cruentus*, S4 *A. retroflexus*, S5 *A.blitum*, S6 *A. spinosus*, and S7 *A. tricolor*, in 12, 24, 36, 48, 60, 72, 84 and 96 DAT. Values represent means of three replicates \pm SE, in a 96 days period. LSD values; S1 = 0.0145, S2 = 0.0157, S3 = 0.0158, S4 = 0.0165, S5 = 0.0218, S6 = 0.0173, S7 = 0.0199.

		Stem	diameter (cm) u					
S	12 DAT	24 DAT	36 DAT	48 DAT	60 DAT	72 DAT	84 DAT	96 DAT
S1	2.70±0.02h	2.85±0.02g	3.77±0.06f	4.16±0.02e	4.84±0.03d	5.14±0.04c	5.38±0.04b	5.71±0.04a
S2	2.69±0.02h	2.83±0.02g	3.76±0.05f	4.14±0.02e	4.82±0.03d	5.12±0.04c	5.37±0.04b	5.69±0.04a
S3	2.67±0.02h	2.80±0.02g	3.72±0.05f	4.13±0.02e	4.81±0.03d	5.09±0.04c	5.36±0.04b	5.67±0.04a
S4	2.66±0.02h	2.79±0.02g	3.70±0.05f	4.11±0.02e	4.79±0.03d	5.07±0.04c	5.34±0.04b	5.65±0.04a
S5	2.65±0.02h	2.76±0.02g	3.68±0.05f	4.10±0.02e	4.76±0.03d	5.05±0.04c	5.33±0.04b	5.62±0.04a
S6	2.62±0.02h	2.76±0.02g	3.64±0.06f	4.08±0.02e	4.77±0.03d	5.03±0.04c	5.31±0.04b	5.60±0.04a
S7	2.61±0.02h	2.76±0.02g	3.64±0.06f	4.07±0.02e	4.75±0.03d	5.01±0.04c	5.30±0.04b	5.57±0.04a

4.1.3 Number of leaves

Soil water deficit generally reduced the number of leaves of the amaranth species (Table 4.1.3.1). There were significant differences in number of leaves among the amaranth species in response to soil water treatments (Table 4.1.3.1). The highest reduction in number of leaves was in T4 treatments followed by T3, T2 and T1 respectively (Table 4.1.3.1). The highest reduction in number of leaves was observed in *Amaranthus tricolor*, followed by *A. spinosus, A. blitum, A. retroflexus, A. cruentus A. hypochondriacus* and *A. albus* respectively (Table 4.1.3.2). There was a significant interaction between soil water treatments and amaranth species (P = 0.0001), appendix 3. There was a significant difference in leaf number (p≤0.05), in 12, 24, 36 and 48 DAT for *Amaranthus albus, A. hypochondriacus, A. cruentus, A. retroflexus and A. blitum,* whereas there was no significant difference in 60, 72, 84 and 96 DAT. *Amaranthus spinosus* and *A. tricolor* were significantly different in all days except in 84 DAT where they were not significantly different (Table 4.1.3.2).

Table 4.1.3.1: Number of leaves for seven Amaranth species namely; S1 *A. albus*, S2 *A. hypochondriacus*, S3 *A. cruentus*, S4 *A. retroflexus*, S5 *A. blitum*, S6 *A. spinosus*, and S7 *A. tricolor*, grown under four watering regimes;- T1-Watering daily, T2-Watering every 3^{rd} and 6^{th} day, T3-Watering every 9^{th} day and T4-Watering every 12^{th} day. Values represent means of three replicates \pm SE, in a 96 days period.

Amaranth species	Number of leave	es under four soil	water treatments	CHIS .		
	T1 (Control)	T2	Т3	T4	Species mean	LSD values
A. albus	82.50±5.99 a	75.92±5.88 b	68.54±5.41 c	61.50±4.78 d	72.11±2.840d	0.8427
A. hypochondriacus	81.21±6.03 a	73.83±5.94b	65.92±5.29 c	59.88±4.76 d	70.21±2.843f	0.8282
A. cruentus	79.17±5.97 a	71.96±5.85 b	63.46±5.03 c	58.08±4.63 d	68.17±2.782e	0.8590
A. retroflexus	77.04±5.95a	69.71±5.87b	60.88±4.96c	55.92±4.56d	65.89±2.770b	0.8261
A. blitum	75.58±5.92 a	68.50±5.89 b	59.46±4.91c	54.29±4.49d	64.46±2.758a	0.8282
A. spinosus	73.75±5.90 a	66.63±5.95b	57.58±4.96 c	52.21±4.49 d	62.54±2.771c	0.7786
A. tricolor	72.67±5.83 a	64.79±5.87 b	55.88±4.93 c	48.96 ±4.15d	60.57±2.739g	0.9232
Treatments mean	77.4±2.220a	70.2±2.205b	61.7±1.911c	55.8±1.720d		
LSD ($P = 0.05$) Species n	mean(S) 0.409		* n=			
LSD ($P = 0.05$) Treatment	nts mean (T) 0.3092		e s			*

Table 4.1.3.2: Number of leaves for the seven amaranth species namely; S1 *A. albus*, S2 *A. hypochondriacus*, S3 *A. cruentus*, S4 *A. retroflexus*, S5 *A.blitum*, S6 *A. spinosus*, and S7 *A. tricolor*, in 12, 24, 36, 48, 60, 72, 84 and 96 DAT. Values represent means of three replicates \pm SE, in a 96 days period. LSD values; S1 = 1.1683, S2 = 1.1011, S3 = 1.1713, S4 = 1.2024, S5 = 1.1918, S6 = 1.2148, S7 = 1.3055.

		Numl	per of leaves und					
S	12 DAT	24 DAT	36 DAT	48 DAT	60 DAT	72 DAT	84 DAT	96 DAT
S1	26.3±1.48f	39.4±1.48e	56.3±2.26d	76.5±2.13c	89.6±1.75b	90.5±3.07b	98.8±3.31a	99.6±3.91a
S2	25.0±1.47f	39.9±1.49e	53.8±2.28d	75.5±2.13c	88.0±1.80b	88.7±3.16b	96.8±3.38a	97.0±4.12a
S3	23.8±1.35f	35.9±1.49e	52.3±2.15d	73.5±2.13c	85.5±1.89b	86.5±2.97b	93.5±3.56a	94.3±4.32a
S4	21.8±1.34f	33.5±1.36e	50.4±2.20d	71.8±2.19c	83.8±1.90b	83.0±3.15b	91.4±3.66a	91.5±4.24a
S5	20.8±1.35g	32.5±1.36f	48.8±2.10e	69.9±2.20d	82.7±1.94b	80.8±3.47c	90.2±3.71a	90.0±4.19a
S6	18.3±1.41g	30.5±1.36f	47.3±2.17e	68.2±2.10d	80.9±1.94b	78.8±3.88c	88.2±3.71a	88.1±4.02a
S7	16.3±1.23f	30.6±1.92e	45.8±2.09d	66.4±2.40c	77.6±2.22b	77.2±4.32b	85.6±3.95a	85.1±4.28a

4.2 Root to shoot ratio

The root to shoot ratio increased with increase in soil water deficit in all the amaranth species from T4, T3, and T2 respectively (Fig 4.2). There were no significant interactions in root: shoot ratio ($p \le 0.05$) between soil water treatments and the amaranth species (P = 0.4501). *Amaranthus tricolor*, had the highest root to shoot ratio followed by *A. hypochondriacus*, *A. cruentus*. *A. retroflexus*, *A. blitum*, *A. spinosus* and *A. albus* respectively (Fig 4.2).

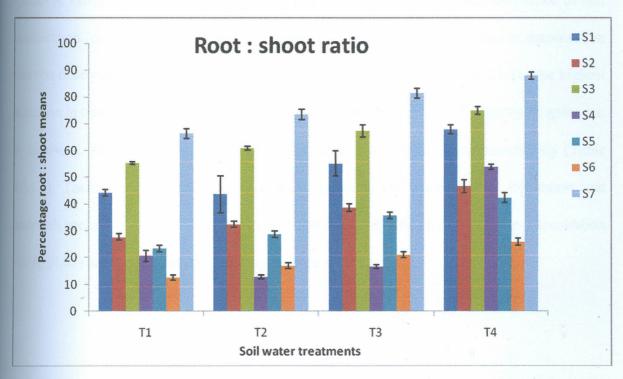


Fig. 4.2 The mean root: shoot ratio of the seven amaranth species namely; S1 *A. blitum*, S2 *A. retroflexus*, S3 *A. spinosus*, S4 *A. albus*, S5 *A. cruentus*, S6 *A. hypochondriacus* S7 *A. tricolor*, grown under four watering regimes; T1-Watering daily, T2-Watering every 3^{rd} and 6^{th} day, T3-Watering every 9^{th} day and T4-Watering every 12^{th} day. Values represent means of three replicates \pm SE, in a 96 days period. LSD values; S1 = 13.913, S2 = 5.3704, S3 = 14.238, S4 = 36.144, S5 = 4.3342, S6 = 3.5802, S7 = 5.5952.

4.3 Gas exchange parameters

4.3.1 Leaf transpiration

Soil water deficit generally reduced leaf transpiration of all the amaranth species (Table 4.3.1.1). There was no significant difference ($p\ge0.05$), in leaf transpiration among amaranth species *A. blitum*, *A. spinosus*, *A. cruentus* and *A. tricolor*, in T2 and T3 treatments, whereas species *A. retroflexus*, *A. albus* and *A. hypochondriacus*, had a significant difference in leaf transpiration ($p\le0.05$) in all treatments (Table 4.3.1.1). The highest reduction in transpiration rate was observed in T4, followed by T3, T2 and T1 respectively (Table 4.3.1.1). The highest reduction in transpiration rate was observed in *Amaranthus tricolor*, followed by *A. spinosus*, *A. blitum*, *A. retroflexus*, *A. cruentus*, *A. hypochondriacus* and *A. albus* respectively (Table 4.3.1.2). There was no significant interaction between soil water deficit treatments and amaranth species (P = 0.2629), appendix 4. There was a significant difference in transpiration rate ($p\le0.05$) among all species in all days (Table 4.3.1.2).

Table 4.3.1.1: Leaf transpiration for seven Amaranth species namely; S1 *A. albus*, S2 *A. hypochondriacus*, S3 *A. cruentus*, S4 *A. retroflexus*, S5 *A. blitum*, S6 *A. spinosus*, and S7 *A. tricolor*, grown under four watering regimes; T1-Watering daily, T2-Watering every 3^{rd} and 6^{th} day, T3-Watering every 9^{th} day and T4-Watering every 12^{th} day. Values represent means of three replicates \pm SE, in a 96 days period.

T1	T2	Т3	T4	Species mean	LSD values
1.00±0.013a	1.0±0.010b	1.0±0.017c	1.0±0.021d	0.96±0.008a	0.0231
1.0±0.009a	0.9±0.012b	0.9±0.024c	0.9±0.025d	0.91±0.010b	0.0202
0.9±0.017a	0.8±0.019ba	0.8±0.026b	0.8±0.027c	0.87±0.011c	0.0243
0.9±0.015a	0.8±0.022c	0.8±0.024b	0.8±0.027d	0.82±0.011d	0.0171
0.83±0.019a	0.79±0.025b	0.79±0.027b	0.75±0.027c	0.79±0.0124e	0.0202
0.8±0.024a	0.8±0.028b	0.8±0.032b	0.7±0.032 c	0.77±0.0145f	0.0159
0.76±0.024a	0.73±0.027b	0.71±0.032b	0.7±0.033c	0.72±0.015g	0.0248
0.9±0.009a	0.8±0.010b	0.8±0.011c	0.8±0.012d		N .
nean(S) 0.0103			5 2		
its mean (T) 0.007	8				
1	(Control) 1.00±0.013a 1.0±0.009a 0.9±0.017a 0.9±0.015a 0.83±0.019a 0.8±0.024a 0.76±0.024a 0.9±0.009a mean(S) 0.0103 nts mean (T) 0.0078	(Control) 1.00±0.013a 1.0±0.010b 1.0±0.009a 0.9±0.012b 0.9±0.017a 0.8±0.019ba 0.9±0.015a 0.8±0.022c 0.83±0.019a 0.79±0.025b 0.8±0.024a 0.73±0.028b 0.76±0.024a 0.73±0.027b 0.9±0.009a 0.8±0.010b mean(S) 0.0103 nts mean (T) 0.0078	(Control) 1.00±0.013a 1.0±0.010b 1.0±0.017c 1.0±0.009a 0.9±0.012b 0.9±0.024c 0.9±0.017a 0.8±0.019ba 0.8±0.026b 0.9±0.015a 0.8±0.022c 0.8±0.024b 0.83±0.019a 0.79±0.025b 0.79±0.027b 0.8±0.024a 0.8±0.028b 0.8±0.032b 0.76±0.024a 0.73±0.027b 0.71±0.032b 0.9±0.009a 0.8±0.010b 0.8±0.011c	(Control) 1.00±0.013a 1.0±0.010b 1.0±0.017c 1.0±0.021d 1.0±0.009a 0.9±0.012b 0.9±0.024c 0.9±0.025d 0.9±0.017a 0.8±0.019ba 0.8±0.026b 0.8±0.027c 0.9±0.015a 0.8±0.022c 0.8±0.024b 0.8±0.027d 0.83±0.019a 0.79±0.025b 0.79±0.027b 0.75±0.027c 0.8±0.024a 0.8±0.028b 0.8±0.032b 0.7±0.032 c 0.76±0.024a 0.73±0.027b 0.71±0.032b 0.7±0.033c 0.9±0.009a 0.8±0.010b 0.8±0.011c 0.8±0.012d mean(S) 0.0103 0.0078	(Control) 1.00±0.013a 1.0±0.010b 1.0±0.017c 1.0±0.021d 0.96±0.008a 1.0±0.009a 0.9±0.012b 0.9±0.024c 0.9±0.025d 0.91±0.010b 0.9±0.017a 0.8±0.019ba 0.8±0.026b 0.8±0.027c 0.87±0.011c 0.9±0.015a 0.8±0.022c 0.8±0.024b 0.8±0.027d 0.82±0.011d 0.83±0.019a 0.79±0.025b 0.79±0.027b 0.75±0.027c 0.79±0.0124e 0.8±0.024a 0.8±0.028b 0.8±0.032b 0.7±0.032 c 0.77±0.0145f 0.76±0.024a 0.73±0.027b 0.71±0.032b 0.7±0.033c 0.72±0.015g 0.9±0.009a 0.8±0.010b 0.8±0.011c 0.8±0.012d

Table 4.3.1.2: Leaf transpiration for the seven amaranth species namely; S1 *A. albus*, S2 *A. hypochondriacus*, S3 *A. cruentus*, S4 *A. retroflexus*, S5 *A.blitum*, S6 *A. spinosus*, and S7 *A. tricolor* in 12, 24, 36, 48, 60, 72, 84 and 96 DAT. Values represent means of three replicates \pm SE, in a 96 days period. LSD values; S1 = 0.0242, S2 = 0.0225, S3 = 0.0285, S4 = 0.0331, S5 = 0.0327, S6 = 0.0343, S7 = 0.035.

,		Leaf tran	nspiration under fo	our soil water treat	tments			
S	12 DAT	24 DAT	36 DAT	48 DAT	60 DAT	72 DAT	84 DAT	96 DAT
		to a second processing the second sec				-		
S1	1.05±0.01a	0.96±0.007c	0.97±0.007c	0.95±0.007c	0.96±0.007c	1.02±0.010b	0.86±0.03d	0.88±0.03d
S2	0.96±0.01b	0.94±0.009b	0.96±0.008b	0.94±0.007b	0.95±0.007b	0.99±0.006a	0.79±0.34c	0.80±0.035c
S3	0.85±0.01a	0.92±0.010b	0.94±0.009b	0.94±0.006b	0.94±0.006b	0.97±0.005a	0.70±0.02d	0.71±0.03d
S4	0.75±0.09f	0.89±0.14b	0.93±0.009a	0.87±0.013e	0.88±0.009c	0.87±0.004d	0.66±0.02h	0.69±0.024g
S5	0.65±0.009c	0.86±0.20b	0.92±0.14a	0.87±0.013b	0.86±0.07b	0.86±0.004b	0.64±0.024c	0.66±0.024c
S6	0.57±0.01d	0.85±0.020b	0.91±0.014a	0.85±0.014b	0.84±0.0008b	0.85±0.009b	0.60±0.022c	0.62±0.024c
S7	0.53±0.010f	0.84±0.020b	0.90±0.014a	0.74±0.27d	0.80±0.11c	0.77±0.05dc	0.58±0.26e	0.59±0.027e

4.3.2 Stomatal conductance

Soil water deficit generally reduced stomatal conductance of all the amaranth species (Table 4.3.2.1). Amaranthus spinosus, and A. hypochondriacus were not significantly different (≥0.05) under T3 and T4 treatments, whereas Amaranthus blitum, A. retroflexus, A. albus, A. cruentus and A. tricolor had a significant difference in stomatal conductance in soil water treatments. The highest reduction in stomatal conductance rate was observed in T4, followed by T3 and T2 respectively (Table 4.3.2.1). The highest reduction in stomatal conductance was observed in Amaranthus tricolor, followed by A. spinosus, A. blitum, A. retroflexus, A. cruentus A. hypochondriacus and A. albus respectively (Table 4.3.2.1). There was no significant interaction between soil water treatments and amaranth species (P = 0.9985), appendix 4. There was no significant difference at (p≥0.05), in stomatal conductance for Amaranthus spinosus and A. tricolor in all days (Table 4.3.2.2). Species A. albus and A. hypochondriacus were significantly different (p≤0.05) in 24, 36, 60, 72 and 96 DAT, while they were not significantly different in 12, 48, and 84 DAT. Stomatal conductance in Amaranthus cruentus was significantly different ($p \le 0.05$) at 24, 60, 72 and 96 DAT, and not significantly different at 12, 36, 48 and 84 DAT. Amaranthus retroflexus was significantly different at (p≤0.05) in 12, 24, 36, 60, 72 and 96 DAT, whereas it was not significantly different in 48 and 84 DAT. Amaranthus blitum was significantly different at (p≤0.05) in 12 and 24 DAT, whereas it was not significantly different in 36, 48, 60, 72, 84 and 86 DAT.



Table 4.3.2.1: Stomatal conductance for seven Amaranth species namely; S1 *A. albus*, S2 *A. hypochondriacus*, S3 *A. cruentus*, S4 *A. retroflexus*, S5 *A.blitum*, S6 *A. spinosus*, and S7 *A. tricolor*, grown under four watering regimes;-T1-Watering daily, T2-Watering every 3rd and 6th day, T3-Watering every 9th day and T4-Watering every 12th day. Values represent means of three replicates ± SE, in a 96 days period.

Amaranth species	Stomatal conducta	ance (mmol m ⁻² s	⁻¹) under four soi	l water treatments		
	T1 (Control)	T2	Т3	T4	Species mean	LSD values
A. albus	19.0±0.59 a	17.8±0.52 b	16.3±0.61 c	14.5±0.71 d	16.88±0.345d	1.1016
A. hypochondriacus	17.8±0.55a	16.6±0.50b	15.0±0.57c	14.0±0.52 c	15.82±0.303f	1.0964
A. cruentus	17.5±0.55 a	15.8±0.46 b	14.0±0.52 c	12.7±0.53 d	14.98±0.315e	0.96
A. retroflexus	16.4±0.48a	14.9±0.47b	13.3±0.52c	12.2±0.53d	14.18±0.296b	1.0933
A. blitum	15.4±0.50 a	13.8±0.48 b	12.3±0.55c	10.8±0.54d	13.09±0.312a	1.0964
A. spinosus	14.6±0.58 a	12.9±0.53 b	11.3±0.60 c	10.3±0.60 c	12.27±0.330c	1.043
A. tricolor	13.5±0.54 a	11.5±0.54b	10.1±0.65 c	9.0±0.66d	11.042±0.343g	1.1199
Treatments mean	16.32±0.243a	14.74±0.242b	13.17±0.263c	11.92±0.261d		
LSD $(P = 0.05)$ Species	mean(S) 0.526					
LSD $(P = 0.05)$ Treatme	ent mean (T) 0.3976	2, -				A

Table 4.3.2.2: Stomatal conductance for the seven amaranth species namely; S1 *A. albus*, S2 *A. hypochondriacus*, S3 *A. cruentus*, S4 *A. retroflexus*, S5 *A.blitum*, S6 *A. spinosus*, and S7 *A. tricolor*, in 12, 24, 36, 48, 60, 72, 84 and 96 DAT. Values represent means of three replicates \pm SE, in a 96 days period. LSD values; S1 = 1.5461, S2 = 1.475, S3 = 1.5506, S4 = 1.5652, S5 = 1.5579, S6 = 1.3576, = S7 1.5837.

r		Stoma	atal conductance					
S	12 DAT	24 DAT	36 DAT	48 DAT	60 DAT	72 DAT	84 DAT	96 DAT
S1	18.7±0.99ba	13.5±0.42d	17.4±0.64b	18.8±0.63ba	13.2±1.04d	15.5±0.69c	18.3±0.51ba	19.8±0.67a
S2	17.0±0.83ba	12.5±0.42d	16.4±0.65b	17.8±0.63ba	13.0±0.62d	14.5±0.69c	17.3±0.51ba	18.2±0.68a
S3	15.5±1.02bc	11.8±0.51d	15.7±0.71bc	17.0±0.72ba	12.7±0.78d	14.5±0.69c	15.5±0.73bc	17.3±0.72a
S4	13.5±1.02d	10.8±0.51g	14.3±0.70c	16.0±0.72ba	13.0±0.62e	13.5±0.69f	16.0±0.49ba	16.3±0.66a
S5	11.5±1.02b	9.7±0.83c	14.6±0.61a	14.8±0.63a	12.0±0.62b	12.4±0.73b	14.5±0.63a	15.3±0.66a
S6	9.6±0.91c	8.5±0.42c	13.8±0.47a	14.0±0.72a	11.0±0.62b	11.5±0.69b	15.0±0.49a	14.8±0.82a
S7	7.9±0.98c	7.5±0.42c	12.8±0.56a	13.6±0.91a	10.2±0.66b	10.3±0.75b	12.5±0.73a	13.5±0.73a

4.3.3 CO₂ assimilation rate

Soil water deficit generally reduced CO₂ assimilation rate of all the amaranth species (Table 4.3.3.1). There was a significant difference ($p \ge 0.05$), in CO₂ assimilation rate among all the soil water treatments and the amaranth species. The highest reduction in CO₂ assimilation rate was observed in T4, followed by T3, T2 and T1 respectively (Table 4.3.3.1). The highest reduction in CO_2 assimilation rate was observed in Amaranthus tricolor, followed by A. spinosus, A. blitum, A. retroflexus, A. cruentus A. hypochondriacus and A. albus respectively (Table 4.3.3.2). There was a significant interaction between soil water deficit treatments and amaranth species (P = 0.001), appendix 4. There was a significant difference ($p \le 0.05$), in CO₂ assimilation rate for Amaranthus retroflexus in all days. Amaranthus albus were significantly different (p≤0.05), in 36, 84 and 96 DAT, whereas it was not significantly different in 12, 24, 48, 60 and 72 DAT. Amaranthus hypochondriacus was significantly different (p \leq 0.05), in 24, 84 and 96 DAT, whereas it was not significantly different in 12, 36, 48 and 60 DAT. Amaranthus cruentus was significantly different (p≤0.05), in DAT 36, 60 and 96, whereas it was not significantly different in 12, 24, 48, 72 and 84 DAT. Amaranthus blitum was significantly different (p≤0.05), in 24 and 96 DAT, whereas it was not significantly different in 12, 24, 36, 48, 60, 72 and 84 DAT. Amaranthus spinosus was significantly different (p≤0.05), in 24 and 72 DAT, whereas it was not significantly different in 12, 36, 48, 60 and 84 DAT. Amaranthus tricolor was significantly different at ($p \le 0.05$), in 96 DAT, whereas it was not significantly different in 12, 24, 36, 48, 60, 72 and 84 DAT (Table 4.3.3.2).

Table 4.3.3.1: CO₂ assimilation rate for seven Amaranth species namely; S1 *A. albus*, S2 *A. hypochondriacus*, S3 *A. cruentus*, S4 *A. retroflexus*, S5 *A.blitum*, S6 *A. spinosus*, and S7 *A. tricolor*, grown under four watering regimes; T1-Watering daily, T2-Watering every 3^{rd} and 6^{th} day, T3-Watering every 9^{th} day and T4-Watering every 12^{th} day. Values represent means of three replicates \pm SE, in a 96 days period. LSD values; S1 = 0.4924, S2 = 0.5394, S3 = 0.5264, S4 = 0.3224, S5 = 0.5394, S6 = 0.4709, S7 = 0.4559.

Amaranth species	CO ₂ assimilation 1	rate (mmol m ⁻² s ⁻¹) under four soil v	water treatments		
	T1 (Control)	T2	Т3	T4	Species mean	LSD values
A. albus	17.75±0.227a	17.04±0.229 b	16.04±0.244c	14.96±0.304d	16.45±0.165d	0.4924
A. hypochondriacus	16.54±0.248a	15.29±0.244b	12.38±0.300c	9.50±0.319d	13.43±0.312f	0.5394
A. cruentus	13.88±0.291a	12.33±0.231b	10.75±0.257c	9.25±0.377 d	11.55±0.229e	0.5264
A. retroflexus	12.46±0.248a	9.67±0.274b	7.71±0.252c	6.08±0.288d	8.98±0.277b	0.3224
A. blitum	9.83±0.274 a	7.00±0.233 b	5.42 ±0.288c	3.75±0.277 d	6.50±0.265a	0.5394
A. spinosus	6.83±0.293 a	5.29±0.244 b	2.96 ±0.292c	1.21±0.233 d	4.07 ±0.257c	0.4709
A. tricolor	6.08±0.329a	4.67±0.214 b	2.71±0.266c	1.13±0.228d	3.65±0.233g	0.4559
Treatments mean	11.91±0.341a	10.19±0.360b	8.30±0.373c	6.60±0.377d		
LSD $(P = 0.05)$ Species	mean(S) 0.2355					
LSD ($P = 0.05$) Treatme	ent mean (T) 0.178					

Table 4.3.3.2: CO_2 assimilation rate for the seven amaranth species namely; S1 *A. albus*, S2 *A. hypochondriacus*, S3 *A. cruentus*, S4 *A. retroflexus*, S5 *A.blitum*, S6 *A. spinosus*, and S7 *A. tricolor*, in 12, 24, 36, 48, 60, 72, 84 and 96 DAT. Values represent means of three replicates \pm SE, in a 96 days period. LSD values; S1 = 0.4559, S2 = 0.6659, S3 = 0.7629, S4 = 0.6342, S5 = 0.6964, S6 = 0.7445, S7 = 0.6448.

		CO ₂ assim	ilation rate under	four soil water tre	atments			
S	12 DAT	24 DAT	36 DAT	48 DAT	60 DAT	72 DAT	84 DAT	96 DAT
S1	17.8±0.25a	17.8±0.32a	16.8±0.32b	15.8±0.33d	15.8±0.32d	16.4±0.31cb	16.3±0.73c	14.8±0.32e
S2	14.8±0.64b	14.8±0.81a	13.8±0.81b	12.3±0.84ed	12.8±0.81cd	13.7±0.88b	13.3±1.22c	11.8±0.81e
S3	12.9±0.43a	13.0±0.49a	12.0±0.49b	10.8±0.51cd	11.0±0.49c	11.3±0.84cb	11.5±0.90cb	10.0±0.49d
S4	10.2±0.65b	10.2±0.78a	9.5±0.67c	8.2±0.68d	8.5±0.67e	8.8±0.86f	9.0±1.07g	7.5±0.67
S5	7.8±0.60a	7.8±0.69a	6.9±0.66b	5.7±0.67ed	5.9±0.66cd	6.5±0.71cb	6.4±1.05cb	5.0±0.68e
S6	5.2±0.60ba	5.3±0.71a	4.5±0.65bc	3.1±0.66fe	3.5±0.65de	4.4±0.65c	4.1±1.02dc	2.6±0.47f
S7	4.9±0.56a	4.8±0.60a	4.0±0.52b	2.8±0.54d	3.1±0.54cd	3.9±0.73b	3.6±0.91cb	2.1±0.48e

4.3.4 Intercellular CO₂

Soil water deficit generally increased intercellular CO₂ concentration of all the amaranth species (Table 4.3.4.1). There was a no significant difference ($p \ge 0.05$), in intercellular CO₂ for Amaranthus albus, and A. hypochondriacus in T3 and T4 treatments, whereas species A. blitum, A. retroflexus, A. spinosus A. cruentus and A. tricolor had a significant difference among all soil water treatments (Table 4.3.4.1). The highest intercellular CO₂ was observed in T1 treatment, followed by T2, T3 and T4 respectively (Table 4.3.4.1). The highest reduction in intercellular CO₂ assimilation rate was observed in Amaranthus tricolor, followed by A. spinosus, A. blitum, A. retroflexus, A. cruentus A. hypochondriacus and A. albus respectively (Table 4.3.4.2). There was a significant interaction between soil water treatments and amaranth species (P = 0.0001), appendix 4. There was a significant difference (p≤0.05), in intercellular CO₂ concentration for Amaranthus spinosus, A. albus, A. cruentus and A. tricolor in all days (Table 4.3.4.2). Amaranthus blitum was significantly different $(p \le 0.05)$, in 12, 24, 48, 60, 72 and 84 DAT, whereas there was no significant difference in 36 and 96 DAT. Amaranthus retroflexus was significantly different ($p \le 0.05$), in 72 DAT, whereas there was no significant difference in 12, 24, 36, 48, 60, 84 and 96 DAT. Amaranthus hypochondriacus was significantly different (p≤0.05), in 96 DAT, whereas there was no significant difference in 12, 24, 36, 48, 60, 72 and 84 DAT (Table 4.3.4.2).

Table 4.3.4.1: Intercellular CO₂ for seven Amaranth species namely; S1 *A. albus*, S2 *A. hypochondriacus*, S3 *A. cruentus*, S4 *A. retroflexus*, S5 *A. blitum*, S6 *A. spinosus*, and S7 *A. tricolor*, grown under four watering regimes;- T1-Watering daily, T2-Watering every 3rd and 6th day, T3-Watering every 9th day and T4-Watering every 12th day. Values represent means of three replicates ± SE, in a 96 days period.

Amaranth species	Intercellular Co					
	T1 (Control)	T2	Т3	T4	Species mean	LSD values
A. albus	13.96±0.523c	15.17±0.488b	15.83±0.488a	16.08±0.727a	15.26±0.290d	0.4854
A. hypochondriacus	8.50±0.507c	11.54±0.521b	14.17±0.488a	14.75±0.725a	12.24±0.377f	0.4709
A. cruentus	8.50±0.507d	9.88±0.501c	11.17±0.488b	12.50±0.507a	10.510±0.290e	0.6528
A. retroflexus	5.13±0.490d	6.79±0.485c	8.50±0.507b	11.17±0.488a	7.90±0.334b	0.6607
A. blitum	2.83±0.445d	4.54±0.521c	5.83±0.488b	8.50±0.507a	5.43±0.322a	0.4709
A. spinosus	1.25±0.382d	2.38±0.429 c	4.17±0.488b	5.50±0.507 a	3.32±0.279c	0.8006
A. tricolor	1.00±0.248d	2.25±0.414c	3.50±0.478 b	4.50±0.507a	2.813±0.248g	0.3992
Treatments mean	5.88±0.378a	7.51±0.394b	9.02±0.393c	10.43±0.384d		
LSD $(P = 0.05)$ Species	mean(S) 0.2874					
LSD $(P = 0.05)$ Treatme	ent mean (T) 0.2173					

Table 4.3.4.2: Intercellular Co_2 assimilation rate for the seven amaranth species namely; S1 *A. albus*, S2 *A. hypochondriacus*, S3 *A. cruentus*, S4 *A. retroflexus*, S5 *A.blitum*, S6 *A. spinosus*, and S7 *A. tricolor*, in 12, 24, 36, 48, 60, 72, 84 and 96 DAT. Values represent means of three replicates \pm SE, in a 96 days period. LSD values; S1 = 0.9344, S2 = 1.1322, S3 = 0.6659, S4 = 0.9337, S5 = 0.6864, S6 = 0.9232, S7 = 0.5646.

	,	Intercellular Co	o ₂ assimilation ra					
S	12 DAT	24 DAT	36 DAT	48 DAT	60 DAT	72 DAT	84 DAT	96 DAT
S1	15.8±0.32c	12.8±0.32f	18.0±0.27a	14.8±0.32d	11.0±0.78g	16.8±0.32b	13.8±0.32e	18.9±0.28a
S2	12.8±0.81dc	9.0±1.00f	14.8±0.80a	11.8±0.80e	8.8±0.80f	13.8±0.80b	10.8±0.80e	15.9±0.80a
S3	11.0±0.49d	8.0±0.49g	13.0±0.49b	10.0±0.49e	7.0±0.49h	12.0±0.49c	9.0±0.49f	14.1±0.48a
S4	8.5±0.67d	5.3±0.78g	10.2±0.77b	7.5±0.66f	4.5±0.67h	9.5±0.67c	6.5±0.67e	11.3±0.76a
S5	5.9±0.65d	2.9±0.65g	7.8±0.65b	4.9±0.65e	2.1±0,59h	6.9±0.65c	3.9±0.66f	8.9±0.69a
S6	3.5±0.64c	1.3±0.39ed	5.3±0.71b	3.3±0.70c	0.7±0.28e	4.5±0.64b	1.8±0.52d	6.3±0.71a
S7	3.0±0.52d	0.8±0.28g	4.8±0.60b	2.1±0.48e	0.4±0.23g	4.0±0.52c	1.5±0.38f	5.9±0.60a

4.4 Chlorophyll fluorescence

4.4.1 Fv/Fm

Soil water deficit generally reduced Fv/Fm of all the amaranth species (Table 4.4.1.1). There was a significant difference (p \leq 0.05) in Fv/Fm among all soil water treatments and amaranth species. The lowest Fv/Fm ratio was observed in T4, followed by T3, T2 and T1 respectively (Table 4.4.1.1). The highest reduction in Fv/Fm was observed in *Amaranthus tricolor*, followed by *A. spinosus*, *A. blitum*, *A. retroflexus*, *A. cruentus A. hypochondriacus* and *A. albus* respectively (Table 4.4.1.2). There was no significant interaction between soil water treatments and amaranth species (P = 0.7171), appendix 5. There was a significant difference (p \leq 0.05), in Fv/Fm for *Amaranthus blitum* in 24, 36 and 60 DAT, whereas there was a significant difference in 12, 48, 72, 84 and 96 DAT. *Amaranthus retroflexus*, *A. spinosus*, *A. albus* and *A. cruentus* were not significantly different (p \geq 0.05), in all days except 36 DAT where they were significantly different. *Amaranthus hypochondriacus* was not significantly different (p \geq 0.05), in all days. *Amaranthus tricolor* was significantly different (p \leq 0.05), in 24, 36 and 84 DAT, whereas it was not significantly different on 12, 48, 60, 72 and 96 DAT, (Table 4.4.1.2).

Table 4.4.1.1: Fv/Fm for seven Amaranth species namely; S1 *A. albus*, S2 *A. hypochondriacus*, S3 *A. cruentus*, S4 *A. retroflexus*, S5 *A.blitum*, S6 *A. spinosus*, and S7 *A. tricolor*, grown under four watering regimes; T1-Watering daily, T2-Watering every 3^{rd} and 6^{th} day, T3-Watering every 9^{th} day and T4-Watering every 12^{th} day. Values represent means of three replicates \pm SE, in a 96 days period. LSD values; S1 = 0.0139, S2 = 0.013, S3 = 0.0284, S4 = 0.0124, S5 = 0.013, S6 = 0.0134, S7 = 0.014.

Amaranth species	Fv/Fm ra	atio under four so	il water treatments				
	T1 (Control)	T2	Т3	T4	Species mean	LSD values	
A. albus	0.94±0.015 a	0.86±0.013b	0.78±0.008 c	0.73±0.008 d	0.83±0.010a	0.0139	
A. hypochondriacus	0.93±0.015a	0.85±0.011b	0.78±0.007 c	0.72±0.008 d	0.82±0.010b	0.013	
A. cruentus	0.91±0.014 a	0.84±0.011b	0.77±0.007 c	0.71±0.007 d	0.81±0.009c	0.0284	
A. retroflexus	0.91±0.013a	0.83±0.011b	0.76±0.008c	0.70±0.007d	0.80±0.009d	0.0124	
A. blitum	0.89±0.013 a	0.82±0.011b	0.75±0.008 c	0.69±0.007 d	0.79±0.009e	0.013	
A. spinosus	0.89±0.013 a	0.79±0.020b	0.75±0.007 c	0.69±0.007 d	0.78±0.010f	0.0134	
A. tricolor	0.88±0.013 a	0.80±0.010b	$0.74 \pm 0.007c$	0.68±0.007d	0.77±0.009f	0.014	
Treatments mean	0.91±0.005a	0.83±0.005b	0.77±0.003c	0.70±0.0030d			
LSD $(P = 0.05)$ Species	mean (S) 0.0081		,			,	
LSD $(P = 0.05)$ Treatme	ent mean (T) 0.0061						

Table 4.4.1.2: Fv/Fm for the seven amaranth species namely; S1 A. albus, S2 A. hypochondriacus, S3 A. cruentus, S4 A. retroflexus, S5 A. blitum, S6 A. spinosus, and S7 A. tricolor, in 12, 24, 36, 48, 60, 72, 84 and 96 DAT. Values represent means of three replicates \pm SE, in a 96 days period. LSD values; S1 = 0.0176, S2 = 0.0189, S3 = 0.0184, S4 = 0.0323, S5 = 0.0197, S6 = 0.0401, S7 = 0.0198.

		FV/FM t	inder four soil w	ater treatments				
S	12 DAT	24 DAT	36 DAT	48 DAT	60 DAT	72 DAT	84 DAT	96 DAT
S1	0.83±0.03c	0.88±0.03b	0.77±0.02f	0.81±0.03dc	0.91±0.03a	0.82±0.02dc	0.80±0.03de	0.80±0.02e
S2	0.82±0.03b	0.87±0.03a	0.76±0.02d	0.81±0.03cb	0.89±0.03a	0.81±0.02cb	0.79±0.03c	0.80±0.02c
S3	0.81±0.03b	0.86±0.03a	0.75±0.02d	0.80±0.03cb	0.87±0.03a	0.80±0.02cb	0.79±0.0.3c	0.79±0.02c
S4	0.80±0.03b	0.85±0.03a	0.75±0.02d	0.79±0.03cb	0.86±0.03a	0.80±0.02cb	0.77±0.02c	0.78±0.02c
S5	0.79±0.03b	0.84±0.03a	0.74±0.02d	0.78±0.03b	0.84±0.03a	0.79±0.02b	0.76±0.03c	0.77±0.02cb
S6	0.78±0.03b	0.82±0.03a	0.73±0.02c	0.78±0.03b	0.84±0.03a	0.75±0.04cb	0.75±0.02cb	0.77±0.02cb
S7	0.77±0.03c	0.81±0.03b	0.73±0.02e	0.78±0.03c	0.83±0.03a	0.78±0.02c	0.75±0.02d	0.77±0.02c

4.4.2 Electron Transport Rate (ETR)

Soil water deficit generally reduced ETR of all the amaranth species (Table 4.4.2.1). There was a significant difference in ETR (p \leq 0.05), among all soil water treatments and amaranth species. The lowest ETR values were observed in T4, followed by T3, T2 and T1 respectively (Table 4.4.2.1). The highest reduction in ETR was observed in *Amaranthus tricolor*, followed by *A. spinosus*, *A. blitum*, *A. retroflexus*, *A. cruentus A. hypochondriacus* and *A. albus* respectively (Table 4.4.2.2). There was no significant interaction between soil water treatments and amaranth species (P = 1.000), appendix 5. There was a significant difference in ETR at (p \leq 0.05), for *Amaranthus albus*, *A. hypochondriacus A. cruentus*, *A. blitum A. spinosus*, and *A. tricolor* in 48 DAT, whereas there was no significant difference in 12, 24, 36, 60, 72, 84 and 96 DAT. *Amaranthus retroflexus* was significantly different at (p \leq 0.05), in 48, 72 and 84 DAT (Table 4.4.2.2).

Table 4.4.2.1: ETR for seven Amaranth species namely; S1 *A. albus*, S2 *A. hypochondriacus*, S3 *A. cruentus*, S4 *A. retroflexus*, S5 *A.blitum*, S6 *A. spinosus*, and S7 *A. tricolor*, grown under four watering regimes;- T1-Watering daily, T2-Watering every 3rd and 6th day, T3-Watering every 9th day and T4-Watering every 12th day. Values represent means of three replicates ± SE, in a 96 days period.

Amaranth species	ETR (μ mol ele	ctrons m ⁻² s ⁻¹) unde	r four soil water trea	atments		
-	T1 (Control)	T2	T3	T4	Species mean	LSD values
A. albus	253.8±19.7a	214.5±17.9 b	180.3±17.0 c	137.2±14.9d	196.43±9.665a	39.882
A. hypochondriacus	248.0±19.4a	209.1±17.7b	175.6±17.0c	132.2±14.5d	191.24±9.540ab	40.056
A. cruentus	243.3±19.3a	206.4 ±17.6b	171.6±16.9c	129.6±14.5d	187.71±9.483cab	39.674
A. retroflexus	226.9±20.6a	203.0±17.3b	167.4±16.8c	125.1±14.0d	180.58±9.399cdb	41.159
A. blitum	234.3±18.9a	199.5±17.2b	162.2±16.9 c	122.2±14.0d	179.58±9.338cdb	40.733
A. spinosus	231.6±18.9a	196.0±17.0b	158.7±16.8c	118.8±13.8d	176.29±9.291cd	40.862
A. tricolor	228.0±18.7a	193.4±16.9b	154.7±16.8c	116.0 ±13.8d	173.01±9.252d	39.666
Treatments mean	237.99±7.233a	203.12±6.468b	167.21±6.302c	125.87±5.309d		
LSD (P = 0.05) Species LSD (P = 0.05) Treatme						

Table 4.4.2.2: ETR for the seven amaranth species namely; S1 *A. albus*, S2 *A. hypochondriacus*, S3 *A. cruentus*, S4 *A. retroflexus*, S5 *A.blitum*, S6 *A. spinosus*, and S7 *A. tricolor*, in 12, 24, 36, 48, 60, 72, 84 and 96 DAT. Values represent means of three replicates \pm SE, in a 96 days period. LSD values; S1 = 41.159, S2 = 40.862, S3 = 40.733, S4 = 40.056, S5 = 39.882, S6 = 39.674, S7 = 39.666.

		ETR 1	under four soil	water treatments				
Species	12 DAT	24 DAT	36 DAT	48 DAT	60 DAT	72 DAT	84 DAT	96 DAT
S1	247.85±25.	220.41	107.69	309.42	263.49	155.23	159.68	107.69
	58cb	±17.09c	±8.23e	±7.39a	±16.84b	±14.87d	±35.25d	±8.23e
S2	240.72±25.	215.50	104.10	303.04	255.70	151.52	155.20	104.10
	68b	±16.96b	±7.89d	±7.27a	±16.63b	±15.02c	±35.07c	±7.89d
S3	235.15±25.	213.21	100.12	299.46	251.56	149.00	153.06	100.12
	23b	±16.80b	±7.73d	±7.29a	±16.16b	±15.22c	±34.91c	±7.73d
S4	204.88±28.	209.75	97.38	294.31	246.37	146.03	148.54	97.38
	54b	±17.01b	±7.80d	±8.06a	±15.64b	±15.18f	±34.32c	±7.80d
S5	223.56±24.	205.48	93.35	290.41	243.29	142.651	144.52	93.35
	60b	±17.02b	±8.05d	±8.05a	±15.47b	±15.16c	±34.05c	±8.05d
S6	219.09±24.	202.85	90.99	286.63	238.29	139.86	141.63	90.98
	95b	±17.05b	±8.00d	±8.30a	±15.54b	±15.11c	±33.82c	±8.00d
S7	215.08±24.	200.06	87.99	283.56	233.36	136.24	139.79	87.99
31	47b	±17.17b	±8.13d	±8.27a	±15.36b	±15.30c	±87.99c	±8.13d

4.5 Biochemical parameter measurements

4.5.1 Chlorophyll content

4.5.1.1 Chlorophyll a

Soil water deficit generally reduced chlorophyll a of all the amaranth species (Table 4.5.1.1.1). There was a significant difference (p \leq 0.05), in chlorophyll a among all soil water treatments and the amaranth species. The lowest chlorophyll a values were in T4, followed by T3, T2 and T1 treatments respectively for the seven species (Table 4.5.1.1.1). The highest reduction in chlorophyll a was observed in Amaranthus tricolor, followed by A. spinosus, A. blitum, A retroflexus, A cruentus A hypochondriacus and A albus respectively (Table 4.5.1.1.2). There was no significant interaction between soil water treatments and amaranth species (P= 1.000), appendix 6. There was a significant difference in chlorophyll a content (p \leq 0.05) among all species in all days (Table 4.5.1.1.2).

Table 4.5.1.1.1: Chlorophyll *a* for seven Amaranth species namely; S1 *A. albus*, S2 *A. hypochondriacus*, S3 *A. cruentus*, S4 *A. retroflexus*, S5 *A. blitum*, S6 *A. spinosus*, and S7 *A. tricolor*, grown under four watering regimes; T1-Watering daily, T2-Watering every 3^{rd} and 6^{th} day, T3-Watering every 9^{th} day and T4-Watering every 12^{th} day. Values represent means of three replicates \pm SE, in a 96 days period. LSD values; S1 = 0.0825, S2 = 0.0794, S3 = 0.0856, S4 = 0.0829, S5 = 0.0794, S6 = 0.1093, S7 = 0.0825.

Amaranth species	Chlorophyll a (r	ng g ⁻¹ leaf tissue) u	nder four soil water	treatments		-
	T1 (Control)	T2	Т3	T4	Species mean	LSD values
A. albus	4.48±0.241 a	3.78±0.225 b	2.97±0.195 c	2.45±0.134 d	3.42±0.128d	0.0825
A. hypochondriacus	4.41±0.242 a	3.70±0.223 b	2.91±0.197 c	2.38±0.132 d	3.35±0.127f	0.0784
A. cruentus	4.33±0.240a	3.63±0.223b	2.78±0.193 c	2.29±0.127 d	3.26±0.127e	0.0856
A. retroflexus	4.23±0.237a	3.55±0.221b	2.72±0.192c	2.20±0.127d	3.18±0.126b	0.0829
A. blitum	4.15±0.233a	3.44±0.215b	2.63±0.190 c	2.11±0.124 d	3.08±0.125a	0.0794
A. spinosus	4.09±0.226 a	3.38±0.216 b	2.56±0.189 c	2.05±0.125 d	3.02±0.124c	0.1093
A. tricolor	4.00±0.228a	3.30±0.215 b	2.46±0.185 c	1.97±0.121d	2.93±0.124g	0.0825
Treatments mean	4.24±0.088a	3.54±0.083b	2.72±0.072c	2.21±0.049d		n
LSD $(P = 0.05)$ Species	mean (S) 0.0424					
LSD $(P = 0.05)$ Treatme	ent mean (T) 0.0321					2009

Table 4.5.1.1.2: Chlorophyll a for the seven amaranth species namely; S1 A. albus, S2 A. hypochondriacus, S3 A. cruentus, S4 A. retroflexus, S5 A. blitum, S6 A. spinosus, and S7 A. tricolor, in 12, 24, 36, 48, 60, 72, 84 and 96 DAT, Values represent means of three replicates \pm SE, in a 96 days period. LSD values; S1 = 0.1167, S2 = 0.1122, S3 = 0.121, S4 = 0.1172, S5 = 0.1123, S6 = 0.1545, S7 = 0.1169.

1		Chlor	ophyll a under t					
Species	12 DAT	24 DAT	36 DAT	48 DAT	60 DAT	72 DAT	84 DAT	96 DAT
S1	2.7±0.15f	3.08±0.27e	4.21±0.31b	2.47±0.10g	2.34±0.25h	5.21±0.33a	3.85±0.26c	3.45±0.28d
S2	2.8±0.16f	3.00±0.28e	4.10±0.32b	2.39±0.09g	2.27±0.24h	5.13±0.33a	3.76±0.26c	3.38±0.28d
S3	2.6±0.15f	2.94±0.28e	4.03±0.32b	2.32±0.10g	2.20±0.24h	5.03±0.33a	3.67±0.26c	3.28±0.28d
S4	2.5±0.15f	2.84±0.27e	3.93±0.32b	2.25±0.11g	2.11±0.24h	4.95±0.33a	3.58±0.25c	3.20±0.28d
S5	2.5±0.16f	2.75±0.28e	3.84±0.32b	2.17±0.11g	2.04±0.23h	4.82±0.33a	3.47±0.25c	3.11±0.27d
S6	2.4±0.17f	2.69±0.28e	3.75±0.32b	2.13±0.12g	1.97±0.23h	4.74±0.32a	3.38±0.26c	3.07±0.27d
S7	2.3±0.16f	2.64±0.28e	3.65±0.33b	2.02±0.11g	1.93±0.23h	4.66±0.32a	3.30±0.26c	2.96±0.27d

4.5.1.2 Chlorophyll b

Soil water deficit generally reduced chlorophyll b of all the amaranth species (Table 4.5.1.2.1). There was a significant difference in chlorophyll b (p \leq 0.05) among all soil water treatments and the amaranth species. The highest reduction in chlorophyll b was in T4, followed by T3, T2 and T1 respectively for the seven species (Table 4.5.1.2.1). The highest reduction in chlorophyll b was observed in *Amaranthus tricolor*, followed by A. *spinosus*, A. *blitum*, A. *retroflexus*, A. *cruentus* A. *hypochondriacus* and A. *albus* respectively (Table 4.5.1.2.2). There was no significant interaction between soil water treatments and amaranth species (P=0.9965), appendix 6. There was a significant difference (p \leq 0.05), in chlorophyll b for *Amaranthus albus*, A. *hypochondriacus* and A. *tricolor* in 36 and 72 DAT, whereas there was no significant difference in 12, 24, 48, 60, 84 and 96 DAT. *Amaranthus cruentus* and A. *retroflexus* were significantly different (p \leq 0.05) in 36, 72, 84 and 96 DAT, whereas there was no significant difference in 12, 24, 36, 48 and 60 DAT. *Amaranthus blitum* and A. *spinosus* were significantly different in 36, 72, 84 and 96 DAT, whereas there was no significant difference in 12, 24, 48 and 60 DAT. *Amaranthus blitum* and A. *spinosus* were significantly different in 36, 72, 84 and 96 DAT, whereas there was no significant

Table 4.5.1.2.1: Chlorophyll b for seven Amaranth species namely; S1 A. albus, S2 A. hypochondriacus, S3 A. cruentus, S4 A. retroflexus, S5 A. blitum, S6 A. spinosus, and S7 A. tricolor, grown under four watering regimes;- T1-Watering daily, T2-Watering every 3rd and 6th day, T3-Watering every 9th day and T4-Watering every 12th day. Values represent means of three replicates \pm SE, in a 96 days period.

Chlorophyll b (m					
T1	T2	Т3	T4	Species mean	LSD values
2.91±0.170a	2.46±0.177b	2.10±0.147c	1.82±0.107d	2.32±0.086d	0.0975
2.84±0.169a	2.34±0.188b	2.00±0.148c	1.80±0.098d	2.24±0.086f	0.0941
2.76±0.168a	2.32±0.174b	1.92±0.147c	1.71±0.093d	2.18±0.084e	0.1104
2.67±0.167a	2.28±0.185b	1.85±0.147c	1.67±0.116d	2.12±0.086b	0.1054
2.58±0.162 a	2.13±0.166b	1.76±0.144c	1.53±0.091d	2.00±0.082a	0.0975
2.52±0.155a	2.07±0.167b	1.73±0.151c	1.47±0.093d	1.95±0.082c	0.1031
2.44±0.158a	1.97±0.169b	1.60±0.140c	1.35±0.097d	1.84±0.082g	0.1232
2.68±0.0621a	2.22±0.066b	1.85±0.056c	1.62±0.039d		
mean (S) 0.0533					
nt mean (T) 0.0403		*			a a
1	T1 (Control) 2.91±0.170a 2.84±0.169a 2.76±0.168a 2.67±0.167a 2.58±0.162 a 2.52±0.155a 2.44±0.158a 2.68±0.0621a mean (S) 0.0533 nt mean (T) 0.0403	T1 (Control) 2.91±0.170a 2.46±0.177b 2.84±0.169a 2.34±0.188b 2.76±0.168a 2.32±0.174b 2.67±0.167a 2.28±0.185b 2.58±0.162 a 2.13±0.166b 2.52±0.155a 2.07±0.167b 2.44±0.158a 1.97±0.169b 2.68±0.0621a 2.22±0.066b mean (S) 0.0533 nt mean (T) 0.0403	T1 (Control)T2T3 $2.91\pm0.170a$ $2.46\pm0.177b$ $2.10\pm0.147c$ $2.84\pm0.169a$ $2.34\pm0.188b$ $2.00\pm0.148c$ $2.76\pm0.168a$ $2.32\pm0.174b$ $1.92\pm0.147c$ $2.67\pm0.167a$ $2.28\pm0.185b$ $1.85\pm0.147c$ 2.58 ± 0.162 a $2.13\pm0.166b$ $1.76\pm0.144c$ $2.52\pm0.155a$ $2.07\pm0.167b$ $1.73\pm0.151c$ $2.44\pm0.158a$ $1.97\pm0.169b$ $1.60\pm0.140c$ $2.68\pm0.0621a$ $2.22\pm0.066b$ $1.85\pm0.056c$ mean (S) 0.0533	(Control) 2.91±0.170a 2.46±0.177b 2.10±0.147c 1.82±0.107d 2.84±0.169a 2.34±0.188b 2.00±0.148c 1.80±0.098d 2.76±0.168a 2.32±0.174b 1.92±0.147c 1.71±0.093d 2.67±0.167a 2.28±0.185b 1.85±0.147c 1.67±0.116d 2.58±0.162 a 2.13±0.166b 1.76±0.144c 1.53±0.091d 2.52±0.155a 2.07±0.167b 1.73±0.151c 1.47±0.093d 2.44±0.158a 1.97±0.169b 1.60±0.140c 1.35±0.097d 2.68±0.0621a 2.22±0.066b 1.85±0.056c 1.62±0.039d mean (S) 0.0533 1.00±0.0403 1.00±0.0403	T1 (Control) T2 T3 T4 Species mean 2.91±0.170a 2.46±0.177b 2.10±0.147c 1.82±0.107d 2.32±0.086d 2.84±0.169a 2.34±0.188b 2.00±0.148c 1.80±0.098d 2.24±0.086f 2.76±0.168a 2.32±0.174b 1.92±0.147c 1.71±0.093d 2.18±0.084e 2.67±0.167a 2.28±0.185b 1.85±0.147c 1.67±0.116d 2.12±0.086b 2.58±0.162 a 2.13±0.166b 1.76±0.144c 1.53±0.091d 2.00±0.082a 2.52±0.155a 2.07±0.167b 1.73±0.151c 1.47±0.093d 1.95±0.082c 2.44±0.158a 1.97±0.169b 1.60±0.140c 1.35±0.097d 1.84±0.082g 2.68±0.0621a 2.22±0.066b 1.85±0.056c 1.62±0.039d

Table 4.5.1.2.2: Chlorophyll b for the seven amaranth species namely; S1 A. albus, S2 A. hypochondriacus, S3 A. cruentus, S4 A. retroflexus, S5 A. blitum, S6 A. spinosus, and S7 A. tricolor, in 12, 24, 36, 48, 60, 72, 84 and 96 DAT. Values represent means of three replicates \pm SE, in a 96 days period. LSD values; S1 = 0.1491, S2 = 0.1458, S3 = 0.1331, S4 = 0.1342, S5 = 0.1378, S6 = 0.1458, S7 = 0.1742.

y .		Chlor						
Species	12 DAT	24 DAT	36 DAT	48 DAT	60 DAT	72 DAT	84 DAT	96 DAT
S1	1.6±0.09e	2.04±0.22d	2.76±0.16b	2.00±0.04d	1.59±0.16e	3.72±0.21a	2.49±0.21c	2.34±0.10c
S2	1.5±0.13f	1.96±0.22e	2.65±0.17b	1.89±0.05e	1.54±0.16f	3.65±0.21a	2.48±0.17c	2.27±0.10d
S3	1.5±0.10f	1.91±0.22e	2.58±0.16b	1.84±0.04e	1.48±0.15f	3.55±0.21a	2.40±0.17c	2.18±0.09d
S4	1.4±0.10f	1.80±0.22e	2.48±0.17b	1.78±0.05e	1.40±0.15f	3.60±0.19a	2.39±0.20c	2.00±0.09d
S5	1.5±0.10f	1.72±0.22e	2.39±0.17b	1.70±0.05e	1.32±0.15f	3.34±0.21a	2.20±0.16c	2.00±0.09d
S6	1.3±0.10e	1.64±0.23d	2.39±0.17b	1.67±0.06d	1.24±1.42e	3.26±0.20a	2.11±0.17c	1.96±0.09d
S7	1.2±0.10e	1.61±0.23dc	2.20±0.18b	1.52±0.06d	1.21±0.14e	3.17±0.20a	2.03±0.17b	1.77±0.14c

4.5.1.3 Total chlorophyll content

Soil water deficit generally reduced total chlorophyll content of all the amaranth species (Table 4.5.1.3.1). There was a significant difference ($p \le 0.05$), in total chlorophyll content for *Amaranthus albus*, *A. hypochondriacus*, *A. cruentus*, *A. blitum*, *A. spinosus* and *A. tricolor* all soil water treatments. *Amaranthus retroflexus* was significantly different ($p \le 0.05$) in T1 and T2, whereas it was not significantly different at T3 and T4. The highest reduction in total chlorophyll content was in T4, followed by T3, T2 and T1 respectively for the seven species (Table 4.5.1.3.1). The highest reduction in total chlorophyll content was observed in *Amaranthus tricolor*, followed by *A. spinosus*, *A. blitum*, *A. retroflexus*, *A. cruentus*, *A. cruentus*, *A. hypochondriacus* and *A. albus* respectively (Table 4.5.1.3.2). There was no significant interaction between soil water treatments and amaranth species (P = 0.9998), appendix 6. There was a significant difference at ($P \le 0.05$), in total chlorophyll content for *Amaranthus albus*, *A. hypochondriacus*, *A. cruentus*, *A. retroflexus*, *A. blitum* and *A. spinosus* in all days except in 12 and 48 DAT. *Amaranthus tricolor* was significantly different at ($P \le 0.05$) in all days (Table 4.5.1.3.2).

Table 4.5.1.3.1: Total chlorophyll content for seven Amaranth species namely; S1 *A. albus*, S2 *A. hypochondriacus*, S3 *A. cruentus*, S4 *A. retroflexus*, S5 *A.blitum*, S6 *A. spinosus*, and S7 *A. tricolor*, grown under four watering regimes;- T1-Watering daily, T2-Watering every 3rd and 6th day, T3-Watering every 9th day and T4-Watering every 12th day. Values represent means of three replicates ± SE, in a 96 days period.

Amaranth species	Total chlorophyll content (mg g ⁻¹ leaf tissue) under four soil water treatments								
	T1 (Control)	T2	Т3	T4	Species mean	LSD values			
A. albus	7.40±0.406a	6.25±0.392b	4.99±0.340c	4.26±0.230d	5.73±0.211a	0.2067			
A.hypochondriacus	7.25±0.406a	6.08±0.392b	4.86±0.339c	4.17±0.226d	5.59±0.210b	0.1721			
A. cruentus	7.10±0.403a	5.96±0.387b	4.74±0.331c	4.00±0.217d	5.45±0.207c	0.1781			
A. retroflexus	6.90±0.398a	5.85±0.393b	4.53±0.344c	3.79±0.222c	5.27±0.210d	0.1807			
A. blitum	6.65±0.398a	5.59±0.370b	4.39±0.329c	3.65±0.212d	5.10±0.202e	0.1721			
A. spinosus	6.61±0.376a	5.48±0.371b	4.26±0.325c	3.51±0.213d	4.96±0.202f	0.1658			
A. tricolor	6.48±0.374a	5.33±0.383b	4.10±0.315c	3.41±0.207d	4.83±0.200g	0.2103			
Treatments mean	6.91±0.149a	5.79±0.145b	4.55±0.125c	3.83±0.084d					
LSD $(P = 0.05)$ Species	mean (S) 0.0916			1	1				
LSD $(P = 0.05)$ Treatme	ent mean (T) 0.0692								

Table 4.5.1.3.2: Total chlorophyll for the seven amaranth species namely; S1 *A. albus*, S2 *A. hypochondriacus*, S3 *A. cruentus*, S4 *A. retroflexus*, S5 *A.blitum*, S6 *A. spinosus*, and S7 *A. tricolor*, in 12, 24, 36, 48, 60, 72, 84 and 96 DAT. Values represent means of three replicates \pm SE, in a 96 days period. LSD values; S1 = 0.2556, S2 = 0.2345, S3 = 0.2433, S4 = 0.2341, S5 = 0.2924, S6 = 0.2345, S7 = 0.2975.

* 10		Total chlor	ophyll content ur					
Species	12 DAT	24 DAT	36 DAT	48 DAT	60 DAT	72 DAT	84 DAT	96 DAT
S1	4.3±0.25f	5.1±0.48e	6.89±0.49b	4.51±0.12f	3.93±0.41g	8.92±0.54a	6.33±0.45c	5.81±0.38d
S2	4.3±0.23f	5.0±0.48e	6.75±0.48b	4.28±0.11f	3.81±0.40g	8.79±0.53a	6.24±0.43c	5.65±0.37d
S3	4.2±0.23f	4.9±0.49e	6.61±0.48b	4.18±0.13f	3.68±0.39g	8.59±0.54a	6.07±0.43c	5.46±0.36d
S4	3.9±0.26f	4.6±0.51e	6.42±0.48b	4.07±0.13f	3.52±0.39g	8.43±0.55a	5.98±0.44c	5.30±0.36d
S5	3.8±0.24f	4.5±0.49e	6.23±0.49b	3.91±0.16f	3.36±0.38g	8.16±0.54a	5.66±0.40c	4.95±0.31d
S6	3.7±0.24f	4.3±0.49e	6.05±0.49b	3.84±0.17f	3.21±0.37g	8.10±0.52a	5.49±0.42c	5.04±0.34d
S7	3.9±0.23f	4.3±0.51e	5.85±0.05b	3.55±0.14g	3.14±0.37h	7.86±0.53a	5.32±0.42c	4.73±0.38d

4.6 Leaf water potential

Soil water deficit treatments generally reduced leaf water potential of all the amaranth species (Table 4.6.1). There was a significant difference in leaf water potential ($p \le 0.05$) among the soil water treatments. There was a significant difference ($p \le 0.05$) in leaf water potential for all species in all treatments, with exception of *A. spinosus* which was not significantly different ($p \ge 0.05$) in T3 and T4 water treatments. The highest reduction in leaf water potential was observed in T4, followed by T3, T2 and T1 respectively (Table 4.6.1). There was no significant interaction between soil water treatments and amaranth species (P = 0.0890), appendix 7.

Table 4.6.1: Leaf water potential for seven Amaranth species grown under four watering regimes;- T1-Watering daily, T2-Watering every 3rd and 6th day, T3-Watering every 9th day and T4-Watering every 12th day. Values represent means of three replicates ± SE, in a 96 days period.

Γ4 0±0.116d 0±0.116d	T3 -0.9±0.109 c	T2	T1 (Control)	Species mean	Species rank
	-0.9±0.109 c	-0.8+0.102b			1
0±0.116d		0.0-0.1020	-0.71±0.097a	-0.9±0.054d	1
	-0.9±0.109c	-0.78±0.104b	-0.7±0.097a	-0.8±0.054cd	2
0±0.115d	-0.9±0.109c	-0.8±0.103b	-0.7±0.097a	-0.8 ±0.054c	3
8±0.14d	-0.9±0.11c	-0.7±0.102b	-0.7±0.096a	-0.8±0.056ab	4
0±0.116d	-0.8±0.109c	-0.7±0.102b	-0.6±0.095 a	-0.8±0.053b	5
9±0.138b	-0.8±0.108 b	-0.7±0.103a	-0.6±0.095a	-0.8±0.056a	6
9±0.116d	-0.8±0.106c	-0.7±0.107b	-0.6±0.093a	-0.8±0.053ab	7
0±0.046a	-0.9±0.040b	-0.8±0.038c	-0.7±0.036d		
) 0.0317 (T) 0.024	2,1				я
	9±0.138b 9±0.116d 9±0.046a 9 0.0317 (T) 0.024	9±0.138b -0.8±0.108 b 9±0.116d -0.8±0.106c 9±0.046a -0.9±0.040b 9 0.0317 (T) 0.024	0±0.138b -0.8±0.108 b -0.7±0.103a 0±0.116d -0.8±0.106c -0.7±0.107b 0±0.046a -0.9±0.040b -0.8±0.038c 0 0.0317 (T) 0.024	0±0.138b -0.8±0.108 b -0.7±0.103a -0.6±0.095a 0±0.116d -0.8±0.106c -0.7±0.107b -0.6±0.093a 0±0.046a -0.9±0.040b -0.8±0.038c -0.7±0.036d 0 0.0317 (T) 0.024	0±0.138b -0.8±0.108 b -0.7±0.103a -0.6±0.095a -0.8±0.056a 0±0.116d -0.8±0.106c -0.7±0.107b -0.6±0.093a -0.8±0.053ab 0±0.046a -0.9±0.040b -0.8±0.038c -0.7±0.036d 0 0.0317

4.7 Relative leaf water content

Soil water deficit generally reduced relative leaf water content of all the amaranth species (Table 4.7.1). There was a significant difference (p \leq 0.05) in relative leaf water content among the soil water treatments and the amaranth species. The highest relative leaf water content was observed in T1, followed by T2, T3 and T4 respectively (Table 4.7.1). The highest reduction in relative leaf water content was observed in *Amaranthus tricolor*, followed by *A. spinosus*, *A. blitum*, *A. retroflexus*, *A. cruentus A. hypochondriacus* and *A. albus* respectively (Table 4.7.2). There was a significant interaction between soil water treatments and amaranth species (P = 0.0220), appendix 7. There was a significant difference (p \leq 0.05), in relative leaf water content for *Amaranthus blitum* and *A. tricolor* in 48 DAT, whereas there was no significant difference in all days. *Amaranthus hypochondriacus*, *A. cruentus* and *A. retroflexus* were significantly different in 48 and 96 DAT, whereas there was no significant difference in 12, 24, 36, 60, 72 and 84 DAT. *Amaranthus albus* was significantly different at (p \leq 0.05), in 12, 48 and 84 DAT, whereas it was not significantly different in 24, 36, 60, 72 and 96 DAT. *Amaranthus spinosus* was not significantly different at (p \geq 0.05), in all days except 48 DAT. *Amaranthus spinosus* was not significantly different at (p \geq 0.05), in all days except 48 DAT (Table 4.7.2).

Table 4.7.1: Relative leaf water content for seven Amaranth species namely; S1 *A. albus*, S2 *A. hypochondriacus*, S3 *A. cruentus*, S4 *A. retroflexus*, S5 *A.blitum*, S6 *A. spinosus*, and S7 *A. tricolor*, grown under four watering regimes;- T1-Watering daily, T2-Watering every 3rd and 6th day, T3-Watering every 9th day and T4-Watering every 12th day. Values represent means of three replicates ± SE, in a 96 days period.

Amaranth species	Relative leaf water content (%) under four soil water treatments						
	T1	T2	Т3	T4	Species mean	LSD values	
	(Control)			W (42)	_	e r	
A. albus	82.5±0.96a	75.7±0.85b	69.7 ±0.99c	64.3±1.07d	73.05±0.844d	0.7063	
A. hypochondriacus	81.4±0.94a	74.0±0.90b	68.4±1.01c	63.2±1.07 d	71.73±0.844f	0.6914	
A. cruentus	79.5±0.98a	72.5±0.88b	66.8±1.02c	61.6±1.14d	70.10±0.844e	0.7088	
A. retroflexus	77.9±1.08a	69.2±1.43b	65.5±0.97c	60.4±1.25d	68.25±0.879b	0.7257	
A. blitum	76.3±1.09a	69.3±0.91b	63.6±0.98 c	59.0±1.21 d	67.05±0.845a	0.6914	
A. spinosus	74.9±1.11a	68.1±0.94b	62.3±0.95 c	57.8 ±1.27d	65.77±0.843c	0.6864	
A. tricolor	73.4±1.08a	66.9±0.95b	61.3±0.87c	56.9±1.27d	64.64±0.819g	0.6659	
Treatments mean	77.98±0.454a	70.81±0.440b	65.38±0.424c	60.46±0.482d			
LSD ($P = 0.05$) Species m	ean (S) 0.4631					4	
LSD $(P = 0.05)$ Treatment	mean (T) 0.3501	, , ,	a la		1 7		

Means with the same letter along the row are not significantly different at ($p \le 0.05$).

Table 4.7.2: Relative leaf water content for the seven amaranth species namely; S1 *A. albus*, S2 *A. hypochondriacus*, S3 *A. cruentus*, S4 *A. retroflexus*, S5 *A.blitum*, S6 *A. spinosus*, and S7 *A. tricolor*, in 12, 24, 36, 48, 60, 72, 84 and 96 DAT. Values represent means of three replicates \pm SE, in a 96 days period. LSD values; S1 = 1.0262, S2 = 0.9707, S3 = 0.9778, S4 = 1.001, S5 = 0.9989, S6 = 1.0023, S7 = 0.9417.

7		Relative leaf	water content und	,				
Species	12 DAT	24 DAT	36 DAT	48 DAT	60 DAT	72 DAT	84 DAT	96 DAT
						- 10 - 10 - 10 - 10 - 10 - 10 - 10 - 10		
S1	67.5±2.71f	71.3±3.17d	71.1±1.82d	81.5±2.24a	75.3±1.92b	74.7±1.69cb	68.8±1.65e	73.9±1.46c
S2	66.3±2.61e	70.3±3.17d	70.2±1.79d	80.4±2.20a	74.0±1.90b	73.6±1.61b	67.1±1.73e	71.9±1.58c
S3	65.2±2.54e	68.6±3.40d	68.4±1.69d	79.2±2.10a	72.1±1.79b	72.2±1.58b	65.3±1.69e	69.9±1.52c
S4	63.6±2.57e	66.6±3.40d	66.4±1.48d	77.6±2.05a	70.9±1.87b	68.8±2.81b	63.8±1.51e	68.5±1.46c
S5	61.8±2.64e	65.2±3.23d	65.9±1.77d	76.3±2.13a	68.7±2.02b	69.2±1.50b	62.1±1.39e	67.4±1.52b
S6	60.3±2.56e	63.7±3.25d	64.9±1.62c	75.3±2.14a	68.0±1.91b	67.6±1.45b	60.7±1.50e	65.8±1.55c
S7	59.3±2.48e	62.8±3.24d	63.7±1.52dc	74.1±2.09a	66.8±1.92b	66.0±1.31b	60.1±1.55e	64.3±1.24c

Means with the same letter along the row are not significantly different at ($p \le 0.05$).

CHAPTER FIVE

DISCUSSION

5.1 Effects of soil water deficit on growth parameters of Amaranthus

5.1.1 Shoot height

The results of this study showed significant reductions in shoot height among the seven species, which could have resulted from a reduction in photosynthetic efficiency. This is in agreement with the results of Castonguay and Markhart (1992), or a reduction in their relative water contents under water deficit conditions. Reduction in shoot height under water deficit could be due to reduction in leaf number. Reduction in leaf number is one of the physiological changes that occur under water deficit stress (Jomo *et al.*, 2014b). A general decline in shoot height with increasing water deficit may further imply that growth allocation may have been diverted to other plant organs like roots, leaves or the stems. Similar results have been reported in rice (*Oryza sativa*) genotypes (Zubaer *et al.*, 2007).

Plant growth is depended on cell division, cell enlargement and differentiation processes which can be delayed by soil water deficit (Thobile *et al.*, 2010). Shoot growth, is generally more sensitive to soil water deficit than root growth. The high soil water deficit treatment, had the lowest shoot height in all amaranth species, probably as a result of reduced cell turgor which affected cell division and expansion (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. At T1 water treatment, shoot height increased the highest, while the contrary was with T4 where shoot 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 an argument also advanced by Mustafa *et al.* (2011).

5.1.2 Stem diameter

The stem diameter reduced with increasing soil water deficit. These results agree with those of Jomo *et al.* (2014b) in African nightshades (*Solanum scabrum* Mill. and *Solanum villosum* Mill.) where increase in water deficit led to a decrease in stem diameter. Reduction in stem diameter with increase in water deficit may have resulted from reduced cell size and cell number due to lower rates of cell division and cell enlargement respectively. While the highest growth during their last stages of development could have been due to resumption of stem cell division, elongation and leaf expansion (Vurayai *et al.*, 2011). These 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).

The stem growth of the seven species of amaranth was inhibited at low soil moisture content. This suggests that growth inhibition may be metabolically regulated possibly serving an adaptive role by restricting the development of the transpiring leaf in water stressed plants (Sharp, 1996). Turgor pressure in growing cells might have also provided the driving force for cell expansion. Hence reduced growth rate under water deficit in all treatments especially in T3 and T4 water levels can be related to reduced cell turgor and reduction in cell wall extensibility. This cell turgor might have decreased with any dehydration-induced decrease in cell water potential. Amaranth results on stem diameter are therefore in agreement with studies in wheat by Moaveni *et al.* (2011), and in sunflower by Mehid *et al.* (2001) which showed to be negatively affected in their cell division and meristematic tissue enlargement.



5.1.3 Leaf number

Soil water deficit reduced the number of leaves in *Amaranthus tricolor*. This was also observed in wheat by Bogale *et al.* (2011) where wilting in mature leaves was associated with carbohydrate depletion due to mobilisation and export followed by senescence. *Amaranthus tricolor*, had least growth especially in the T4 soil water deficit and this might have enhanced nucleic acid destruction of the polysomal mRNAs in the zone of elongation of the hypocotyls (Hsiao, 1973). At the start of water deficit, changes in the leaf number were more visible, where T1 had the highest number of leaves, whereas T4 had the least number of leaves. Decrease in leaf number, may be due to reduction in cell division and cell expansion as a result of reduced photosynthesis which was common at T4 soil water deficit (Jomo *et al.*, 2013).

The highest number of leaves in *Amaranthus albus* and all the amaranth species especially in 94 DAT implied 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 leaf number. The effects of soil water deficit on leaf number might be considered as an adaptive mechanism which helps plants to reduce water loss. 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).

The significant decrease in leaf numbers with increasing water deficit results were concormittant to those observed in pigweed by Moran and Showeler (2005), in maize plants by Sah and Zamora (2005) where reduced leaf numbers reduced light interception by the plant and eventually reduced biomass production. *A. albus* had the highest number of leaves and this detaching of old leaves for the formation of new leaves with smaller leaf area could

have been another way of stress avoidance that was aimed at reducing plant water consumption and hence conserving water during water deficit.

5.1.4 Root to shoot ratio

The root to shoot ratio of the species increased with increase in water deficit. The increase in root length according to Jomo *et al.* (2014b), might have helped plants to grow even under low water deficit conditions besides leaf number reduction. Roots tend to grow until the plants demand for water is met (Jomo *et al.*, 2014b). In spider plant for instance, AVRDC, (2003) showed that severe drought resulted in increased root length. Bogale *et al.* (2011), while investigating Ethiopian durum wheat genotypes subjected to water deficit showed increased dry matter in root to shoot ratio. However, this is not always true because root and shoot growth are also controlled by nutrient availability, growth stages and most importantly the plant species (Dhindsa and Cleland, 1975; Jomo *et al.*, 2014b).

The differential sensitivity of roots and shoots with root growth being less sensitive to water deficits could have led to the increase in the root to shoot ratio under water deficit conditions because increased root surface area allows more water to be absorbed from the soil. Besides differential sensitivity, the observed increase in root: shoot ratio with increase in water deficit, 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). Jomo, (2013) showed root: shoot ratio of two African nightshades species to have increased under soil water deficit. Root: Shoot ratios of many crops and pasture species increased under water deficit condition (Zhang *et al.*, 2004), which was attributed to relative greater decrease in shoot biomass. Masinde *et al.* (2005), reported similar results in *Cleome gynandra*, and attributed this to differential sensitivity of the root and shoot biomass production to soil water deficit.

Under low soil water content, the roots grow deeper in search for water. Water deficit usually changes the source-sink relationship thus altering assimilate partitioning, and under water stress, the roots become the stronger sink. Bogale *et al.* (2011), 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 might have aided the crops in the ability 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 causes a decline in the growing zones while increased root surface area allows more water to be absorbed from the soil and could be an adaptive response by *A. tricolor* 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 as a result of differential sensitivity, and a reduction in shoot growth coupled with continued root growth would result in an improved plant water status under low water deficit conditions (Bogale *et al.*, 2011). Root growth may have been reduced by the use of pots and this might have had a negative consequence on shoot growth an argument also supported by Jomo *et al.* (2014b). Results of root to shoot ratio were also in agreement with those of Nikolaev *et al.* (2010) in wheat cultivars where growth continued at very low water potential. *Amaranthus tricolor* had the highest root to shoot ratio which may have conferred more tolerance to water deficit.

5.2 Effects of soil water deficit on gas exchange parameters of Amaranth species.

The significant difference in gas exchange between the seven species in the initial stages (12 and 24 DAT) and later stages (84 and 96 DAT) may have been due to less water being acquired for growth hence low rates of transipiration. The seven amaranth species especially *A. albus*, might have minimized water loss through the reduction of the transpiring surface area by the plants in a form of reduced leaf area or changed leaf shape by rolling the leaves as a response to soil water deficits. Therefore the reduced number of leaves might have had a favorable energy budget producing lower transpiration rates (Simpson, 1981). This was also further supported by Schimidit (1983), Tuinstra *et al.* (1997 and Mitra (2001) who stated that lower transpiration rates could only be achieved by minimizing radiation and absorption through increased epiticular wax load, leaf orientation and leaf rolling.

The accelerated reduction in leaf numbers, might have been the morphological plant feature that helped them to adapt to soil water deficit. Reduced leaf number in T4 soil water deficit might have further reduced the radiant heat load in leaves and subsequently increasing the reflectance of the leaf surface and reducing the absorption of photosynthetic active radiation (Simpson, 1981; Schimidit, 1983; Jomo *et al.* 2014c). Transpiration results were concomitant to those obtained by Kirnak, (2001) in eggplants, Sharp and Davis, (1985) in maize, Imana *et al.*, (2010) in tomato, Bogale *et al.*, (2011) in wheat, El Hafid *et al.*, (1998) in pears, Ma *et al.* (2006) in peach, and Demir *et al.* (2006). Where there was a decrease in transpiration with increasing water deficit. However, in amaranth species there was a significant decrease in transpiration with increasing water deficit as shown in table (4.3.1) probably due to the high leaf temperatures.

Stomatal conductance in water stressed plants was generally lower as compared to the well-watered plants. A decline in stomatal conductance with increase in water deficit might

have helped plants to avoid dessication because severe water deficit lead to increased ABA concentrations that help regulate the opening and closing of the stomata. The reduction in stomatal conductance and leaf transpiration rates in stressed plants as observed in the T4 soil water deficit treatment, might have been further accelerated by the decreased rate of water flow into the plant thereby decreasing the number of leaves. The reduced stomatal conductance might have decreased the intercellular CO₂ concentration in turn reducing the CO₂ assimilation rate (Zhao *et al.*, 2010).

The amaranth species 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). Reduction in stomatal conductance decreases transpiration and limits CO₂ assimilation rate (Tezera *et al.*, 2002). Different results were obtained in sunflower where stomatal closure had a minor effect on photosynthesis because the direct effects on the photosynthetic activity of the 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 a reduction in photosynthetic pigment concentration could have reduced the functional activity of PSII thereby decreasing the rates of photosynthesis among the seven amaranth species. A reduced carboxylation efficiency as observed by Jia and Gray (2004), might have reduced ribulose-1,5-bisphospate (RuBP) regeneration, thereby significantly decreasing photosynthesis an argument also advanced by Jomo *et al.* (2014c).

According to Cornic and Fresneau (2002), stomatal closure caused by soil water deficit may lead to reduction in photosynthetic rates. Further decrease in net photosynthetic rate observed under water deficit might have been as a result of reduced internal CO₂ concentration (Ci) which limits photosynthesis at the acceptor site of ribulose-1-5-bisphosphate carboxylase/oxygenase (Rubsco) or by the direct inhibition of photosynthetic enzymes like Rubisco as also noted by Haupt- Herting and Fock, (2000) or ATP synthase

(Nogués and Baker, 2000; Tezara et al., 2002). 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 were slightly higher as compared to T3 and T4 this might have resulted to an increased CO₂ diffusion into the leaves to attain higher photosynthetic rates which favoured higher biomass in T1 and T2 (Siddique et al., 2000). The decrease in the photosynthetic rates in the species therefore can be explained by the clear decline in the stomatal conductance and possibly the main reason for low photosynthesis rates under increasing water deficit, as photosynthesis can be recovered by the supply of enough CO₂ to the leaves that could in turn help in attaining higher photosynthetic rates favouring higher plant biomass.

According to Danda and Behl (2004), the initial response of any plant to drought stress is an increase in abscissic acid (ABA) levels, that in turn regulate the closure of the stomata in an attempt to reduce water loss. Therefore this might have had an effect on gas exchange and especially CO₂ absorption that adversely affect the photosynthesis. This decline in photosynthetic rate due to water deficit can be ascribed to reduced chloroplasts activities and the weakening of carbon assimilation Genty *et al.*, (1987).

Studies by Merah, (2001) are in agreement with the results of the current study. The ability of a plant to survive under water deficit is depended on its ability to restrict water loss through the epidermis after the stomata has attained a minimum aperture, thereby helping it prevent development of lethal water deficit which may lead to lethal temperatures under high sun intensity as observed by Silva *et al.* (2007). Thus *Amaranthus albus*, followed by *Amaranthus hypochondriacus* maintained a high relative leaf water content, possibly by minimising water loss to sustain transpiration cooling, consequently sustaining a constant

CO₂ influx into the chloroplast thereby allowing a greater photosynthetic rate and an ultimate crop yield performance (Silva *et al.*, 2007).

CO₂ assimilation rates on the other hand, reduced with increasing water deficit. This is in agreement with previous studies by Demir *et al.*, (2006); and Ma *et al.*, (2006) in peach and pear trees respectively. Increasing water deficit make cells to lose their turgidity hence causing stomatal closure. This in turn might have limited the rate of CO₂ diffusion through the stomata causing a decline in photosynthesis. CO₂ assimilation was higher in well watered plants as compared to the stressed ones, and this can be attributed to both stomatal and non stomatal factors.

In amaranth low CO₂ assimilation occurred in T3 and T4 soil water deficit treatments and this could be attributed to the reduced stomatal conductance as a result of stomatal closure which in turn reduced photosynthesis. Despite increased water deficit in stressed plants as compared to well watered plants, CO₂ assimilation was not significantly different among all species in most DAT, indicating that the stressed plants might have had higher water use efficiency (WUE). This can be further confirmed because amaranth species were minimizing water loss through transpiration and carbon acquisition which was probably an adaptation for this species. Similar results were observed by Cornic *et al.* (1989), in bean (*Phaseolus vulgaris* L. plants, where drought stress progressively reduced CO₂ assimilation rate due to a decrease in stomatal conductance, which directly affected the rate of photosynthesis and further stimulated the reduction of photosynthetic activities. This argument was supported partially by Pieters and El-souki (2005), although they pointed out reduction in photosynthetic activities to be as a result of reduced concentrations of CO₂ at the sites of carboxylation and impairments of mesophyll metabolism.

There was no corresponding decline in intercellular CO₂ (Ci) as CO₂ assimilation increased slowly under water deficit possibly due to non stomatal effects in the

photosynthetic processes which might have been as a result of increase in mesophyll resistance as suggested by Cornic, (1994). This was also reported in wheat by Danda and Behl (2004) where a reduction in Ci occurred in presence of the enzyme Rubisco which had a higher affinity for oxygen when it was low. Whereas according to Pierce *et al.*, (2007), Ci tends to remain constant over a range of environmental conditions, in this study the results also confirmed a similarity by not being significantly different among species *A. blitum*, *A. albus*, and *A. hypochondriacus*.

5.3 Effects of soil water deficit on chlorophyll fluorescence of Amaranthus.

In severe water deficit T4, Fv/Fm ratio decreased indicating a reduction in efficiency of PSII reaction centers, or possibly due to their damage. 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 from treatment T1 to T4 indicates to some extent, the occurrence of photoinhibition due to photoinactivation of PSII centers (Bjorkman and Powles, 1984). The non significant Fv/Fm values for species Amaranthus retroflexus, A. spinosus, A. albus and A. cruentus in all days except 36 DAT is an indication that there is no loss in the yield of PSII photochemistry; this is an indication of resistance of the photosynthetic machinery to water deficit stress (Chaves et al, 2002). High Fv/Fm values in T1 water treatment among all amaranth species, may lead to high dry matter production, because of the normal photosynthetic rates. The higher Fv/Fm values maintained by Amaranthus albus in T1 water treatment could be an indicator that photochemistry of PSII, and light driven electron transport and enzymatic reaction indeed required ATP from the chloroplasts and that they were not severely affected as compared to other amaranth species. The higher 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 amaranth ranged from 0.98 to 0.84 for T1 water level, 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. According to Sikuku *et al.* (2012), a decrease in Fv/Fm 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 describes 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.

The Fv/Fm ratio was indicative of the thyllakoid membrane integrity and the relative efficiency of electron transport from PSII to PSI (Johnson et al., 2002). On the other hand water deficit reduced photosynthesis directly thereby dehydrating the protoplasm which might have had a lowered photosynthetic capacity. The general reduction in Fv/Fm ratio with increasing water deficit observed among the seven amaranth species could have been as a result of metabolism which might have been faster during the initial DAT possibly because plants were absorbing and utilizing more water as compared to T4. There were significant differences among all species with *Amaranthus albus* recording the highest values in Fv/Fm ratio under an increasing water deficit. This is indicative of their photosytems ability to use incident light thereby giving an indication of their overall photosynthetic capacity, an observation also noted by Flexas et al. (2004).

The patterns of changes in fluorescence parameters observed in this study are consistent with those reported under water deficit conditions for barley plants according to Mamnouie et al. (2006) and Bambara groundnuts (Vurayai et al., 2011). Estimates of ETR describe the ability of photosystems 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 (Bimpong et al., 2011; Flexas et al., 2004). Although there was no significant difference in ETR between species, Amaranthus blitum and Amaranthus retroflexus, Amaranthus retroflexus had higher ETR rates with respect to an increase in water deficit, implying that it depicted more tolerance to water deficit, since low ETR under water deficit suggests low tolerance to water deficit (Blum et al., 2009). The reduction in ETR as earlier noted, with increasing water deficit imposition may also have been due to a possible increase in the excitation energy quenching process in the PSII antennae which could be indicative of down regulation of the electron transport.

5.4 Effects of soil water deficit on chlorophyll content of Amaranthus.

There was a general reduction in chlorophyll *a*, *b* and total chlorophyll content in all species and this could be alluded to an increased water deficit stress inhibiting chlorophyll synthesis. The general reduction in chlorophyll contents from T1, T2, T3 and T4 respectively, among the seven amaranth species was similar to results reported in maize by Anajum *et al.* (2011) and in barley by Kuroda *et al.* (1990). Nikolaev *et al.* (2010), found a decline in chlorophyll content 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. Low water deficit may have led 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 may include a decrease in the electron transport rate and photophosphorylation and this may be associated with changes in conformation of the thylakoids and of the coupling factor (ATP-synthetas- a sub unit of the thylakoids) (Jomo *et al.*, 2013). Dehydration of leaves could be as a result of photosynthetic pigments such as chlorophyll not being resistant to stress, hence chlorophyll *a* 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 soil 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 and in 48 and 60 DAT, might be attributed to the increased degradation of chlorophyll pigments due to stress induced metabolic imbalance (Steinke and Stier, 2003).

Among the seven species of Amaranth Amaranthus albus, had the highest chlorophyll content in all treatments followed by Amaranthus hypochondriacus, Amaranthus cruentus. Amaranthus retroflexus, Amaranthus blitum, Amaranthus spinosus and Amaranthus tricolor respectively and this might have further implied that the production of reactive oxygen species was mainly driven by excess energy absorption in the photosynthetic apparatus, which could be avoided by degrading the absorbing pigments (Farudiddin et al., 2009), this in turn indicates their tolerance levels an observation made by Chen et al. (2007) in wheat and maize varieties where the said tolerant varieties had higher chlorophyll contents than sensitive varieties under water deficit. 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 out 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 (Fariduddin *et al.*, 2009).

5.5 Effects of soil water deficit on leaf water potential of Amaranthus.

Water deficit decreased leaf water potential. According to Montagu et al. (1999), drought resistant plants maintain higher potentials than drought sensitive plants. Therefore based on the findings from this study, Amaranthus albus having maintained a relatively higher leaf water potential could be indicative of its drought tolerance and resistance. Studies on groundnut (Arachis hypogaea L.) generally exhibited more avoidance of drought stress than cowpea, mungbean {VLgna radiate (L.) Wilkz.), and soybean (Glycine max (L.) Merr.), (Zlatev and Yardanov, 2004). This was primarily attributed to maintenance of higher leaf water potentials and cooler canopy temperatures, which maintained physiological functions favourable for higher seed yield and dry matter production. Similarly, in this study, Amaranthus albus, Amaranthus hypochondriacus, Amaranthus cruentus and Amaranthus blitum recorded -1.0 Mpa respectively, possibly due to a maintained leaf turgor pressure in an

attempt to regulate leaf water potential. In *Amaranthus albus*, solute accumulation could have also played a more important role in maintaining leaf water potential. However further reduction of water potential might have also been effected by a reduction in leaf area primarily, and presumably, low resistances to water flow through diminished stomatal conductance an observation also made by Kura-Hotta *et al.* (1987) who further stated genotypic differences in water potential between two distinct groups of chickpea lines, one with high and one with low osmoregulation. Water potential declined from approximately - 0.8 MPa at the start to -1.6 MPa and -2.0 MPa at the end of the drying period for high and low groups, respectively.

Leaf water potential decrease in T3 and T4 treatments in comparison with T1 and T2 water application levels, might have resulted in a decline in photosynthesis rate as a result of stomatal closure. However, chloroplasts activity might have been more limiting than stomatal closure at low water potential in T3 and T4 treatments which is in agreement with results of Gou and Al-katib, (2003). At low water potentials translocation of photosynthates especially sucrose might have continued from the source (leaves) to the sink (roots) despite loss of photosynthetic activity in the leaves (Zlatev and Yardanov, 2004). For instance beet root results conducted both in the laboratory and under field conditions revealed that the lowest leaf water potential values can result in the ceasation of dry matter accumulation by the whole plant, nevertheless this might further allow the accumulation of dry matter in other parts of the plant, including developing leaves. Losses in photosynthetic activity at low water potentials in T4 may have further reduced the assimilate supply because of inhibition of photosynthesis which in turn might have reduced the general plant growth.

5.6 Effects of soil water deficit on relative leaf water content of Amaranthus.

Relative leaf water content can be a good indicator of water deficit stress and probably a more useful integrator of plant water balance than the leaf water potential (Levit et al., 1980). There was a decrease in relative leaf water content in all amaranth species (Table 4.7.1). This might have been caused by water loss through evapotranspiration and decreased water absorption by the roots as soil moisture content was limiting. These results are in agreement with those reported by Bogale et al. (2011), in durum wheat and Chaves et al. (2002). The low relative leaf water content observed in all species and more specifically in Amaranthus tricolor might have predisposed the plant leaves to photoinhibition (Bjorkman and Powles, 1984). This may further inhibit photosynthetic activity, leaf growth rate and leaf area development. Whereas high relative leaf water content under water deficit conditions were observed in T1 treatment for Amaranthus albus, could be due to improved photosynthesis that increase in relative leaf water content and can help in selecting the most tolerant amaranthus species that can maintain cell turgor, cell enlargement and development under water deficit environments.

The most tolerant species *Amaranthus albus* might have been able to maintain protoplast hydration for a longer duration under water deficit stress condition hence ensuring higher productivity. Siddidue *et al.* (2000) reported relative water content reductions among water stressed wheat to be between 25-39%, this suggests that *Amaranthus albus* which was able to maintain higher relative water content than other species at T4 soil water deficit could further maintain protoplast hydration for a longer duration under water deficit stress conditions.

CHAPTER SIX

CONCLUSIONS, RECOMMENDATIONS AND SUGGESTIONS FOR FURTHER STUDIES

6.1 Conclusions

- Shoot height, stem diameter and leaf number significantly reduced with increasing soil
 water deficit and during their initial growth stages as compared to their last growth
 stages possibly due to complete maintenance of turgor in the growing regions as a
 result of osmotic adjustment.
- The root: shoot ratio increased with increase in water deficit. *Amaranthus tricolor* had higher root: shoot ratio compared to other species in all treatments. This indicates that *Amaranthus tricolor* is more tolerant and well adapted to water deficient regions as compared to other amaranth species.
- Transpiration rate and stomatal conductance reduced significantly with increasing water deficit from treatment T1, T2, T3 and T4 respectively. *Amaranthus albus* had the highest transpiration rate stomatal conductance followed by *Amaranthus hypochondriacus*, *Amaranthus cruentus*, *Amaranthus retroflexus*, *Amaranthus blitum*, *Amaranthus spinosus* and *Amaranthus tricolor*.
- Soil water deficit treatments caused a significant reduction in Fv/Fm ratio, in all the
 Amaranthus species
- Generally chlorophyll a and chlorophyll b decreased significantly with increase in soil
 water deficit in all Amaranthus species.

- Soil moisture content and leaf relative water content were highest in the control (T1), followed by T2, T3 and T4 respectively.
- Based on the results obtained from this study, for optimum growth of the amaranth species, soil water should be maintained at T1 soil water treatment. *Amaranthus albus* responded better to soil water deficit than the other species.
- Soil water deficit decreased the growth of Amaranthus tricolor, followed by A.
 spinosus, A. blitum, A. retroflexus, A. cruentus A. hypochondriacus and A. albus
 respectively.

6.2 Recommendations

From the results obtained it can be recommended that:-

- 1. Soil water deficit significantly reduced the growth of the seven Amaranth species;

 Amaranthus albus, Amaranthus hypochondriacus, Amaranthus cruentus, Amaranthus retroflexus, Amaranthus blitum, Amaranthus spinosus and Amaranthus tricolor.

 Therefore this study recommends growth measurements as suitable parameters for determining the effects of water deficit on the different indigenous vegetables, and that among the seven species the most tolerant and well adapted to water deficient regions were Amaranthus albus, Amaranthus hypochondriacus, Amaranthus cruentus Amaranthus retroflexus, Amaranthus blitum, Amaranthus spinosus and Amaranthus tricolor respectively.
- 2. The significant reduction in CO₂ assimilation and intercellular CO₂ with increasing water deficit confirmed the overall photosynthesis among the *Amaranthus spp*. Hence a similar comparison should also be done to ascertain the same trend among other plant species.

- 3. Soil water deficit significantly reduced chlorophyll fluorescence of amaranthus *spp*. The significant differences among the seven species suggests their photosynthetic efficiency and can be recommended in future studies to help understand how chlorophyll fluorescence indirectly monitors photosynthetic efficiency.
- 4. Chlorophyll contents of the seven amaranth species decreased significantly with increase in soil water deficit this suggests that chlorophyll content is also suitable in determining the effects of water deficit on the amaranthus vegetables and therefore they should be adopted for future physiological studies.
- Leaf water potential and relative leaf water content serve as indicators of plant water status and are therefore recommended to evaluate plants water status under increasing soil water deficit.

6.3 Suggestions for further studies

- Future studies should be conducted under field conditions and climatic and soil water deficit conditions in order to predict the growth and physiology of Amaranth species for future sustainable production.
- 2. Determination of other pigments content such as carotenoids other than chlorophyll *a* and *b* could be an effective means of monitoring amaranthus growth and estimating their photosynthetic productivity. These should be studied in future to help understand better the overall photosynthesis of Amaranth species to water deficit.
- 3. Future studies should focus on the extent to which solutes accumulation could lower the osmotic potential thereby helping in the maintainance of turgor and turgor-driven processes, such as stomatal opening and expansion that might have continued even under lower water potentials.

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