ISSN: 2350-0328
International Journal of AdvancedResearch in Science, Engineering and Technology
Vol. 3, Issue 2 , February 2016

Drought inhibition of chlorophyll content among seven *Amaranthus* species.

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ABSTRACT Drought as a result of soil water deficit stress destroys chlorophyll content and further inhibits its synthesis in plants. Limitations to water resulting in soil water deficit, may be a consequence of different chlorophyll contents in plants. 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 consumedmicronutrient rich leafy vegetables in Kenya; yet, information regarding drought inhibition of their chlorophyll content is lacking which is representative of their nutritional value. This research was therefore designed to evaluate the reduction of the 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 chlorophyll contents. 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: 100%, 75%, 50% and 25% available water capacity. Chlorophyll content was determined through extraction and absorbance of chlorophyll solution read spectrophotometrically. Data was subjected to analysis of variance and separation of means using the Least Significant Difference at 5% level. Results showed that the seven species of amaranth were significantly ($p \leq 0.05$) affected by soil water deficit. Chlorophylls *a*, *b* and total chlorophyll also showed a general decrease with increasing soil water deficit. From the results obtained, it can be concluded that among the seven species of amaranthus evaluated, *A albus,* was ranked to be more tolerant to soil water deficit and therefore can be recommended to be grown in water deficient regions followed by *A. hypochondriacus*, *A. cruentus, A. retroflexus,* *A. blitum*, *A. spinosus,* and *A. tricolor* respectively. The results of this study can also be used to recommend better management plant strategies to drought, as it considered the effects of drought on the chlorophyll contents.

KEYWORDS : Drought, amaranth, chlorophyll content.

I. INTRODUCTION

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, (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.

Sullivan and Ross (1979), and Mitra (2001) stated that 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 and physiological characteristics but also on the biochemical contents such as chlorophyll. However information regarding amaranth chlorophyll contents is conspicuously lacking yet it could help in identifying their tolerance to drought stress and their photosynthetic capacity during drought conditions. There is need therefore to investigate drought inhibition of chlorophyll contents of the promising amaranth species, because water-stress tolerant vegetables will ensure 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

ISSN: 2350-0328
International Journal of AdvancedResearch in Science, Engineering and Technology
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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), all of which are elements of chlorophyll content.

Reports by Zanella*et al.* (2004) reported that in Jack bean plant, 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 behaviour occurs among the seven amaranth species commonly cultivated and if it occurs, its implication 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 hence an associated loss of chlorophyll an argument partially supported by Jafar *et al.* (2004).

A. Water deficit on chlorophyll content

According to Moaveni *et al.* (2011) water deficit conditions caused reductions in chlorophyll content in wheat varieties. Similar observations were also made by Alireza *et al.* (2011) in *Matricaria chamomilla* L. a medicinal plant. Studies by Randall *et al.* (1977) on the consequence of drought stress on the organization of chlorophyll into photosynthetic units and on the chlorophyll-protein composition of mesophyll and bundle sheath chloroplast of *Zea mays* found out that most of the chlorophyll lost in response to water deficit occurs in the mesophyll cells with a lesser amount being lost from the bundle sheath cells. All of the chlorophyll loss can be accounted for by reduction on the lamellar content of the light harvesting chlorophyll a/b protein (Randall *et al.*, 1977). Studies by Kura- Hotta *et al.* (1987) on rice seedlings showed that 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 Levitt (1980), 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),while drought stress produces changes in the ratio of chlorophylls *a* and *b* (Anajum *et al.,* 2011). Manivannan *et al.* (2007) reported a large decline in the chlorophylls *a, b* and total chlorophyll content in different sunflower varieties caused by soil water deficit, on the other hand Shamshi (2010) while working on wheat cultivars reported that drought stress reduced concentrations of chlorophyll *b* more than chlorophyll *a*.

II. MATERIALS AND METHODS

A. Study site

The experiment was set up in a glasshouse at Kenya Agricultural and Livestock Research Organisation (KALRO), Kisii Centre. The research site was 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 (FAO/UNESCO, 1974). The mean annual day temperature was 20^{o}C with the average maximum daily temperature not exceeding 31^{o}C and the average minimum night temperature not dropping below 15^{o}C.

B. Soil moisture content determination

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

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International Journal of AdvancedResearch in Science, Engineering and Technology
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$$W = \frac{W1-W2}{W1} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{eqn 1.}$$

Where;
W1 = fresh weight
W2 = dry weight
W = percentage soil moisture content

The treatments were: 100% available water capacity (no water stress/control), 75% available water capacity (slight), 50% available water capacity (moderate) and 25% available water capacity (low), according to (Vanassche and Laker 1989 and Neluheni *et al.*,2007), where 25% was the lowest water level applied for plant survival.
The determination of field capacity was done gravimetrically. The upper limit of field capacity was determined by watering soil thoroughly to drainage and then allowed to drain for 24 - 48 hours then soil samples were collected at 10 cm. The scooped samples were immediately placed in polythene tubes (non-perforated) to avoid any moisture loss. The fresh weights (W_1) were taken using an electronic weighing balance. Samples were then dried in an oven for 48 hours at 72^{o}C and the dry weight (W1) obtained, and the percentage water content (W) was calculated as shown in equation (1). The lower limit for plant water extraction (permanent wilting point) was determined by growing plants to flowering without limiting water intake, after which water intake was limited by stopping irrigation until permanent wilting was achieved. The percentage water content by mass was calculated at the permanent wilting point. The levels of moisture deficit imposition for each treatment in terms of percentage were calculated according to Nguyen *et al*. (2013).

AWC=FC-WP...eqn 2.

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

Where;
AWC = available water content
WP = wilting point
FC = field capacity
T1 = treatments
Before initiating treatments plants were irrigated with normal tap water using a hand sprinkler to full saturation for two weeks in order to improve root development (Imana *et al.,* 2010). After which 500 ml of water was applied to each pot and this was able to wet the soil to full saturation.

C. Chlorophyll content determination

Chlorophyll content was determined using methods of Arnon (1949) and Coombs *et al.* (1987) as described by Jomo (2014). The 4th 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. 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
mg *Chl b* / g leaf tissue =22.9 (D645)-4.68 (D663) x V / 1000xW
mg *tChl* / g leaf tissue =20.2 (D645)+8.02 (D663) x V / 1000xW

Where; D= absorbance measured at wavelengths 645nm and 663nm.

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International Journal of AdvancedResearch in Science, Engineering and Technology
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V= volume (ml) of the acetone extract.
W= fresh weight (g) of leaf tissue from which the extract was made.

D. Statistical analysis of data

Data were analyzed using the (SAS, 2003) statistical program. Differences between soil water deficit treatments as well as the amaranths 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).

III. RESULTS

Soil water deficit generally reduced soil moisture content of all the amaranth species (Table 1). There was a significant difference in soil moisture content ($p \leq 0.05$) among all treatment means. *Amaranth blitum, A. retroflexus, A. cruentus* and *A. tricolor* were not significantly different at ($p \geq 0.05$) in their overall means, while *A. spinosus, A. albus* and *A. hypochondriacus* were significantly different at ($p \leq 0.05$) in their overall means. The highest soil moisture content was observed in 100%, followed by 75%, 50% and 25% (Table 4.8). *A. albus*, had the highest soil moisture content followed by *A.hypochondriacus*, *A.cruentus*. *A. retroflexus*, *A. blitum*, *A. spinosus* and *A.tricolor* respectively. There was a significant interaction between soil water deficit treatments and amaranth species ($P=0.001$). The reduction in soil moisture content at 25% soil water deficit was 44% of the control treatment for *Amaranthus blitum,* 44% for *A. retroflexusus*, 44% for *A. spinosus*, 43% for *A. tricolor*, 42% for *A. albus,* 42% for *A. cruentus* and 42% for *A. hypochondriacus.*

Table 1: Soil moisture content for seven Amaranthus (*spp*) grown under four levels of water application; 100% available water capacity (no water stress), 75% available water capacity (slight), 50% available water capacity (moderate) and 25% available water capacity (low).

Amaranthus (*spp*)	Soil moisture content by weight (%) under four soil water deficit treatments					
	100 % (Control)	**75 %**	**50 %**	**25 %**	**Overall species mean**	**Species rank**
A. blitum	**29.36±0.09a**	**22.85±0.54 b**	**18.36±0.89 c**	**12.97±1.34 d**	20.9±0.745**cb**	5
A. retroflexus	**29.32±0.13a**	**23.22±0.54b**	**18.13±0.94c**	**12.76±1.29d**	20.9±0.754**cb**	4
A. spinosus	**29.99±0.21a**	**23.85±0.53b**	**18.35±1.02c**	**13.07±1.40 d**	21.3±0.786**a**	6
A. albus	**30.35±0.23 a**	**23.21±0.56 b**	**17.71±0.93 c**	**12.84±1.35 d**	21.0±0.794**b**	1
A. cruentus	**30.00±0.20 a**	**22.77±0.54 b**	**18.01±0.89c**	**12.45±1.37 d**	20.8±0.785**cb**	3
A. hypochondriacus	**29.58±0.19 a**	**23.02±0.61 b**	**18.01±0.97 c**	**12.49±1.32 d**	20.8±0.779**c**	2
A. tricolor	**29.55±0.18 a**	**22.99±0.56 b**	**17.86±0.95 c**	**12.83±1.34 d**	20.8±0.767**cb**	7
Overall treatments mean	**29.7±0.07a**	**23.1±0.21b**	**18.1±0.35c**	**12.8±0.50d**		
CV (%) 3.779491						
LSD (P = 0.05) Species (S) **0.2244**						
LSD (P = 0.05) water level (T) **0.1696**						

KEY.
CV Coefficient of Variation
LSD Least Significant Difference
Values represent means of three replicates ± SE, in a 96 days period. Means with the same letter are not significantly different between the columns and the rows.

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A. Chlorophyll *a*

Soil water deficit generally reduced chlorophyll *a* of all the amaranth species (Figure 1). There was a significant difference in chlorophyll *a* ($p \leq 0.05$) among all soil water deficit treatments and among amaranth species means. The highest reduction in chlorophyll *a* was in 25%, followed by 50%, 75% and 100% respectively for the seven species. *A. albus*, had the highest chlorophyll *a* followed by *A.hypochondriacus*, *A.cruentus*. *A. retroflexus*, *A. blitum*, *A. spinosus* and *A.tricolor* respectively. There was no significant interaction between soil water deficit treatments and amaranth species (P= 1.000). The reduction in chlorophyll *a* at 25% soil water deficit was 51% of the control treatment for *Amaranthus blitum*, 52% for *A. retroflexusus*, 50% for *A. spinosus*, 55% for *A. albus*, 53% for *A. cruentus*, 54% for *A. hypochondriacus* and 49% for *A. tricolor.*

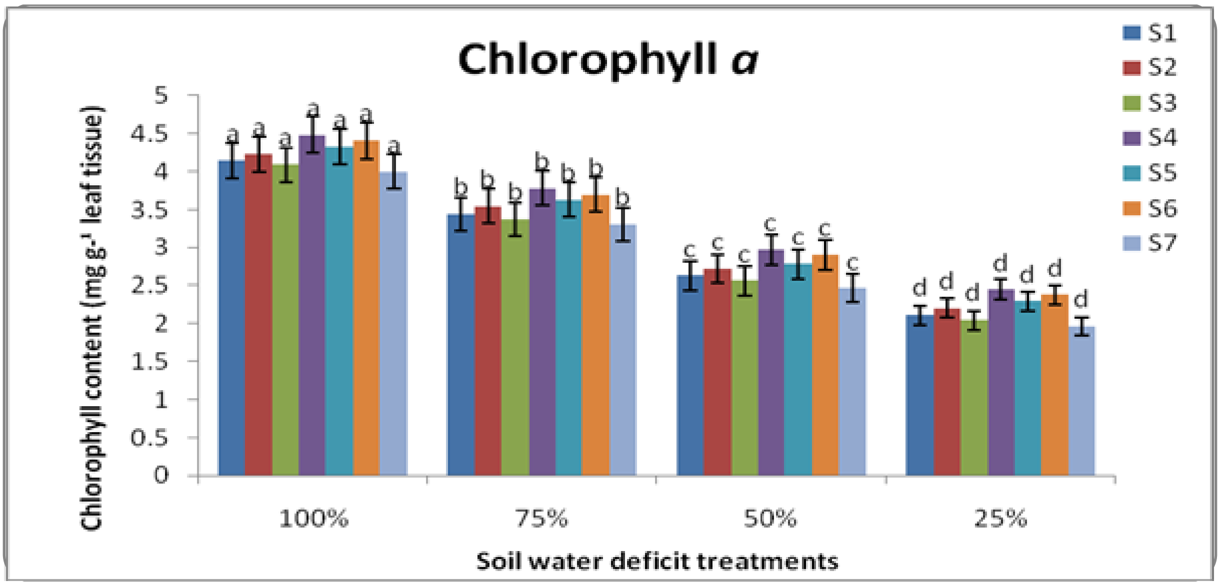

Fig: 1 The mean chlorophyll *a* content of the seven amaranth species namely; S1 *A. blitum*, S2 *A. retroflexus*, S3 *A. spinosus*, S4 *A. albus*, S5 *A. cruentus*, S6 *A. hypochonriacus* S7 *A. tricolor*, grown under four soil water deficit treatments.Means with the same letter are not significantly different.

B.Chlorophyll *b*

Soil water deficit generally reduced chlorophyll *b* of all the amaranth species (Figure 2). There was a significant difference in chlorophyll *b* ($p \leq 0.05$) among all soil water deficit treatments and among amaranth species means. The highest reduction in chlorophyll *b* was in 25%, followed by 50%, 75% and 100% respectively for the seven species. *A. albus*, had the highest chlorophyll *b* followed by *A.hypochondriacus*, *A.cruentus*. *A. retroflexus*, *A. blitum*, *A. spinosus* and *A.tricolor* respectively. There was no significant interaction between soil water deficit treatments and amaranth species (P= 0.9965). The reduction in chlorophyll *b* at 25% soil water deficit was 59% of the control treatment for *Amaranthus blitum*, 63% for *A. retroflexusus*, 58% for *A. spinosus*, 63% for *A. albus*, 62% for *A. cruentus*, 63% for *A. hypochondriacus* and 55% for *A. tricolor.*

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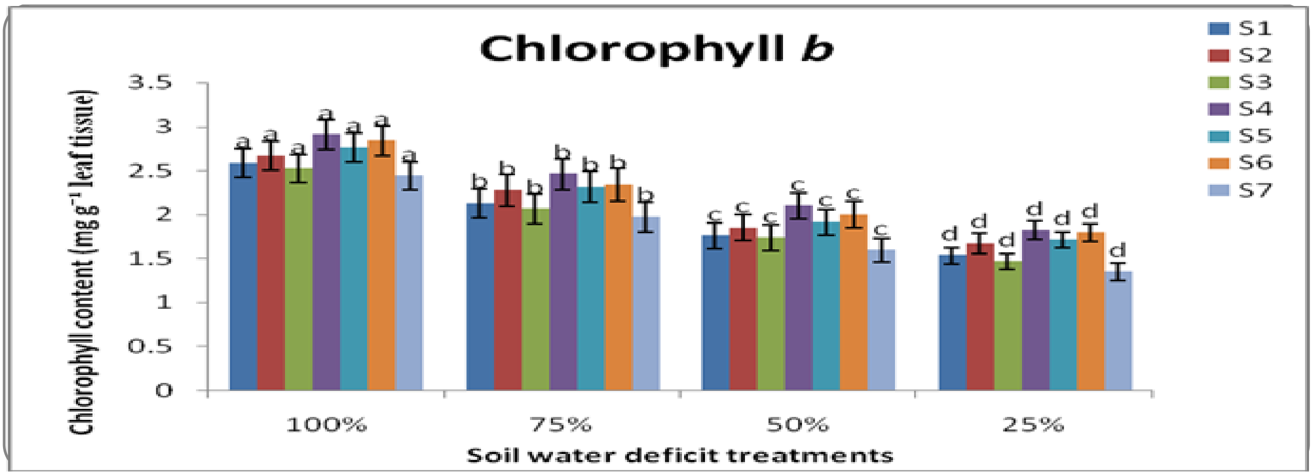

Fig: 2 The mean chlorophyll *b* content of the seven amaranth species namely; S1 *A. blitum*, S2 *A. retroflexus*, S3 *A. spinosus*, S4 *A. albus*, S5 *A. cruentus*, S6 *A. hypochonriacus* S7 *A. tricolor*, grown under four soil water deficit treatments.Means with the same letter are not significantly different.

C.Total chlorophyll content

Soil water deficit generally reduced total chlorophyll content of all the amaranth species (Figure 3). There was a significant difference in total chlorophyll ($p \leq 0.05$) among all soil water deficit treatments and among amaranth species means. The highest reduction in total chlorophyll content was in 25%, followed by 50%, 75% and 100% respectively for the seven species. *A. albus*, had the highest total chlorophyllfollowed by *A.hypochondriacus*, *A.cruentus*. *A. retroflexus*, *A. blitum*, *A. spinosus* and *A.tricolor* respectively. There was no significant interaction between soil water deficit treatments and amaranth species ($P= 0.9998$). The reduction in total chlorophyll content at 25% soil water deficit was 55% of the control treatment for *Amaranthus blitum*, 55% for *A. retroflexusus*, 53% for *A. spinosus*, 58% for *A. albus*, 56% for *A. cruentus*, 58% for *A. hypochondriacus* and 53% for *A. tricolor.*

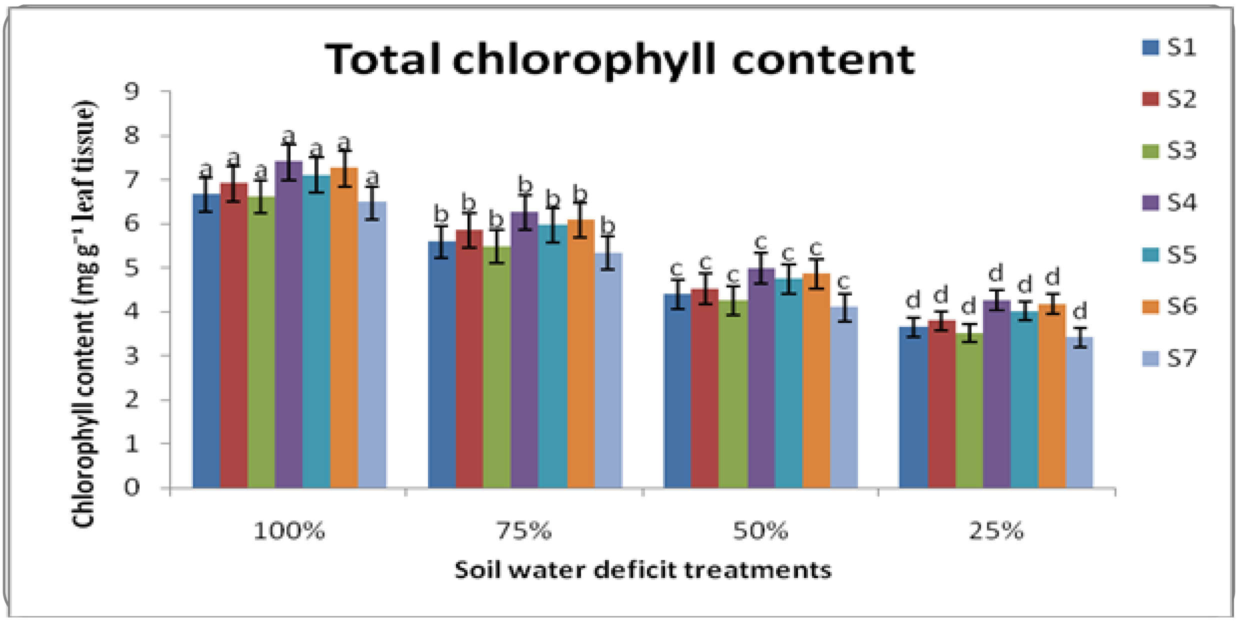

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Fig: 3 The mean total chlorophyll content of the seven amaranth species namely; S1 *A. blitum*, S2 *A. retroflexus,* S3 *A. spinosus*, S4 *A. albus*, S5 *A. cruentus*, S6 *A. hypochonriacus* S7 *A. tricolor*, grown under four soil water deficit treatments.Means with the same letter are not significantly different.

IV. DISCUSSION

A. Soil moisture content

Soil moisture content in amaranth decreased with decreasing soil water deficit (table 1). This is in agreement with results of Martim *et al.* (2009), on grapevine and Zhao *et al.,* (2010) on *Betula Platyphylla* seedling plant. However soil moisture content was not significantly different in *A. blitum, A. retroflexus, A. cruentus,* and *A.tricolor,* species possibly because plants were adapting to their water deficit environment and an indication that when water was not limiting the species of amaranth might have had the same water absorption, utilization and water loss in sustaining their physiological processes. Whereas according to Thobile, (2010) moisture requirements for plants differ with the species, stage of development and plant age, further losses of moisture from the soil may be attributed to surface evaporation, transpiration through the leaves and water absorbed by the roots (Luvaha *et al.*, 2008). The significant differences in soil moisture contents imply variations as a result of metabolism among the amaranth species.

B. Chlorophyll content

Water deficit caused a general reduction in chlorophyll *a*,*b*and total chlorophyll content in all species as observed in Figures (1, 2 and 3) respectively. This could be alluded to an increased water deficit stress inhibiting chlorophyll synthesis which is said to occur at four consecutive stages: (I) the formation of 5-aminole-vuliniuc acid (ALA); (II) ALA condensation into porphobilinogen and primary tetrapyrrol, which is transformed into protochlorophyllide; (III) light-dependent conversion of protochlorophyllide into chlorophyllide; and (IV) synthesis of chlorophylls *a* and *b* along with their inclusion into developing pigment–protein complexes of the photosynthetic apparatus (Liu*et al*., 2004). The general reduction in chlorophyll contents among the seven amaranth species was similar to results observed by Anajum *et al.* (2011) and by Kuroda *et al.* (1990) in barley.

Nikolaev *et al.* (2010), observed a decline in chlorophyll content from 15% to 13% in water stressed wheat as compared with well watered plants in three varieties of wheat. Chlorophyll content is one of the indices of photosynthetic activity (Bojovic and Stojanovic, 2005), and according to Montagu and Woo (1999), water deficit can destroy chlorophyll and inhibit its synthesis. Extreme water deficit (25%) lead 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 might be associated with changes in conformation of the thylakoids and of the coupling factor (ATP-synthetas- a sub unit of the thylakoids) and decreased substrate binding by coupling factor (Vieira and Necchi 2006). Dehydration of leaves could be as a result of chlorophyll pigments not being resistant to stress, hence the reduction in chlorophyll *a* wasless as compared to chlorophyll *b*in all species, possibly due to the inhibition of biosynthesis of precursors of chlorophyll under soil water deficit as also reported by Moaveni *et al.* (2011). While the reduction in chlorophyll *b*was higher than in chlorophyll *a* in all species, probably due to increased protein synthesis, and increased nitrogen metabolism (Singh*et al.*, 2008). The significant decrease in total chlorophyll content under soil water deficit might be attributed to the increased degradation of chlorophyll pigments due to stress induced metabolic imbalance (Steinke and Stier, 2003). According to Chen *et al.* (2007), in wheat and maize varieties, tolerant varieties have higher chlorophyll contents and among the seven species, *Amaranthus albus*, had the highest chlorophyll content followed by *Amaranthushypochondriacus*, *AmaranthuscruentusAmaranthus retroflexus*, *Amaranthusblitum*, *Amaranthus spinosus* and *Amaranthustricolor* respectively and this further implied that the production of reactive oxygen species was mainly driven by excess energy absorption bychlorophyll content, which could have been avoided by degrading the absorbing pigments (Farididdin *et al.*, 2009).According to Colom and Vazzana, (2003) water deficit causes large reductions in chlorophyll and carotenoid content, which directly affects photosynthesis due to poor light absorption and conversion into useful energy. Kirnak *et al.* (2001) found that water deficit resulted in significant decrease in chlorophyll content, among other parameters for plant growth under high water stress, which resulted in less fruit yield and quality. Steinberg *et al.* (1990) reported a reduction of chlorophyll concentration in peach trees subjected to different levels of water stress, and was in agreement with the results of this study, that showed water deficit in the pot grown indigenous vegetables produced a reduction in total chlorophyll content subjected to different levels of water deficit. The reduction

ISSN: 2350-0328
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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 could have been an adaptive defence mechanism of the chloroplast (Singh*et al.*, 2008) to drought. On the other hand, a possible reduction instomatal conductance leading to a decrease in carbon assimilation might have contributed to a decreased photosynthetic rate, as a result of the inhibitory effect of decreased water content on leaf development (Fariduddin *et al.,* 2009; Vurayai *et al.*, 2011).

V.CONCLUSION

Chlorophyll *a* and chlorophyll *b* decreased significantly with increase in water deficit, possibly due to increased protein synthesis, and increased nitrogen metabolism. There was also a significant difference in total chlorophyll concentration with increase in water deficit and this could be attributed to an increase in oxidative stress and subsequent deterioration in chloroplast structure and an associated loss of chlorophyll. Chlorophyll *b* concentration was more reduced than chlorophyll *a* in all the soil water deficit treatments, implying that chlorophyll *a* is more drought tolerant than *b*. A*maranthus albus* having maintained higher chlorophyll *a, b* and total chlorophyll contents in all soil water deficit treatments makes it the most tolerant species followed by *Amaranthushypochondriacus*, *Amaranthuscruentus,Amaranthus retroflexus*, *Amaranthusblitum*, *Amaranthusspinosus* and *Amaranthustricolor* respectively.

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VII. ACKNOWLEDGEMENTS

The authors wish to thank Kenya Agricultural and Livestock Research Organisation, Kisii Centre and Maseno University for creating an enabling environment for this research work.

ISSN: 2350-0328
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