



# **Eco-physiological Responses of *Amaranthus cruentus* L. to Deficit Irrigation under Different NPK 20:10:10 Fertilization Rates**

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## **Authors' contributions**

*This work was carried out in collaboration between both authors. Authors PTT and MPM conceived the research, produced the experimental plan, implemented the research and collected field data. Author PTT analysed the data. Both authors read and approved the final manuscript.*

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## **ABSTRACT**

The aim of this research was to investigate the responses of *Amaranthus cruentus* L. to deficit irrigation under fertilization, in a 2 by 3 factorial experiment with two levels of irrigation (1.5 litre/week and 0.75 litre/week) corresponding to 2600 and 1300 mm/year respectively and three levels of NPK 20:10:10 (0, 138, 275 kg ha<sup>-1</sup>). This experiment was conducted in a screen house in Cameroon, and lasted for 12 weeks after the nursery phase. Growth parameters and chlorophyll fluorescence were measured weekly for 8 weeks. Destructive sampling was done at 12 WAT to determine biomass partitioning, water use efficiency and the root/shoot ratio. Data were analyzed for variance and relationships in the MINITAB Version 17 statistical package. Within each irrigation level, plant mass decreased as fertilizer rates decreased, while root: Shoot ratio increased instead. Plant mass expressed higher values at the higher irrigation levels while root: Shoot ratio was lower compared to that at lower irrigation levels. This shows a strategy for resource re-allocation to roots under both water and nutrient deficit. Harvest index was statistically similar across irrigation and fertilizer levels. Within the higher irrigation levels, WUE of plants decreased with a decrease in fertilizer rates but not for plants subjected to deficit irrigation. While chlorophyll fluorescence values differed significantly

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across treatments, all values were below 0.8, indicative of stress. Factor analysis showed that growth of *A. cruentus* was highly fertilizer-dependent, while chlorophyll fluorescence was irrigation-dependent. This suggest that fertilizer application is essential in ameliorating the effects of deficit irrigation, and will be essential in the production of this crop under deficit irrigation.

**Keywords:** Leafy vegetable; deficit irrigation; biomass partitioning; chlorophyll fluorescence; factor analysis.

## 1. INTRODUCTION

Across the world, food insecurity is a persistent problem and has been centre stage in policy cycles for decades. A search of the terms '*global food insecurity*' on Google Scholar yields 458,000 results in 0.22 seconds. Ensuring food security is one of the sustainable development goal adopted by the United Nations as part of its Agenda 2030 for sustainable development. According to [1], over 239 million people suffer from food insecurity in sub-Saharan Africa alone. In addition to food insufficiency, sub Saharan African countries also rank among those with highest levels of nutritional deficiencies, so called hidden hunger (Muthayya et al. [2]), with the situation constantly deteriorating in Africa [3]. There are several causes of food insecurity namely political instability, war and civil strife, macroeconomic imbalances and trade dislocations to environmental degradation, poverty, population growth, gender inequality, inadequate education, and poor health [4]. Of these, the most important to the crop ecophysiological is environmental degradation.

Global environmental change driven by deforestation, urbanization and global warming, has led to degradation of water resources as well as quality of arable soils [5-7]. This has rendered irrigation water scarce, and the soils typically unsuitable for cultivation of most crops in sustainable quantities. Water scarcity in the growing season, but most especially in the off-season, results in drought. Drought stress occurs in plants when the levels of water available in the soil are insufficient for normal functioning of the plants. Typically, plant cells lose turgidity, guard cells close to restrict water loss and in consequence the rate of photosynthesis drops as CO<sub>2</sub> cannot diffuse in adequately, leaf fall occurs to reduce transpiration, further reducing the area and photo systems available for photosynthesis; reactive oxygen species accumulate in cell cytoplasm and if these reach toxic concentration cell death occurs; there is reduced growth ultimately, as available photosynthate is diverted from plant growth to defense response [8-10].

Similarly, global change alters nutrient uptake patterns by plants [11] and soil degradation typically leads to nutrients depletion in arable soils. Soil nutrient depletion can also be attributed to insufficient and unbalanced fertilizer use, leading to nutrient depletion induced soil fertility problems [12]. The result is that available nutrient concentrations are either low or insufficient for plant use, or they are fixed in the soil and plants cannot effectively take these up to power growth and development. Therefore it is possible to alleviate some of the effects of drought stress in crop plants by augmenting soil nitrogen concentration through fertilizer use, as has been shown for some varieties of potato [10]. Using nitrogen to alleviate drought stress has thresholds for each species, and this has to be studied carefully in the context of sustainable food production, for instance it has been shown that in sugar beet, nitrogen application serves to ameliorate the effects of drought stress, but once thresholds are exceeded it contributes to worsening the stress, as seen through up-regulated proline concentrations [13]. Similar findings have been reported for soybean, where application of nitrogen fertilizer under drought conditions alleviated drought stress, but decreased yields when applied in well irrigated conditions [14]. The action of nitrogen in stress tolerance is typically through synthesis of proline and other quaternary ammonium compounds that constitute compatible osmolytes [15] and act against reactive oxygen species, stabilizing membranes, proteins and DNA. It is also essential in the synthesis of essential structural proteins that are necessary for growth and development.

Species responses to water deficit are essential in crafting strategies for alleviating hidden hunger and improving food security. This is especially true for vegetable crops which are high value short duration crops and hence highly susceptible to even brief periods of stress. *Amaranthus cruentus* L. is highly popular in most African diets. It has been shown that this species is mildly tolerant of drought stress, with decrease in leaf area per root dry mass and specific leaf

area [16] but few researchers have investigated how this species responds to nitrogen fertilization under drought stress, for example [17]. The general objective of this research was to evaluate the ecophysiological responses of *Amaranthus cruentus* to soil moisture variations under NPK fertilization. The results would aid in guiding sustainable production under appropriate fertilization regimes, in various soil moisture scenarios.

## 2. MATERIALS AND METHODS

### 2.1 Description of the Study Site

The experiment was conducted in a screen house at SOWEFCU, Kumba (South West, Cameroon). This site is located at latitude N 04.62°58' and longitude E 09.44°98', at an elevation of about 237 m above sea level and it is found in the South West Region of Cameroon, which lies in the Humid Forest Agro-ecological zone with monomodal rainfall regime. The annual rainfall in the area is about 2600 mm per year and temperature varies between 20 to 35°C with a mean monthly temperature of 26°C for the whole year (IRAD, 2013). This region has a distinct dry season from November to March and the rainy season lasts from April to October. During the experiment, temperature inside the screen house varied from 33°C to 40°C while the relative humidity varied from 45 to 50%.

### 2.2 Experimental Design and Treatments

A factorial experiment was laid out with 3 replicates for each treatment under screen house conditions. The treatments consisted of three levels of NPK (20-10-10) compound fertilizer namely 0, 138, 275 kg ha<sup>-1</sup> as inorganic fertilizer and two levels of irrigation (2600mm and 1300mm per year) that were combined in a 3×2 factorial experiment. The fertilizer rates were determined from [18] who established a threshold of 275 kg ha<sup>-1</sup> for leafy vegetables. The irrigation levels were determined from the mean annual rainfall for Kumba of about 2600 mm, and half of this represented a deficit irrigation level for the region. Based on the surface area of the pots used, both fertilizer and irrigation rates were then calculated for weekly application rates (Table 1).

### 2.3 Agronomic Practices

The seeds of *Amaranthus cruentus* were sown on a prepared nursery bed (with the dimensions 1cm×2cm, done under a shade, approximately 50% of luminosity) and watered regularly using a

watering can. Seedlings emerged after 5 days, and the nursery bed was weeded regularly. On the 22<sup>th</sup> May (11 days after sowing), seedlings were treated with an insecticide, Kumfu 5%WP at 0.5g per liter to control insects. On the 27<sup>th</sup> May Cacaocides 2010 75WP (fungicide) was applied at 5 g per 5 litres of water to the fields to prevent infection of fungal disease. The top soil samples were collected from the top 30 cm, air dried, crushed and sieved to pass through 2 mm sieve for pre-planting soil analysis at the Plant and Soil Laboratory of the Faculty of Agronomy and Agricultural Sciences (FASA), of the University of Dschang, for the following parameters: Soil texture, pH, organic carbon, total N, extractable P, exchangeable levels of Ca, Mg, Na and K, and cation exchange capacity. All analyses were done using standard methods of the APHA (2005). The results of these analyses are reported in [19].

The pots used had a volume of 6 L, and each was filled with 5.5 Kg of top soil. The pots were perforated (10 holes of 2mm each) at the bottom to avoid waterlogging. The pots were then labeled according to the different treatments. The three-week old seedlings were transplanted into different plastic pots, 3 seedlings per pot. The plants were grown inside the screen house for 8 weeks with irrigation and fertilization as defined in Table 1.

### 2.4 Data Collection

#### 2.4.1 Growth measurements

Growth data including plant height, number of leaves, number of branches, leaf area, collar diameter, as well as chlorophyll fluorescence (FV/FM) were collected weekly from 2 WAT to 8 WAT. Plant biomass partitions were determined at the end of the experiment.

Plants height was measured from the base of the stem to its apex using a millimeter rule. The total number of mature leaves on both the stem and branches were also counted and recorded. Collar diameter of plants from each pot was determined at 2 cm above the soil surface (the ground level) using a Vernier Caliper. Leaf area of the plants was determined by measuring the individual leaf length and width and multiplied by a factor of 0.64, which is a regression constant derived for the species [20] as shown below:

$$LA = 0.64(LI \times WI) \quad (1)$$

Where, LI is the leaf length and WI is the leaf width.

**Table 1. Fertilizer and irrigation rates of the different treatments for application**

Treatment Combination	Irrigation as per rainfall (mm)	Equivalent Irrigation/pot/week (L)	NPK 20:10:10 fertilizer rate (Kg $ha^{-1}$ )	Equivalent weekly application rate/pot (g)
I1F1	2600	1.5	275	7.4
I1F2	2600	1.5	134	3.7
I1F3	2600	1.5	0	0
I2F1	1300	0.75	275	7.4
I2F2	1300	0.75	134	3.7
I2F3	1300	0.75	0	0

I= Irrigation, F= Fertilizer, N=Nitrogen, P=Phosphorous, K=Potassium.

### 2.4.2 Chlorophyll fluorescence

Chlorophyll fluorescence was measured as a ratio of the variable to maximum fluorescence (fv/fm). This measures the quantum yield potential of photosynthesis, or maximal photochemical efficiency of PSII. This was measured using a pocket Plant Efficiency Analyzer (Hansatech Instruments, U.K.). Measurements were done on leaves (second fully expanded leaves from the top of the plants), which had been initially dark adapted for 20 seconds using leaf clips.

### 2.4.3 Biomass partitioning

At the end of the experiment, plants were harvested carefully from the pots, and separated into flowers, shoots and roots, then weighed separately using an electronic balance (Salter, China) to have the fresh mass (FM). Then the roots, shoots and flowers were packaged separately in well-labelled paper envelopes and dried in an air-flow oven at 60°C to constant mass for 48 hours. The dry masses (DM) were then determined using an electronic balance.

Root: shoot ratio was calculated for each treatment as follows [21]:

$$\text{Root: Shoot Ratio} = \frac{\text{Root mass}}{\text{Shoot mass}} \quad (2)$$

The water use efficiency was calculated using the following formula [21]:

$$\text{Water Use Efficiency} = Mb/Cw \text{ (kg/m}^3\text{)} \quad (3)$$

Where, WUE=water use efficiency, Mb=Sum of weight (dry weight) of plant (flowers, shoots and roots) in Kg; and Cw= cumulative amount of

water applied during the experiment per treatment (m<sup>3</sup>).

The harvest index was calculated for each treatment by using the following formula [21]:

$$\text{Harvest index} = \frac{\text{Economic yield}}{\text{Biological yield}} = \frac{\text{Fresh shoot mass}}{\text{Total plant dry mass}} \quad (4)$$

## 2.5 Data Analysis

Data were subjected to Analysis of variance through the GLM Approach, with natural log transformation following tests for normality and homogeneity of variance. Means were separated through Tukey HSD test at  $\alpha = 0.05$ . Spearman rank correlation was done to determine relationships between variables, and Factor analysis to determine key drivers of the observed responses. All analyses were conducted in the MINITAB Version 17 statistical package (Minitab Inc., PA, USA), and significance was set at  $\alpha = 0.05$ .

## 3. RESULTS

### 3.1 Effect Inorganic Fertilizer (NPK 20:10:10) Application and Irrigation on Growth of *Amaranthus cruentus*

#### 3.1.1 Height

Fertilizer, irrigation and time had a significant effect on plant height, number of leaves and leaf area of *Amaranthus cruentus* (Table 2). Fertilizer significantly influenced collar diameter, but the effect of irrigation on collar diameter was not significant. The interaction between fertilizer and irrigation were not statistically significant for any of the growth parameters.

**Table 2. Table of significance following ANOVA tests on growth parameters of *A. cruentus***

Factor	Height	Number of leaves	Leaf area	Collar diameter
Fertilizer (F)	0.000	0.000	0.000	0.014
Irrigation (I)	0.000	0.000	0.001	0.131
Time (T)	0.000	0.000	0.000	0.000
F*I	0.177	0.079	0.214	0.056
F*T	0.096	0.000	0.000	0.202
I*T	0.882	0.627	0.449	0.521
F*I*T	1.000	0.996	0.988	0.423

Values in table represent p-values showing levels of significance. P-values less than 0.05 are not statistically significant

**Table 3. Effect of irrigation and fertilizer rates on growth of *A. cruentus* in screenhouse**

Fertilizer (Kg/ha)	Height (cm)	Number of leaves	Leaf area (cm <sup>2</sup> )	Collar diameter (cm)
0	29.42a	13.11a	384.72a	0.56a
138	37.05b	16.98b	940.58b	0.61ab
275	36.65b	17.49b	1019.05b	0.66b
<b>Irrigation (mm/yr)</b>				
1300	32.93a	15.14a	658.59a	0.59a
2600	35.48b	16.33b	780.80b	0.63a

Values in the table represent means. Means separated through ANOVA with Tukey HSD test a  $\alpha = 0.05$ . Means with the same letter within the column for each main effect are not statistically different.

As fertilizer rate increased above the control, plant height (29.42 to 37.05cm), number of leaves (13.11 to 17.49), leaf area (384.72 to 1019.05 cm<sup>2</sup>) and collar diameter (0.56 to 0.66 cm) all increased. Doubling irrigation rate significantly increased plant height, number of leaves and leaf area, but there was no significant effect on collar diameter (Table 3).

Fig. 1 shows further, that plants that were fertilized performed better in terms of growth. Over time, all plants grew to flowering and senescence (Figs. 1A to B). Plants that were fertilized maintained their chlorophyll green coloration while those that were not fertilized were clearly chlorotic (Fig. 1 C). As fertilizer rates decreased, plant growth and chlorophyll concentration clearly drops (Fig. 1 D).

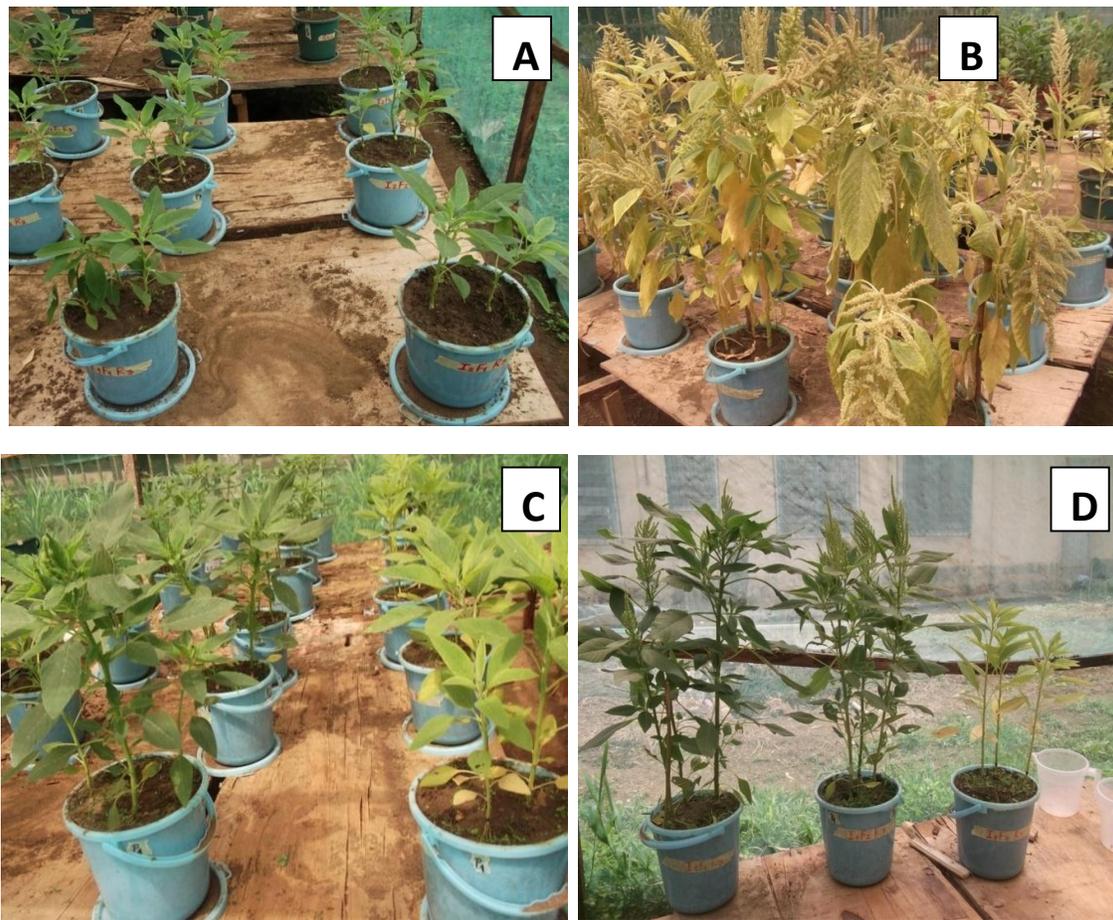
### 3.2 Number of Flowers

The number of panicles was significantly influenced by the fertilizer treatments, time and the interaction between fertilizer and time ( $p < 0.0001$ ). Fig. 2 shows the number of flowers of *Amaranthus cruentus* as influenced by fertilizer rates. Mean number of flowers increased from 0.76 in the control to 2.51 in plants that received the highest fertilizer rate.

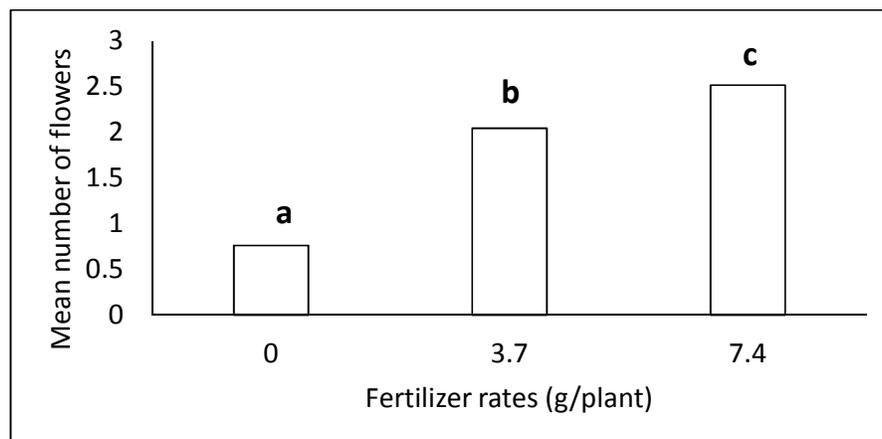
With respect to irrigation, the number of panicles (1.56 to 1.58) was not statistically different.

### 3.3 Effect of Treatments on Biomass Partitioning, Harvest Index, Water Use Efficiency and Chlorophyll Fluorescence

The interaction between irrigation and fertilizer rates on biomass partitions, yield, WUE and chlorophyll fluorescence (Table 4) significantly influenced the expression of these parameters. Within each irrigation range, plant mass decreased as fertilizer rates decreased, while root: shoot ratio increased. Plant mass expressed higher values at the higher irrigation levels while root: Shoot ratio was lower compared to that at lower irrigation levels. Harvest index was statistically similar across irrigation and fertilizer levels. Within the higher irrigation levels, WUE of plants decreased with a decrease in fertilizer rates but not for plants subjected to the deficit level of irrigation. For both irrigation levels, plants treated with higher levels of fertilizer had higher values of chlorophyll fluorescence, but these were all below 0.8.



**Fig. 1.** Visual observations on *A. cruentus* plants during the experiment. A: plants at 2WAT; B: Plants at 9WAT; C: Difference between plants that received fertilizer and those that did not. Plants not fertilized show clear signs of chlorosis. D: Difference between plants treated with 7.4g (at left), 3.7 g (in the middle) and 0g (at right) of NPK 20:10:10.



**Fig. 2.** Number of flowers as influenced by fertilization rates in screenhouse. Values represent means. Means separated through Tukey HSD test at  $\alpha=0.05$ . Bars with the same letter are not statistically significant

**Table 4. Effect of irrigation and fertilizer rates on biomass partitions, harvest index, WUE and chlorophyll fluorescence of *A. cruentus* in greenhouse**

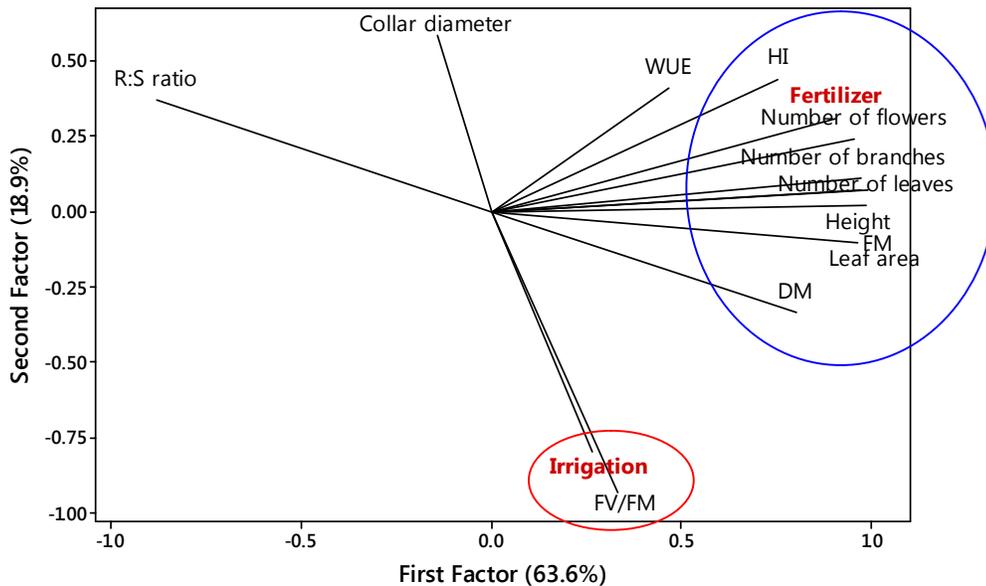
Fertilizer (Kg/Ha)	Irrigation (mm/m <sup>2</sup> /year)	FM (g)	DM (g)	R:S ratio	HI	WUE (g/L)	Fv/fm
275	2600	190a	53.7ab	0.101b	3.55a	3.40ab	0.64a
138	2600	150b	79a	0.102b	2.56ab	3.82a	0.63bc
0	2600	55d	30.3c	0.204ab	2.06b	2.66b	0.62c
275	1300	130.3bc	45b	0.118b	2.98ab	5.58a	0.65a
138	1300	117.7c	45b	0.155ab	2.47ab	5.71a	0.64ab
0	1300	58d	24c	0.411a	2.46ab	3.04ab	0.62c

Values in the table represent means. Means separated through ANOVA with Tukey HSD test a  $\alpha = 0.05$ . Means with the same letter within the column for each main effect are not statistically different.

**3.4 Correlation between Treatments and All Parameters**

At  $\alpha = 0.05$ , there were strong positive correlations between fertilizer rates and height ( $\rho = 0.717$ ), number of leaves ( $\rho = 0.837$ ), number of branches ( $\rho = 0.837$ ) and leaf area ( $\rho = 0.837$ ). There were also strong positive correlations between fertilizer rates and yield parameters like number of flowers and harvest index ( $\rho = 0.956$  for both variables). Fertilizer rates also correlated positively with biomass fractions like fresh mass ( $\rho = 0.837$ ) and dry mass ( $\rho = 0.667$ ), as well as ecophysiological variables like WUE ( $\rho = 0.598$ ). There was a strong negative correlation between fertilizer rates and root: shoot ratio ( $\rho = -0.837$ ). While

Irrigation also correlated with some growth parameters, these correlations were weaker than the fertilizer effect. Irrigation correlated positively with chlorophyll fluorescence ( $\rho = 0.683$ ) and negatively with root: shoot ratio ( $\rho = -0.488$ ) and WUE ( $\rho = -0.488$ ). Factor analysis of the correlation matrix is presented in Fig. 3 and shows the close positive association of fertilizer rates with growth parameters, WUE, biomass partitions and harvest index. It also shows the relationship between irrigation and these parameters. Overall, the treatment effects explain 82.5% of the observed variability in the data, with the fertilizer effect contributing 63.6% as seen from the first factor, and irrigation accounting for 18.9% as seen from the second factor.



**Fig. 3. Factor analysis of the relationship between treatments and the measured variables. Variables closely associated are positively correlated and negatively correlated to those on the opposite end of the same axis**

#### 4. DISCUSSION

Water and soil nutrient availability are two major factors that limit crop growth and yield, but which farmers also have the possibilities of modifying for maintained yield sustainability [22]. In the current experiment, all growth parameters decreased in the lower irrigation regime which represents a deficit irrigation scenario, compared to the higher one which represents the norm. This is possibly a result of water deficit stress; the deficit irrigation treatment corresponded to 1300 mm/m<sup>2</sup>/year, which is a 50% reduction in irrigation or the region, and this in turn resulted in a 31.42% reduction in shoot yield for the highest levels of NPK 20:10:10 fertilized plants. Water deficit causes plants to close stomata so as to reduce water loss, and in consequence gaseous exchange is reduced and the rate of photosynthesis drops, ultimately resulting in reduced production of biomolecules for growth and development [23,24]. There is reduced turgor, which limits the rate of cell elongation that is a key physiological stage in plant growth [24]. In plants treated with NPK 20:10:10 fertilizer, the growth variables were significantly higher even under the deficit irrigation scenario compared to those that did not receive nitrogen. Plants fertilized with 275 Kg ha<sup>-1</sup> exhibited 19.7% more growth in height compared to those that did not receive fertilizer. This suggests that nitrogen fertilization is essential in ameliorating growth under drought stress, possibly by alleviating nutrient deficiency due to water deficit, and hence providing essential nitrogen for biosynthesis of cell components and compatible osmolytes like proline and glycinebetaine, consistent with findings of [14,25]. Biomass partitioning revealed a strategy of resource re-allocation for drought tolerance that involves shifting photosynthate below ground for root formation at the expense of shoot growth. This was seen from the increased root:shoot ratio in plants under deficit irrigation, and no- or lower nitrogen fertilization rates. This strategy enables the plants to forage better for scarce nutrients and moisture as an adaptation to drought stress [26]. In this species, soil nitrogen availability is a better determinant of growth compared to a 50% reduction in irrigation water, as tested in this experiment, seen from the Factor Analysis.

On the other hand, we found a strong association between chlorophyll fluorescence and irrigation levels. Water availability is essential to the turgor and hence health of leaves. Kim et al. [27] have demonstrated that leaf dehydration decreases

leaf water potential and cell turgor pressure. Also, photolysis of water is one of the very early steps in photosynthesis, without which the very essence of plant production would be moot. Recent studies have shown that far from being just a passive solvent, water can be considered an integral part of Photosystem II [28]. Drought stress has been shown to suppress leaf chlorophyll concentrations [29]. When this occurs, photosynthesis rates and efficiency also drop and this is one of the central reasons for growth reduction in crop plants under drought stress.

#### 5. CONCLUSION

The results show that growth of *A. cruentus* reduces by up to 31.42% under 50% deficit irrigation corresponding to 1300 mm/m<sup>2</sup>/year but this can be ameliorated with nitrogen fertilization of 275 Kg Ha<sup>-1</sup>. Therefore in a future where both irrigation water unavailability and ambient temperatures are predicted to increase, farm managers should consider decreasing irrigation regimes while simultaneously fertilizing with nitrogen to maintain yields.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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