POSITION OF AMARANTHUS SEED ON THE PLANT IN RELATION TO SEED QUALITY AND PRODUCTIVITY UNDER VARYING WATER REGIMES

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PREFACE

The research contained in this thesis was completed by the candidate while based in the Discipline of Crop Science, School of Agricultural, Earth and Environmental Sciences, in the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg Campus, South Africa. The research was financially supported by the South African Department of Agriculture, Forestry, and Fisheries (DAFF) and the Water Research Commision (WRC) Project: K5/2493//4.

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14 August 2018

Signed: Prof A. T. Modi (Supervisor)

Date

DECLARATION

I, Nkosinathi Hlatshwayo, declare that:

(i) the research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work;

(ii) this dissertation has not been submitted in full or in part for any degree or examination to any other university;

(iii) This dissertation does not contain other persons' data, pictures, graphs or other information unless specifically acknowledged as being sourced from other persons;

(iv) this dissertation does not contain other persons' writing unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

a) Their words have been re-written, but the general information attributed to them has been referenced;

b) Where their exact words have been used, their writing has been placed inside quotation marks, and referenced;

(v) Where I have used a material for which publications followed, I have indicated in detail my role in the work;

(vi) this dissertation is primarily a collection of material, prepared by myself, published as journal articles or presented as a poster and oral presentations at conferences. In some cases, additional material has been included;

(vii) This dissertation does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the dissertation and in the References sections

NHlatshwayo *14 August 2019*

Signed: N. Hlatshwayo Date

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GENERAL ABSTRACT

Amaranth is one of the important vegetables that are highly nutritious in protein, iron, vitamins A, C, and K. Its nutritional value can be used to reduce food insecurity, poverty and malnutrition problems in South Africa because it occurs naturally and it has the ability to tolerate drought and produce reasonable yield in infertile soils. Amaranth occurs in different species and landraces. In commercial agriculture, this is a serious weed plant. However, it is also utilised as a leafy vegetable crop in many developing countries. In South Africa, it is considered as an underutilized traditional crop with limited cultivation compared to other vegetable crops. The aim of the study was to determine seed quality, growth, and development of two amaranth landraces collected from three agriculture locations in KwaZulu-Natal, South Africa, where the plant is utilised for food security when it is available during the cropping season. Three positions on the plant were defined as equal thirdiles of the seed filled portion of the plant referred to as top, middle and bottom. Seed quality of the harvested original material was determined in terms of physical attributes (seed weight) and germination parameters, namely vigour, rate, and total. Then the original seeds were planted to produce plants under controlled environment conditions differing in water regimes from the seedling establishment to harvest maturity. The water regimes were 100% field capacity (FC), 50% FC and 30% FC to create adequate and stressed conditions. First-generation plant growth and development was determined in terms of morphology (plant height, leaf number, leaf area and aboveground biomass). This response was correlated with physiological parameter namely stomatal conductance and chlorophyll content index. Seed quality results showed that seed weight parameters namely, seed mass per landrace, seed mass per part, and thousand seed mass were significantly affected ($p<0.05$) by site, landrace, and position on the plant. Seed water content was significantly affected by landrace and water activity was significantly affected by sites only. Germination was significantly affected ($p<0.05$) by landrace, position, and site. Controlled tunnel results showed that plant height and leaf area were significantly ($p<0.05$) affected by site, landrace water regimes and the interaction of site, water regime, position, and landraces. However, in leaf number, site, landrace, position and water regimes were significantly ($p<0.05$) affected but the interaction of site, water regime, position, and landrace were not significantly ($p>0.05$) affected. Both physiological parameters (chlorophyll content index and stomatal conductance) were not significantly ($p>0.05$) affected by site, landrace, position and their interaction, However, they were significantly ($p<0.05$) affected by water regimes. Leaf yield was significantly ($p<0.05$) affected by water regimes and the interaction of site and water regimes only. For both parent and first generations, seed quality differed with the position on the plant, where middle and bottom seed performed better than top located seed. Water stress reduced seed quality. It is concluded that seed quality of amaranthus is associated with a position on the plant, regardless of the location of harvested material. Water stress reduces plant growth and seed yield.

Keywords: Amaranthus, position on the plant; seed quality, crop performance

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LIST OF ABBREVIATIONS

KOK - Kokstad

SWA- Sway mane

UMB- Umbumbulu

TSW-Thousand seed weight

FC- Field capacity

CCL- Chlorophyll content index

SC- Stomatal conductance

LN-Leaf number

LA-Leaf area

PH-Plant height

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Chapter 1

1. Background

Internationally, prevalence's of malnutrition is approximately about 2 billion people suffer from undernutrition (WHO and FAO, 2006). With the population of South Africa continuing to increase to reach a population of 65.5 million by 2050 (STATSSA, 2017), there will be an increase in the population suffering from malnutrition. Currently, in South Africa, the common nutritional problems people face include energy and macronutrient (protein) status, micronutrient malnutrition, dietary diversity, and nutrient density (Kinabo et al., 2013). However, the most prevalent nutritional deficiencies, particularly in people living in household levels includes micronutrients (iron (Fe) and zinc (Zn)), vitamin A and vitamin C (Wenhold et al., 2007) because they tend to rely mostly on staple crops such as maize, wheat, potato, and ignore meats and other vegetables due to high cost of it. Thus, there is a demand to increase the production of food accompanied by an increase in nutrient density. The production can be improved by using underutilised crops such as amaranth (*Amaranthus* spp**.**) since it has been confirmed that these crops contain high nutritional value and adapt easily to unfavourable conditions (Ebert et al., 2013).

Although amaranths are also known for its high nutritional value which is needed for the human diet, it can be advantageous to people residing in developing countries where food is expensive (Mnkeni et al., 2007; Caselato-Sousa and Amaya-Farfán, 2012; Achigan-Dako, Sogbohossou and Maundu, 2014). Studies of Bahta and Bauer (2007) and Gorinstein et al., (2007) reported that that both seed and leaves of some amaranths were known to be denser in micronutrients, such as β carotene, vitamin C, protein, calcium, and iron, compared to cabbage and lettuce (Muriuki, 2015). Moreover, amaranths with its ability to tolerate drought and produce a yield in infertile soils can be used to reduce food insecurity, poverty and malnutrition problems. The crop originated in South America and consists of approximately 50-70 species that can be cultivated as a leafy vegetable, grain, and ornamental plant depending on the use (Achigan-Dakoe et al., 2014; GC and GA, 2016). However, grain amaranths are the most common one and it is used in different countries due to its adaptability to unfavorable conditions (Alemayehu, Bendevis and Jacobsen, 2015). Worldwide, the largest producers of amaranth have been reported to be China, where 150,000 ha are reportedly grown for forage use (Myers, 1996). Amaranths are grown principally for vegetable use and have better tasting leaves than the grain types of amaranths, although the crop in South Africa is considered as an underutilized crop. However, there are areas where the crop is produced for human consumption and those include Limpopo, North West, Mpumalanga and KwaZulu-Natal provinces (DAFF, 2014). They produce fresh leaf yield of up to 40 ton/ha and the grain yield is highly variable but can be up to 1000 kg/ha (DAFF, 2014). In such areas, the crop is usually not planted but occurs naturally after the first rain and harvested from the wild. Considering the above issues, water plays a crucial role in crop growth and production, however, recently, water is becoming scarcer due to climate change. The study of Jaleel et al., (2009) showed that water scarcity is one of the limiting factors to agricultural production. South Africa with the rainfall of 464 mm and with its high evaporation of 1100 to 3000 mm compared to world's average rainfall and evaporation of 846 mm (Grab and Craparo, 2011) and 1130 mm (Babkin, 2009), this makes it vulnerable to drought. Due to this, the International Water Management Institute points out, that South Africa is a drought-prone country. Under these changing environmental conditions, there is a need to develop drought-tolerant crops and understand their water use. Though there are reports pointing out that amaranths are tolerant to drought, improvement of this crop is needed, and it could contribute to food security. Knowledge about amaranth landraces performance under drought conditions, water use, and its agronomy is not well understood in South Africa.

The significance of the study

Findings of the study will help fill the knowledge gap by providing knowledge on the responses of amaranth landraces to water stress. It will also help small-scale farmers and plant breeders in selecting the best landraces for crop improvement, specifically drought tolerance and nutritional value.

Research aim

Considering the above matter, the study aimed to examine the effect of sites (locations) and position of seed on seed quality, growth and development parameters of amaranthus landraces under water-stress conditions

Specific objectives were:

- To compare seed quality of amaranth landraces by determining the vigour (moisture content, water activity), physical attributes (seed weight) and viability (germination) based on seed positional effect.

- To determine morphological, yield and water use responses of amaranth landraces under different water regimes based on seed positional effect.

Chapter 2

Literature Review

2.1. Origin, taxonomy and classification

Amaranths are originated in South and Central America and belong to the family Amaranthaceae that contains about 800 species of dicotyledonous of either annual or perennial growth (Uusiku et al., 2010). In the genus *Amaranthus,* most species are viewed as the weedy type and few species are cultivated with only about 50 to 70 species cultivated. Mlakar et al., (2010) point out that there are three-principal species of grain amaranth namely: *A. caudatus* L., *A. cruentus* L., and *A. hypochondriacus* while, the leaf vegetable amaranths are known as *Amaranthus tricolor* L. which is grown particularly in East Asia (Kochhar, 1986; Khandaker et al., 2008). The leaves of A. *cruentus* L are dark and used in part of Africa as vegetable amaranth (Kauffman & Weber, 1990). The difference between a vegetable and grain amaranth lies in the colour of seeds. Vegetable amaranths contain dark seeds whereas grains contain light seeds. Seeds produced in a gram of amaranth are almost up to 3000 seeds (Department of Agriculture, 2014). The colour of the seeds varies with species and range from black to white. However, the stem and leaves colour range from red to green (Kochhar, 1986). Steckel et al., (2004) reported that the major amaranths considered as weeds are *A. spinosus. A. retroflexus,* and *A. hybridus*.

Amaranths are annual herbaceous plants that are known as pseudocereal (not true grass cereal) because of their cooking and flavour is similar to that of grains (Leder, 2009; Amicarelli, Vera, and Camaggio, 2012). The plants have the height ranges from 0.5 to 2 m depending on the species and growth habit (Grubben, 1976). In terms of growth habit, amaranths are recognized to have indeterminate growth. The inflorescence of amaranths can be terminally or axillary and they vary from being erect or decumbent (Fig. 2.1). According to Brenner et al., (2000), amaranths have monoecious flowers with the colour that is white, green, pink and/or purplish.

Amaranths have been known by many names depending on the country of origin. In Africa, principally in South Africa it is also called Pigweed and Cockscomb (in English) Misbredie, Hanekam and Varkbossie (in Afrikaans), Imfino, Imbuya, Isheke (in isiZulu), Imbuya, Umifino Umtyuthu (in isiXhosa), Thepe, Theepe (in IsiPedi), Sesotho and Setswana, Umbuya, Isheke (in siSwati) , Vowa, Theebe (in Tshivenda), and Imbuya, Tyutu in Pondo ((Fox and Norwood Young, 1982; Van Wyk and Gericke., 2000; Vorster et al., 2002).

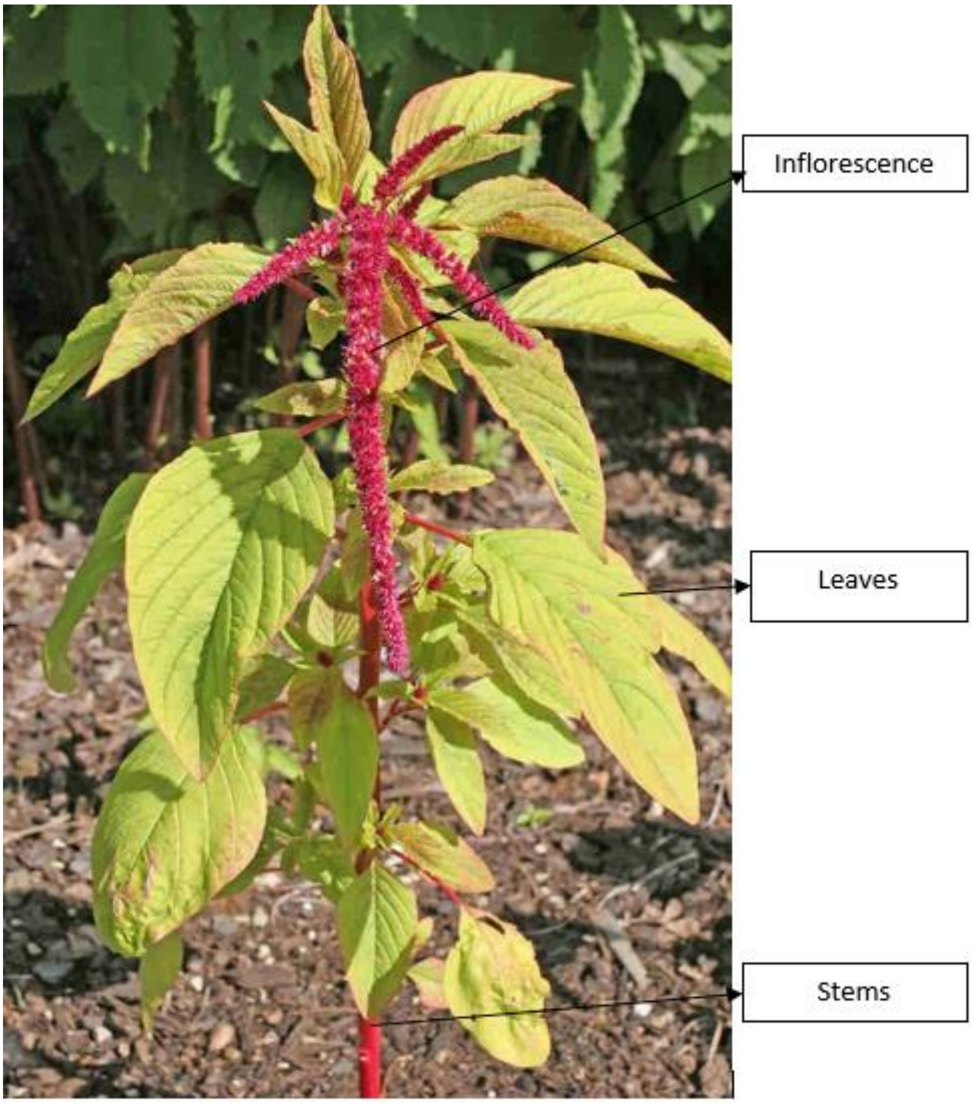

Figure 2. 1: Description of amaranths plant showing stems, leaves, and inflorescences. (Taken on July 6, 2018).

2.2. Uses of amaranths

Amaranths can be described as a multipurpose crop because it can be used in diverse ways. The leaves are cooked and eaten as a stew or as a potherb (boiled greens) or as a soup (Mlakar et al., 2009). Palada and Crosssman (1999) point out that the leaves can also be used as salads. The seeds and grains are edible, when heated they taste like nutty-flavoured popcorn. Grain amaranths can be milled to make flour (Tosi et al., 2001). Furthermore, Valcárcel-Yamani, B. and da Silva Lannes (2012), reported that grain amaranths can also be used to make pancakes, bread, muffin, and dumpling. Weedy plants can be used as fodder and forage for animals. Several studies have reported that the total ration of broilers increases after they have been given cooked amaranths (Alegbejo, 2013). In terms of medicinal value, vegetable amaranths are preferable for lactating mothers, young children and people suffering from anemia, fever, kidney, and haemorrhage (IFPRI, 2016). Moreover, it has been speculated that amaranths also can supplement the dietary for those individuals that suffer from HIV/AIDS. How?, for example, as described by Kelly and Martin (1983), ‘'if the patient receives a nutrient-rich diet, the anti-retroviral drug effectiveness can increase the body mass index of people ‘'.

2.3. Nutritional values

A recent study by Schönfeldt and Pretorius (2011) reported that malnutrition is caused by food and nutrition insecurity, at household levels and the statistics show that about one out of two children is suffering from hunger in South Africa. Food and nutrition insecurity is defined as the lack of individual to access to sufficient, nutritious and safe food to meet their dietary needs at all time (FAO, 1996). According to National Food Consumption Survey-Fortification Baseline (NFCS-FB-1) (2008), the number of South Africans that are at risk of hunger is one third and the number of people who are food insecure is one out of five. Furthermore, nutrients deficiencies such as vitamin (A, C), iron (Fe), and zinc (Zn) are considered as the major.

Previous studies have reported that indigenous crops such as amaranths, cowpea (*Vigna unguiculata)*, kale (*Brassica oleracea var. sabellica)*, and amadumbe (*Colocasia esculenta*) can contribute to minimizing the effect of nutrition deficiency as these crops are known to be nutritious. These crops are considered for high nutritional value and have large levels of vitamins and minerals and anti-oxidants (Abukutsa-Onyango, 2003). Amaranth is one the most highly nutritious crops, providing as a source of protein, energy, minerals, and vitamins for human consumption and livestock feed compared to other leafy vegetables (Venskutonis and Kraujalis, 2013) (Table 2.1)

Table 2. 1. The comparison of amaranths with other vegetables leaves in terms of nutrient composition (100 g portions) (Colonna et al., 2016).

Components	Dry matter (g)	Food energy (cal)	Protein (g)	Fats (g)	Carbohydrates Ash (g) Fiber (g) Total (g)	Calcium (mg)	Iron (mg)	Zinc (mg)	Potassium (mg)	Vitamin A (mg)	Vitamin C (mg)	Rinoflavin (mg)	Niacin (mg)	Thiamine (mg)
Amaranth	13.10	36	3.50	0.50	2.60 1.30 6.50	267	2.32	0.90	411	6.10	80	0.16	1.40	0.08
Spinach	9.30	26	3.20	0.30	1.50 0.60 4.30	93	2.71	0.53	470	8.100	51	0.2	0.60	0.10
Chard	8.90	25	2.40	0.30	1.60 0.80 4.60	88	1.80	0.36	550	6,500	32	0.17	0.50	0.06

2.4.1. Vitamins and minerals

Amaranth is a reliable source of vitamin A, vitamin B6, vitamin K, vitamin C, folate, and riboflavin. Mineral contents such as calcium, potassium, iron, copper, magnesium, phosphorus and especially manganese are also found in amaranth. Ogedegbe et al., (2013) reported that blindness and death of young children can be suppressed by consuming 50 to 100 g of vegetable amaranths per day as it contains vitamin A. Amaranths contain three times the content of, calcium and niacin when comparing it with spinach (Guillet, 2004) (Table 2.1). Guillet (2004) further reported that amaranths contain about eighteen times of vitamin A, thirteen times more vitamin C, twenty times of Ca and seven times of Fe compared to lettuce.

2.4.2. Lipids

Detailed research about amaranth oil has been studied for the last two to three decades by different scientists (Lyon and Becker, 1987; Pogojeva et al., 2006). Many authors have reported that 6 to 10 % of oil have been found in amaranths grain and the oil contain about 50% of linoleic acid which is required for human nutrition (Whelton, 1997). Additionally, the oil of amaranths is similar to the oil produced from other cereal such as barley, oats, and wheat (Leon-Camacho et al., 2001). Amaranths oil also contain squalene that ranges from 1.9 to 8.7 % depending on the species compared to other plant species which typically range from 0.001 to 0.4 % (He and Corke, 2003). Squalene is best known as the ingredient for skin cosmetic.

2.4.3. Proteins

The protein content of amaranths is high in both grain amaranths and vegetable amaranths compared to that of wheat and maize (Venskutonis and Kraujalis, 2013). However, when comparing the two (grain amaranths and vegetable amaranths), grain amaranth tends to have high proteins 15, 0 g than vegetable amaranths 3.5 g. According to the Lewandowski (1984) grain amaranths proteins contain amino acid lysine levels that are twice that of wheat protein and 3 times that of corn. However, amaranths protein contains a low level of leucine (Bejosano, Feliciano and Corke, 1998), thus, it is regarded as a good complement to cereal grains.

2.4.4. Anti-nutritional components

Leaves of amaranths comprise nitrates, oxalic acid, and a large number of nitrates in human can cause the development of gastric cancer in human (Mirvish, 1983; Santamaria, 2006). According to McKnight et al., (1999), the elevated level of nitrates can also cause problems for individuals that suffer from anaemia because they can make an individual more susceptible to methemoglobinaemia. Minerals in the body can be reduced by the excessive amount of oxalic acid particularly calcium. Other anti-nutritional components found in amaranths include polyphenol, phytohemagglutinins, phytic acid, saponins, tannins, and phenolic and protease inhibitors (Repo-Carrasco-Valencia et al., 2009).

2.5. Seed quality

Seeds are considered as one of the important inputs in crop productions. The productivity of crops can be increased by planting a quality seed. Balkaya et al., (2004) point out that uniform and rapid germination, genetic stability, high seed vigour and freedom from pest and diseases are characteristics of high-quality seeds. Additionally, Krishnotar et al., (2009) emphasized that the requirements for increased yield and quality of crop are a rapid and uniform emergence of a seedling. According to Odindo (2007), the two most important indicators of seed quality are seed viability (germination capacity) and seed vigour. Seed viability is defined as the ability of the seed to germinate under favourable conditions given that dormancy in the seeds is broken. On the other hand, seed vigour is defined as the indication of seed that gives the seed the ability for rapid and uniform emergence and development of normal seedlings under a wide range of field conditions. Wang et al., (2010) emphasizes that the properties that affect seed vigour are genetic background, storage and environment factors (temperature, photoperiod, water deficits, and soil fertility) during seed development. Furthermore, Pervez et al., (2009) reported that drought stress can also affect seed quality.

2.5.1. Seed position

Seed position in the mother plant can be regarded as one of the factors or components that can cause variation in seed quality, seed growth and yield apart from environmental conditions. Such effects in seeds have been reported in different crops including maize (Miya, 2015), Ash Gourd (Vanangamudi, 2017), carrots (Gray, Steckel, and Ward, 1985), pumpkin (Kumar et la., 2015), tomato (Pet and Garretsen, 1983). In amaranth, Jha et al., (2010), reported that the top and middle portion inflorescence have greater germination and seedling emergence compared to the bottom inflorescence, in none stressed crops. Likewise, in carrots, Szafiroska, (1992); Corbineau et al., (1995) have indicated that first and second umbels germinated higher and were heavier than the seeds of the third and quaternary umbels. According to Wang (2010), this is partly due to the resources not being fully distributed equally in the mother plant. With all these studies being done, there is little knowledge about the effect of water stress on seed quality. Therefore, research is still needed to be conducted to determine the effect of seed position on drought condition to measure seed quality and crop performance (yield).

2.6. Water stress and its effect on plant growth and development

Water stress (drought) can be defined in several ways depending on the disciplinary. However, for the sake of this study, we will focus on agricultural drought. According to Mabhaudhi, (2009), drought occurs when there is not enough water to support plant growth and development. This is attributed to poor rainfall distribution, meteorological drought and poor cultural practices that decrease soil water resulting in plants being water-stressed. Pourdad and Beg (2003), point out that drought is one of the crucial factor affecting the growth and development of crops including amaranths in many regions of the world. Closure of stomata, loss of turgor, and reduction of water content, cell enlargement, and growth decreases are the major characteristics of water stress in plants. Furthermore, previous studies of (Miyashita et la., 2009) have reported that stomatal conductance, transpiration, and photosynthesis are also affected by water stress.

Impaired germination and poor stand establishment are the primary effects of drought in many crops including amaranths because this stage is dependent on external factors (Harris et al., 2002). Furthermore, cell growth has also been reported as the most sensitive stage during drought stress. According to (Taiz and Zeiger, 2006), cell growth in the leaves compared to roots and shoots in many plants are most affected due to a reduction in turgor pressure in the leaves. It has been shown that as the turgor pressure is decreased by water stress, so does the cell division, expansion and differentiation become reduced. Previous studies have reported that cell elongation in higher plants, as water stress becomes more severe can be inhibited by interruption of water flow from the xylem to the surrounding elongating cells (Zinhle Ntombela, 2012). The morphological and biochemical changes in plants (including amaranths) such as reduced plant height, leaf area, reduced seedling, and crop growth have been observed under drought conditions. For instance, several studies have reported that water stress significantly reduces the stem length, leaf area expansion in different crops including soybean

(Specht et al., 2001), parsley (Petropoulos et al., 2008) and potato (Heuer, Bruria, and Nadler, 1995). Moreover, leaf production and expansion become significantly reduced under water stress, thus these leads to abscission and senescence of leaves (Ohashi et al., 2006; Nelson et la., 2007). Reduced leaf and stem length, plant height and leaf number have also been reported to decrease in soybean (Zhang et al., 2004), cowpea and sunflower (Manivannan et al., 2007), and amaranths (Jomo et al., 2015). This is due to inhibition of mitosis, cell elongation and expansion in plants (Nonami, 1998; Kaya et al., 2006; Hussain et al., 2008).

Roots are important in plants life since they are recognized as the main carrier of water to the crop. They are also known as the plant organ responsible for adaptation to drought stress and absorb mineral nutrients from the soil. According to Vadez et al., (2008) root density and depth are considered as one of the morphological parameters that are crucial for evaluating drought tolerance in crops. Jomo et al., (2014) further added that under extreme water deficit, increased roots length assist plants to grow. Furthermore, as described by Modi and Mabhaudhi (2013) under drought stress, increased in root length is closely associated with an increase in root: shoot ratio. In the studies conducted by Jomo (2013) and Jomo et al., (2015) in amaranths and in African nightshades, the dry matter roots: shoot ratio increased more under drought stress conditions. However, Blum (2005) reported that reduced assimilates in the leaves and leaf senescence under water stress conditions occur because of an increased in root: shoot dry matter ratio, not because of increased dry matter partitioning to the roots.

With all farmers, the main reason for growing crops is to get high yield (Jaleel et al., 2009). Yield can be defined as the harvestable part of the crop. Jaleel et al., (2009) further explained that the harvestable yield of the crops is different under water-stressed conditions. According to Farooq et al., (2009) under drought stress conditions, there are many yields determining factors that are affected. For instance, reproductive growth stage has been reported to be affected more compared to the vegetative stage. This is because at reproductive growth stage dry matter partitioning to sinks is mostly affected leading to reduced yield. Flexas et al., (2004) and Farooq et al., (2009) point out that reduced rate of photosynthesis and disturbed assimilate partitioning are also one of the factors responsible for reduced yield in crops experiencing drought stress.

2.7. Mechanisms responsible for drought tolerance and amaranthus response to water stress

Plants responses to drought stress differ and are dependent on the intensity and duration of the stress. Mechanisms evolved by plants to survive and adapt to water stress are: escape, avoidance, and tolerance (Mitra, 2001). However, the negative effect of these mechanisms is that the plant quality is easily decreased (O'Brien and Price, 1998). Lawlor and Cornic,(2002) reported that plant quality decreased because morphological, biochemical, physiological and molecular processes become altered.

Drought escape can be defined as the ability of the plant to complete its life cycle before water stress becomes terminal. This mechanism involves the occurrence of rapid phenological development (early maturity) and developmental plasticity. The study of Araus et al., (2002) reported that drought escape makes the time of flowering in plants a very crucial trait, as it is associated with drought adaptation. For example, early maturing plants can survive a drought before it becomes terminal. The underutilized crop that has been reported to have this mechanism is cowpea (Singh, 1994). No reports have been recently published in amaranth having this kind of mechanism. The disadvantage of drought escape is that it decreases the production of crops yield particularly those that are a long season.

Drought avoidance is defined as the ability to stand drought stress while maintaining high tissue water potential (Hall and Schulze, 1980; Blum, 2005). The mechanisms involved are a reduction of water loss through (epidermal resistance, reduction in solar radiation absorbed by aerial parts of plant and reduction in the evaporative surface) and maintenance of water uptake through (increased rooting and low resistance to water flow). Studies of Turner et al., (2001) and Kavar et al., (2008), have reported that these mechanisms can also be characterised by stomatal regulation and rooting from the soil. According to Lawlor and Cornic (2002); and Efeoglu et al., (2009) amaranths regulate loss of water (transpiration) by closing their stomata. However, this leads to a negative effect on the plant's yield parameters. For instance, Mitchell et al., (1998) reported that plant height, leaf number, leaf area index and leaf area becomes reduced. This reduction is attributed to plant reducing water loss. Likewise, in the studies of Masarirambi et al., (2012) reported that plant height, leaf number, leaf area and leaf yields of amaranths cultivar particularly A. *hybidus* and A. *tricolor* decreased as the water regimes decreased from 80% and 40% FC. Traits responsible for avoidance mechanism are root characters such as biomass, depth, density, and length. Hence these root characteristics are responsible for the final yield. As stated from the above, the negative effect of these mechanisms is that it leads to reduced yield.

Drought tolerance is defined as the ability of the plant to sustain a period of drought stress (Mitra, 2001). This kind of mechanism is hardly found in plants and it involves biochemical mechanisms. Antioxidation, osmoprotection (proline), and solute accumulation (osmotic adjustment) are the major defence mechanisms responsible for drought tolerance (Farooq et al., 2009). These mechanisms function during seed embryo development and are lost during germination (Blum, 2005). The study of Liu and Stutzel (2002) have shown that vegetable amaranths defend itself by making solute accumulation (osmotic adjustment) to delay wilting and death. However, in the study of Slabber et al., (2004) mechanisms used by amaranths to cope with drought stress is the osmotic, metabolic and photosynthetic adjustment. The advantage of solute accumulation in plants including amaranths is that it assists in cell enlargement, stomata opening in the leaves, photosynthesis and lastly in plant growth (Kusaka et al., 2005). Unlike drought escape and drought avoidance where a reduction in yield is observed, in drought tolerance no solid reduction in yield is observed however it is crucial crop mechanism for dealing for with stress (Blum, 2005).

2.8. Physiological responses to water stress

2.8.1. Stomatal conductance

Stomatal conductance is known to measure the rate of diffusion of carbon dioxide (CO_2) and water vapour in and out of the leaf (Chaves et al., 2002). Plants under drought stress conditions, close their stomata to reduce transpiration water loss. The study of Modi & Mabhaudhi, (2013) have shown the closure of stomata leads to reduced gases exchange like CO_2 in the plant leaves which consequently, decreases the net photosynthesis leading to reduced plant yield. Cornic, Gabriel, and Massacci (1996) point out that the first mechanism almost every plant uses to respond to water stress is the closure of stomata. Other studies have also stated that stomatal control in the leaves has been associated with abscisic acid (ABA) signaling from drying roots. For instance, Hussain et al., (2012) reported that as the stomatal conductance reduces during a water stress condition, the ABA concentration increases in the leaf and causes slow growth in plants. Stomatal response to water stress has been assessed in different crops; vegetable amaranth (Liu and Stützel, 2002); maize (Tardieu et al., 1991); clover (Socias et al., 1997).

2.8.2. Chlorophyll content

The chlorophyll content is recognized as one of the photosynthetic pigments found in plants and responsible for absorbing energy from light. According to Gitelson et al., (2003), valuable information is provided by this pigment regarding the physiology status of plants. These pigments are very sensitive to drought stress. Anjum et al., (2003) confirmed that photosynthetic pigments such as chlorophyll content and carotenoids were affected due to water stress. Similarly, Iturbe-Ormaetxe et al., (1998) point out that typical under severe water stress, photosynthesis is inhibited, this causes a change in chlorophyll content, chlorophyll components and photosynthetic apparatus which result in reduced yield. Smirnoff (1995) point out that the reason for the decrease in chlorophyll content of different crops is caused by reactive oxygen species (ROS) which damages the chloroplasts.

2.8.3. Other mechanisms plant uses to respond to drought

Osmotic adjustment is considered as an important response that plants use for stress tolerance. The process of osmotic adjustment involves the maintenance of cell turgor by increasing the concentration of compatible solutes in the plant cells to respond to water stress (Martinez et al., 2004). Martinez et al., (2005), point out that the solutes that contribute to these processes are sugars, glycerol, proline or glycine betaine organic acids, calcium, potassium, chloride ions, etc. Farooq et al., (2009) emphasized that these solutes are not toxic even at high cytosolic concentration and are of low molecular weight. Lang (2007) reported that the osmoprotectant mechanisms only function under severe osmotic environmental conditions (severe drought stress) to helps plants cope.

Proline accumulation is considered as the first response that the plants use to cope with biotic and abiotic stresses. Its role is to reduce proteins denaturation and keep the structure and the activities of enzymes intact (Rajendrakumar et al., 1994). The other roles of proline according to Raymond and Smirnoff (2002) is that it can be used in the stabilization the macromolecules, can serve as a store of carbon and nitrogen for use after water stress has decreased. Hare et al., (1998) point out that many plants generally adapt to water stress by accumulating free proline. However, this raised questions among scientists about whether proline accumulation is an indicator of stress or is it a sign of adaptation.

Reactive oxygen species (ROS) can be defined as the agents that can damage cell components and cause them to dysfunction. Anjum et al., (2011) reported that when plants are affected by environmental stress i.e. water stress, ROS accumulate in the plants. The ROS are produced in chloroplast, mitochondria, and perisomes and causes oxidative stress. These agents are hydrogen peroxide, superoxide anion, singlet oxygen and hydroxyl radical (HO^-) (Foyer and Noctor, 2000; Chaves, Maroco, and Pereira, 2003; Brou et al., 2007). High level of ROS can cause oxidative damage, which interns will affect photosynthesis, cellular membranes and lastly it can cause cell death. Furthermore, Loka et al., (1994) also reported that proteins, carbohydrates, nucleic acids, and lipids are other cellular components can be affected by the accumulation of ROS under water stress.

The oxidative damage caused by ROS under water stress can be alleviated using antioxidants systems (Elstner, 1982). Srivalli et al., (2003) point out that these antioxidants present in the chloroplasts are enzymatic and non-enzymatic and directly detoxify ROS. The non-enzymatic antioxidants include Vitamin C, glutathione, Vitamin E, ascorbate (ASC), flavonoids and β-carotene (Gill and Tuteja, 2010) while the enzymatic antioxidant are Superoxide dismutase (SOD), Catalase (CAT) and Peroxidase (POD), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), glutathione-s-transferase (GST) (Vardharajula et al., 2011; Yadav et al., 2014). These enzymatic and non-enzymatic systems protect the cell by controlling the ROS concentration. It has been reported that amaranths contain antioxidants (polyphenols) comparable to that of soybean and rice, such antioxidant in amaranths help minimize the build-up of ROS (Pazinatto, et al., 2013).

2.9. Opportunities for neglected underutilised crop species

Neglected underutilised crop species (NUCS) are described as the crop species that receive little attention in terms of research, the cultivation of it is mainly confined to small scale farming (Chivenge et al., 2015). Their level of utilisation is generally low and their potential value is mostly underexploited and underestimated. Neglected and underutilised species are a subset of agrobiodiversity that are well known for tolerating adverse conditions such as those observed under climate variability and change, and marginal croplands (Chivenge et al., 2015; Mabhaudhi et al., 2016).

The utilisation of NUCS crops will not only ensure household food and nutrition security however it will allow farmers to use less water as water is one of the limiting factors disturbing crop production particularly in developing countries including South Africa. Annandale et al., (2011) point out that water availability in South Africa might be expected to drop below the benchmark of 1000 m^3 in a year per person in the next coming as per the date of this publication it will now be within the next 10 to 20 years. Therefore, there is a need to understand or develop modern technologies/strategies that can be used to increase crop production using limited water resources or a drop of water.

So far, strategies developed by plant researchers are to generate drought-tolerant crops and screening for high water use efficiency (WUE). Water use efficiency is defined in different ways depending on the disciplines. Generally, WUE is the amount of yield produced per unit of water used. Karatassiou et al., (2009) emphasized that WUE is an important measure between water consumption and biomass production. Previous studies of Molden et al., (2010) and Blum, (2005) point out that high yield determinant under drought stress is WUE. Conversely, Modi and Mabhaudhi, (2013) have revealed that there is a linear relationship between yield and water use by crop. Some of the NUCS and their water use and WUE have been shown in Table 2. 2.

Table 2. 2. Water use, and water use efficiency of some NUS crops and their yield (Chibarabada, et al., 2017; Johnson, Burton L, Henderson, 2002)

Species	**Amaranths**	**Chickpea**	**Cowpea**	**Bambara groundnut**	**Lentil**
Water use (mm)	143-308	150–340	78-258	300-638	160-308
Water use efficiency (WUE) (kg dry matter ha-1mm^{-1})	2.8-11.8	1.9–3.6	0.11-0.2	0.1-0.12	2.3-4.5
Yield (Kg ha^{-1})	3000-5020	358-1357	1020-1340	500-2400	339-1657

2.10. Nutritional water productivity

Nutritional Water Productivity (NWP) is a concept that was introduced by Renault and Wallander (2000). NWP has been defined as a measure of yield and the nutritional content per volume of water consumed. Chibarabada et al., (2017) point out that, NWP is a useful metric to quantify water food-nutrition nexus because, it combines the knowledge of the composition of food particularly nutrients such as protein and fats, carbohydrates with the knowledge of water productivity of food products altogether. According to Steduto et al., (2007) crop water productivity is defined as the amount of water needed to per unit total biomass produced. The concept of NWP in South Africa rarely done, therefore, limited data or research has been done to calculate the NWP of different food crops and especially in NUCS. However, few vegetable crops have been studied and those are sweet potato (Masango, 2014), spinach (Nyathi, Beletse and Van Halsema, 2012); cabbage (Jovanovic and Annandale, 1999). The purpose of this concept NWP is to promote the production of nutritious food to minimize the gap of nutrients (malnutrition) in the vulnerable South African population while using less water. This could be helping people who reside in at a household level because they suffer from lack of proteins, micronutrients and minerals deficiencies (Diskin, 1994; Baker et al., 1996; Bourne et al., 2002; Blum, 2011).

Conclusion

In conclusion, this study has shown that amaranths remain as the crop of choice for cultivation and consumption, due to its ability to adapt easily to various environmental conditions give the crop advantage to be used. Furthermore, it has been shown also that amaranths have been recognised as a nutritious crop, which means it can reduce food and nutrition insecurity in developing countries such as South Africa. Drought affects crop production. Therefore, understanding of morphological and physiological responses that are involved in amaranths under water stress conditions can allow plant breeders to select the genotypes of amaranths that are drought tolerant to increase the yield.

Chapter 3

Effect of seed position on seed quality of Amaranthus landraces

3.1. Introduction

Amaranth (*Amaranthus* spp.) is fast becoming one of the vegetables that are essentially needed for human diet and normal health and developments. It is produced in Africa, India, China, Russia, and United States (Kolodziejek, 2017). The exact production of amaranths in South Africa is not known, however, it has been reported that the produced yield (fresh leaves) have been up to 40 tons per hectare (DAFF, 2014). The low production of amaranths in South Africa is caused by lack of improved varieties of amaranths. Therefore, landraces have been used for production. Amaranth landraces are crops that have not been improved in terms of breeding, however, historically and their background are known. Moreover, farmers especially small-scale farmers face a challenge of accessing high seed quality of amaranth landraces.

Seed quality can be defined using the quality components which include the genetic quality (varietal purity), physical quality (purity and moisture content), physiological (germination capacity, viability, and vigour), pathological quality (seed health) (Tripp and Louwaars, 1997; Bishaw et al., 2012). According to Odindo (2007), physiological qualities such as viability and vigor are the most crucial indicators of seed quality because they are essential properties of the seed. Germination capacity is the indicator of the seed's ability to develop into a normal plant. Whereas, viability is a measure of how many seeds are alive and could produce normal seedling under favorable conditions. However, seed vigor is defined as the ability of the seed to germinate and emerge in the field or green-house rapidly and uniformly under diverse environmental conditions (Ventura et al., 2012). Elias (2006) points out that high-quality seeds are very vital because they can produce uniform, rapid and excessive yielding plants under different field conditions. Likewise, Penaloza et al., (2005) and Elias (2006) added that high-quality seeds also ensure the good seedling establishment and optimum plant population.

The seed quality can be affected by several factors, for instance, climatic factors (such as water availability, light, temperature, rainfall, wind, etc.) and variation between and within plants (Perez-Rea and Antezana-Gomez, 2017). Factors affecting variation within the plants include the position of the seed, nutrient supply in the parent plant and the maturity of the plant. A number of studies have been conducted on nutrient supply and age of the mother plant (Modi, 2007; Mao et al., 2014; Paneru et al., 2017). However, there is very little knowledge or studies done about the effect of the position on the mother plant on seed quality and performance of progeny. Though, there have been studies done on different crops such as carrot (Thomas et al.,1979), parsnip (Gray, Steckel and Ward, 1985), lettuce (Smith et al., 1973), Florence fennel (Thomas, 1994). However, it still remains unclear whether germination and vigour in seeds are influenced by the position of the seeds on the mother plants of amaranths. Moreover, the effect of seed position in different parts of the plants has not been studied extensively in amaranths landraces in South Africa. Therefore, it is vital to evaluate amaranth seed quality before they are planted in the field. In this study, it was hypothesized that seed position of amaranths has no influence on seed quality. Therefore, this research was undertaken to determine the effect of seed positions of two amaranths landraces on seed quality.

3.2. Method and materials

3.2.1. Plant material

Seeds of amaranths landraces (landrace A (orange) and landrace B (green)) were used in this study. Both Amaranth landraces were sourced from three locations from local farmers in KwaZulu-Natal (KZN) namely; (Umbumbulu (UMB), Swayimane (SWA) and Kokstad (KOK). The seeds were collected from the top third, middle third and bottom third in each branch per plant. In each subgroup, seeds were shelled into a labeled container (landrace A (Umbumbulu) Top third; landrace A (Umbumbulu) middle third; landrace A (Umbumbulu) bottom third; landrace B (Umbumbulu) Top third; etc).

Figure 3. 1: Positions where seeds were collected (A) and the landraces that were collected from different sites (B).

3.2.2. Site description

The study was conducted in the seed technology laboratory at the University of KwaZulu-Natal (UKZN) Pietermaritzburg. (29°37'12"S; 30°23'49"E).

3.2.3. Experimental design

The experimental design used was a factorial experiment laid out in a randomized complete block design (RCBD), replicated three times. Sites (Umbumbulu (UMB), Kokstad (KOK), and Swayimani (SWA)), landraces (landrace A, landrace B); and position (top third, middle third and bottom third) were the factors. The treatment structure was 3*2*3 replicated three times.

The seed tests included the water activity test, moisture content test, seed mass per species, length per part, seed mass per part, 1000 seed mass per sample and standard germination test.

3.2.4. Determination of seed water activity and seed moisture content

Seed water activity and moisture content were determined for each of the three groups (top, middle, and bottom) using aqua lab (CX2) water activity meter Decagon Devices, Inc.and use an intelligent grain moisture meter (KM – 21G) (ISTA, 1985).

$$SWC = \frac{Fw - Dw}{Fw} \qquad \text{(Equation 3.1)}$$

Where: SWC = seed water content (g water^{-1} g seed)

Fw = Fresh weight

Dw = Dry weight

3.2.5. Determination of seed mass per species, length per part, seed mass per part and 1000 seed mass per sample

Seed mass per species and seed mass per part was measured using a digital precision balance (RADWAG WTC 2000). For 1000 seed mass per sample, seeds were counted and weighed. Length per part was measured using a ruler.

3.2.6. Standard germination test

Twenty-five seeds per sample were collected. The seeds were placed in a standard petri dish on two pieces of moistened filter paper and incubated in a germination chamber set at 25-30 ^{0}C for 14 days for standard germination (AOSA, 1992). Germination was assessed daily by counting seeds that show the radicle protrusion for up to 12 days. Final germination recording was on the 14th day and be based on the normal and abnormal seedling (ISTA, 1966).

3.3. Description of statistical analysis

Data collected in this study were subjected to analysis of variance (ANOVA), GenStat 14th edition. Means of significant different variables were separated using LSD at 5% probability levels (Steel, Torrie, and Dickey, 1980)

3.4. Results and discussion

3.4.1. Seed mass per landrace

The results of seed mass per landrace were significantly ($P<0.001$) affected by site, landraces, and interactions between site and landraces. The mean values between the interactions showed that in landrace A, seed mass was higher at KOK followed by seeds produced at SWA and UMB. However, in landrace B, seeds produced at SWA had the highest seed mass followed by seeds produced at UMB and KOK (Figure 3. 2). This finding corroborates the study of Kolodziejek (2017), these differences could be caused by differences in environmental conditions and soil fertility which influences the genetic makeup of species.

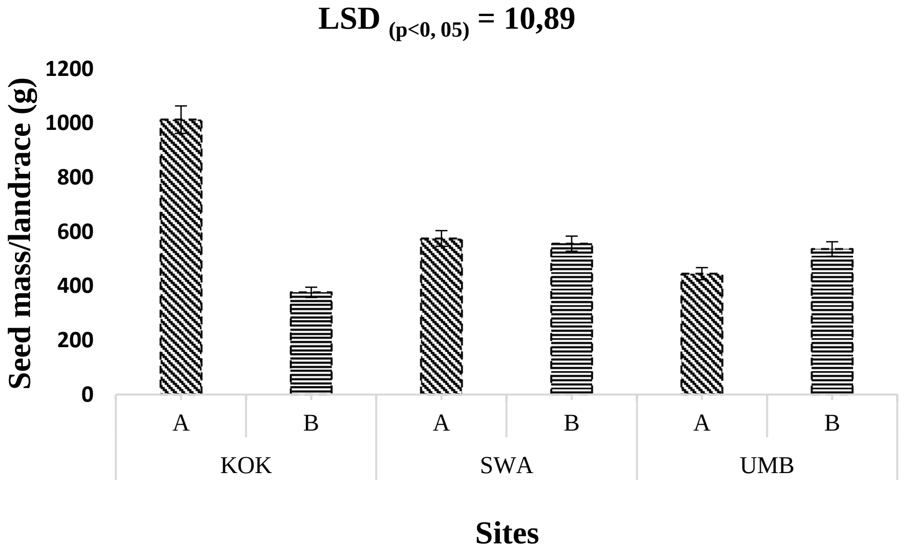

Figure 3. 2: Seed mass of amaranth landraces (A and B) produced at different sites (KOK-Kokstad, SWA-Swayimane, and UMB-Umbumbulu)

Table 3. 1. The interaction between site, landraces, and position of seeds on seed quality parameters of amaranths landraces

Site	**Landraces**	**Position of seeds**	**Length per part (cm)**	**Seed mass/ part (g)**	**1000 seed weight (g)**	**Seed water content %**	**Water activity (a_w)**
Swayimane	**Landrace A**	**Top**	20.67	168.3	2.110	10.59	0.696
		Middle	17.33	153.3	1.997	5.98	0.639
		bottom	11.67	181.7	2.113	9.35	0.513
	Landrace B	**Top**	14.00	141.7	2.293	10.08	0.629
		Middle	13.00	153.3	1.790	9.03	0.604
		bottom	10.67	218.3	2.107	8.71	0.651
Kokstad	**Landrace A**	**Top**	19.00	421.0	2.040	7.56	0.715
		Middle	15.00	322.7	2.107	10.77	0.678
		bottom	12.87	282.7	2.08	3.92	0.625
	Landrace B	**Top**	18.00	131.7	1.353	9.80	0.629
		Middle	11.33	120.0	1.730	5.87	0.604
		bottom	10.67	143.3	1.833	10.01	0.651
Umbululu	**Landrace A**	**Top**	15.00	195.0	2.177	8.25	0.459
		Middle	15.67	265.0	2.293	8.21	0.462
		bottom	12.33	211.7	2.380	6.38	0.515
	Landrace B	**Top**	16.67	143.3	1.920	9.88	0.582
		Middle	15.00	158.3	1.747	10.08	0.594
		bottom	13.00	257.0	1.930	10.82	0.596
Mean			**14.55**	**203.8**	**1.995**	**8.63**	**0.611**
LSD $_{(p<0.05)}$			**4.832**^{NS}	**12.46****	**0.2900**^{NS}	**3.618***	**0.1593**^{NS}

*, ** and NS indicating significant differences at 5%, 1%, and no significant differences, respectively

3.4.2. Length per part

Length per part of landraces differed significantly (P<0.001) in the landraces, and position of seed. However, regarding the site and the interactions, there were no significant differences (P>0.05). The mean values between the interactions showed that in landrace A, higher length per portion was observed in SWA at the top position and the lowest also was observed in SWA at the bottom positions. However, in landrace B, the highest length per portion was observed in KOK at the top position and the lowest length per part was observed at the bottom position in both KOK and SWA (Table 3. 1).

3.4.3. Seed mass per part

Regarding seed mass per part, significant differences (P<0.001) were observed in a site, landraces, and position. There was a highly significant difference (P<0.001) between the interactions site x position; landraces x position; site x landraces x position. Mean separation showed that in landrace A, seed mass per part was higher in seeds produced in the top position (421 g) at KOK, however, the lowest seed mass per part was observed in the middle position (153.3 g) at SWA. On the other hand, in landrace B, seeds produced at UMB in the bottom position (257 g) had higher seed mass per part, however, the lowest seed mass per part was observed in the middle position (120 g) at KOK (Table 3. 1). This could be caused by differences in resources allocation within the species. Moreover, Harper et al., (1970), alluded that there is competition for available nutrients in the inflorescences of plants. Therefore, this means that top positions were more dominant compared to bottom positions seeds in a landrace A and in landrace B, bottom position seeds were able to compete better to photoassimilates.

3.4.4. Thousand seed weight

The results of thousand seed weight differed significantly (P<0.001) in sites, landraces, however, the position was not significantly different. There was a significant difference (P< 0.05) in interactions between site, landraces, and landraces, position. Mean separation showed that in landrace A, seeds produced at UMB in the top position (2.38 g) had higher 1000 seed weight, however, the lowest 1000 seed weight was observed in seeds produced SWA in the middle position (~2.00 g). On the other hand, in landrace B, seeds produced at SWA in the top position (2.29 g) had higher 1000 seed weight, interestingly, the lowest 1000 seed weight was observed in the top position (1.35 g) in the seeds produced at KOK (Table 3. 1). Overall, landrace A had higher seed weight compared to landrace B. The results of TSW concurred with the previous reports by Smiciklas et al., (1992) in soybean and panayoto (2010), in carrots that higher seed and ambels mass were found at the top and middle portion compared to the bottom portion. This suggests that higher rate of the partitioning of assimilates from sink and source ratios during growth was more in the top position as it had higher seed size (weight) compared to the middle and bottom position which had smaller seed size (weight).

3.4.5. Seed water content

The seed water content was showed no significant differences ($P > 0.05$) in sites and position. However, significant differences in terms of seed water content were observed between landraces. Landrace A (7.89 %) had lower seed water content compared to landrace B (9.36%) (Figure 3. 3). The variation between species of the same family in seed water content based on the position of seed or fruit has also been reported by Alan (2007). Regardless of the fact that in most of the interactions there were no significant differences with respect to seed water content, however, between sites, landraces and position there were significant differences ($p < 0.05$). In landrace A, seeds produced at KOK in the middle position (10.77 %) had higher seed water content, however, the lowest seed water content was observed in the bottom position (3.92 %) also at KOK. whereas in landrace B, highest seed water content was observed in seeds produced at UMB in the bottom position (10.01 %), however, the lowest seed water content was observed in the middle position (5.87 %) at KOK (Table 3. 1). Odindo (2007), alluded that seed water content may differ because of differences in stage of physiological development. Hence, suggesting that bottom and middle position seeds were more mature compared to top position seeds.

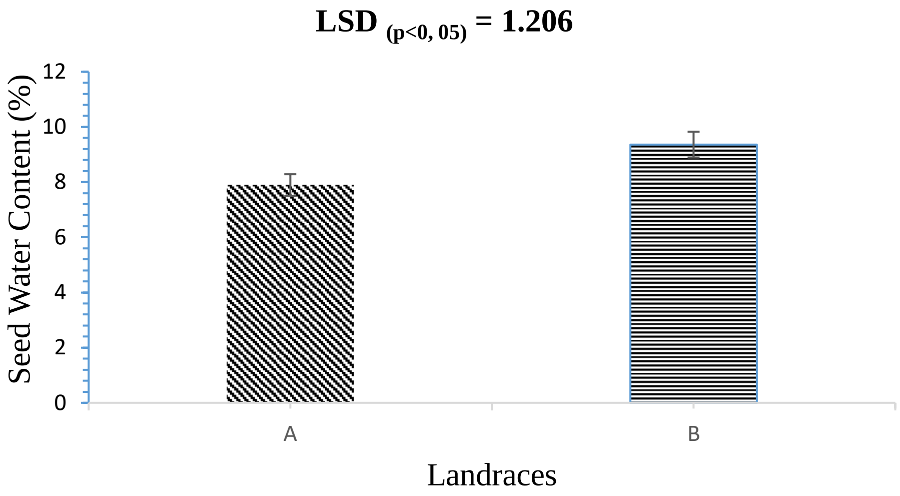

Figure 3. 3: Seed water content of amaranth landraces (A and B)

3.4.6. Water activity

Water activity measures the availability of water to participate in biological, chemical and physical processes. It can be used to determine if the moisture in the seed is at a level safe for storage. The current results showed that water activity had significant ($p<0.05$) difference in sites, however, in landraces, positions significant ($P>0.05$) differences were not observed. The site, KOK produced seeds with high (0.0677) water activity followed by SWA (0.622) lastly UMB (0.535) (Figure 3. 4). The interactions also were not significantly ($P>0.05$), however, based on mean values, in landrace A, seeds produced at KOK in the top position (0.715) had higher water activity, and likewise, the lowest water activity was observed in the top position (0.459) however at UMB. Whereas in landrace B, highest water activity was observed in seeds produced at UMB and SWA in the bottom position (0.651), however, the lowest seed water content was observed in the top position (0.582) at UMB (Table 3. 1). These results suggest that water activity was at a safe level for storage.

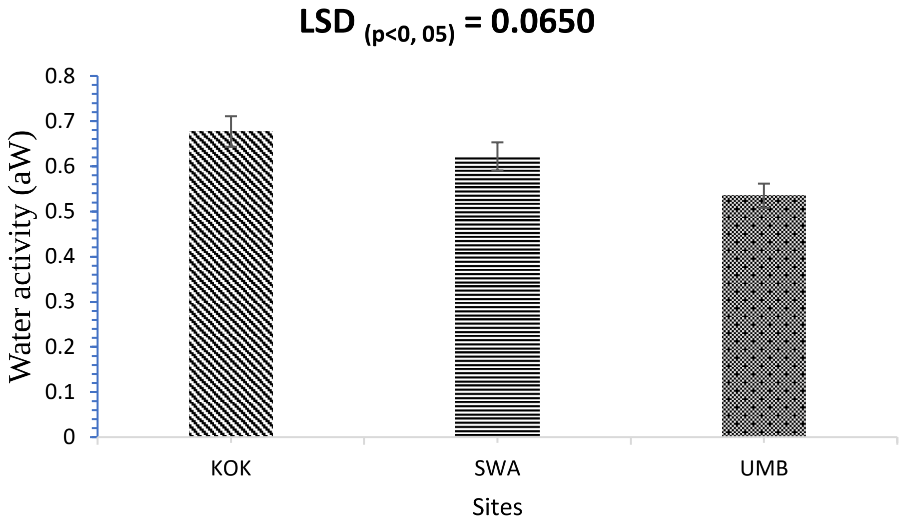

Figure 3. 4: Water activity of amaranth landraces based on site

3.4.7. Germination

There were highly significant differences ($p < 0.001$) in position, landrace, site and time (days) with respect to germination. The interaction significantly ($p<0.05$) differed between day and landrace, Landrace A germinated higher over time compared to landrace B (Figure 3. 5).

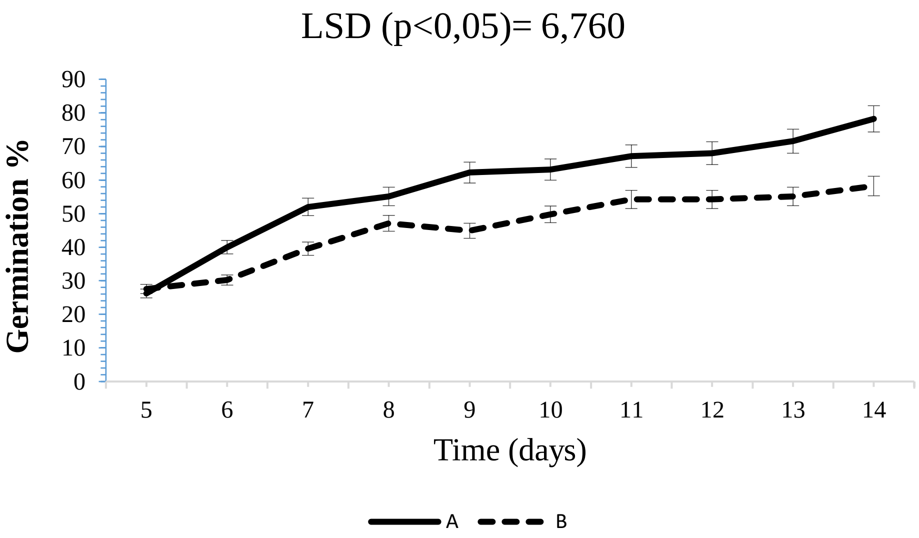

Figure 3. 5: Germination percentage (%) of amaranths landraces (A and B) over time (days)

The interactions between position, landrace, and site were also highly significant ($p<0.001$), Based on mean values, in landrace A, seeds produced at SWA in the bottom position (84%)

had higher germination %, while the lowest seed germination % was observed in the top position at KOK. However, in landrace B, the highest germination % was observed in the seeds produced at UMB in the middle position (65.6%), while the lowest seed germination % was observed from the bottom position at UMB (Figure 3. 6). These findings of the current study do not support the previous research reported by Jha (2010), where it was found that seed from the top followed by middle germinated higher than the bottom seeds in palm amaranth. However, it should be reported that this study also evaluated the effect of shading the mother plant, therefore this might have an influence on the germination. Higher germination % between seeds may be attributed to the size of seed and seed coat thickness and permeability (Beninger et al., 1998). Thereby, middle and bottom seeds had smaller weight and thinner seeds coat, which implies that they had a higher surface area to volume ratio (Fowler and Bianchetti, 2000). Therefore, seeds had relatively higher ability to absorb water and germination at a faster rate (Dolan, 1984). While, top position seeds due to their heaviness in seed weight, they accumulated higher resources and store it in the seeds, as a result, they had a lower ability to absorb water for germination at a faster rate. Moreover, Seeds from the UMB and SWA compared to KOK. This implies that differences in ecological conditions and the genetic characteristics of individual landrace have an influence on germination %. Other factors that influence differences in germination according to Gray and Thomas (1982) are seed development, maturation, and harvesting. Surprisingly, there was a correlation between seed water content and germination %, this suggests that seed water content can be used to predict seed viability.

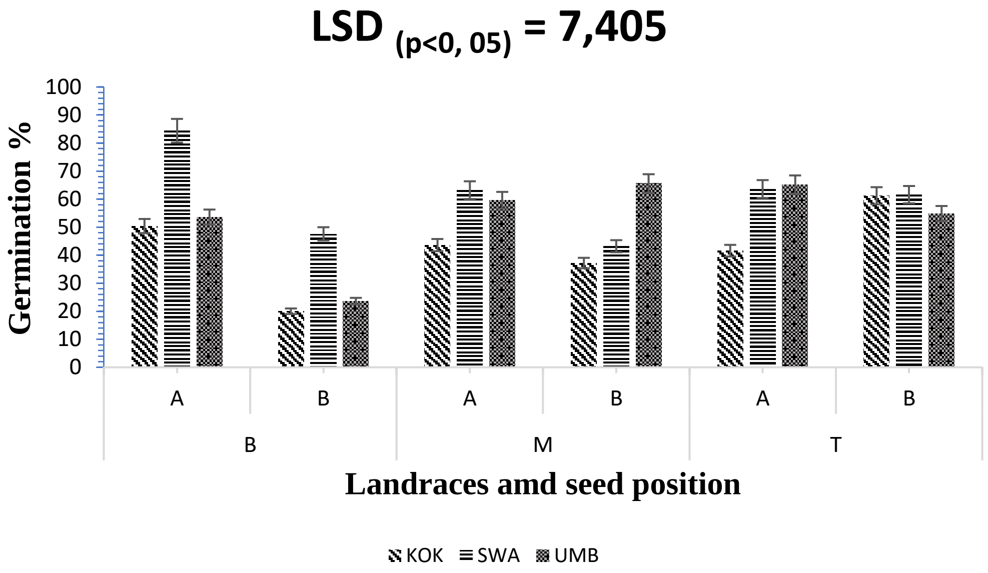

Figure 3. 6: Germination percentage of amaranths (A and B) based of seed positions (Bottom, Middle, and Top) from different sites (Swayimane (SWA), Kokstad (KOK) and Umbumbulu (UMB).

3.5. Conclusion

The present study indicated seed position has an influence on seed quality, aspect of seed quality investigated include seed mass per landraces, seed mass per part, SWC, seed water content, water activity, and germination. Bottom and middle position seeds showed high seed water content and germination %. This suggests that bottom and middle position seeds from SWA and UMB had high seed qualities compared to top position seeds from KOK. Landrace A had higher seed quality compared with landrace B. Hence, it can be suggested that small scale farmers should harvest seeds in the bottom and middle positions seed for higher seed quality depending on the landraces.

Chapter 4

Effect of water stress on amaranth crop morphology and physiology based on seed position on the plant

4.1. Introduction

Food security is a condition related to whether all individuals at all-time have access physically and economically, to safe, enough and nutritious food for active and healthy life (FAO, 1996; Pérez-Escamilla, 2017). In Sub Saharan Africa, about 239 million people are reported to suffer from micronutrient deficiency particularly iron (Fe), iodine, zinc (Zn) and vitamin A (Smuts et al., 2004; Muhanji et al., 2011; AfariSefa et al., 2012; Graef et al., 2014; Njume et al., 2014). Regardless of the fact that South Africa is considered to be a food secure country an estimate of 28.3 % of the population are at risk of hunger and 26 % of are experiencing it and others, (Shisana et al.,2014). Labadarios et al., (2003), reported that South African children suffer most from vitamin A, calcium, iron, zinc, folate, vitamin B6, niacin, riboflavin, vitamin C and vitamin E deficiencies and this effect is mostly observed in rural areas. One of the reasons that families residing in part of South Africa are food and nutritional insecure to Savy et al., (2005), is that they tend to depend on food that contains a lot of starch while ignoring the food that contains protein and other nutrients in their diets.

Amaranths are one the most important vegetable crop that is known to have high protein quality (16% to 18%) compared to other cereal (Becker et al., 1981; Akin-Idowu et al., 2013). The minerals found in amaranths such as calcium, iron, phosphorus, and magnesium are higher than those found in animal products such as meat and milk. Furthermore, folate (B9), riboflavin (B2), niacin (B3), vitamin A, B, C, and E have also been reported in amaranths (FAO 2004). Amaranths originated in Central and South America. Its cultivation dates back to 5,000 to 7,000 years ago in South America and is usually consumed as both grain and leafy vegetable worldwide (Smitha, 2010). Based on the aforementioned potential of the crop, amaranth can be regarded as one of the crops that can be used to alleviate food insecurity. Moreover, the crop can compete with commercial crops. However, there are several factors that affect its production, these include drought and seed position on the parent plant.

Generally, drought is a shortage of water for the plant to grow and develop. The occurrence of drought will carry on increasing due to global climate change and drought is one of the most prevalent factors that limit food production (Bray, 1997; Dai, 2013). On the other hand, the positional effect in seeds is also one of the factors that can cause within variation in yields and performance. Several studies have reported the effect of seed position on different crops (Thomas et al., 1977; Rocha and Stephenson, 1990; Botwright et al., 2001; Baydar and Erbas, 2005; Alan and Eser, 2007; Donath and Eckstein, 2010), however, few have linked it with drought stress. For example, the study done by Ghassemi-Golezani (2010) reported that there was no significant interaction between seed position and water stress (water regimes). Moreover, most of the researchers tend to focus on the seed quality (germination and vigour) and forget about crop growth and its performance.

A number of researchers have reported that the functioning of physiological, biochemical and morphological (growth) processes in crops including amaranths are triggered by drought stress (Fischer and Maurer, 1978; Ludlow and Muchow, 1990). The effect of water stress physiologically, is that it affects the translocation of nutrients and metabolites. Hall et al., (1990) point out that photosynthesis, gas exchange and respiration of crops are also affected by drought stress. Furthermore, according to Šircelj et al., (2005), the metabolism of organic compounds, proteins, amino acids and carbohydrates changes due to water stress. Morphological features such as plant height, leaf area, and leaf number are reduced due to drought (Specht et al., 2001; Wullschleger et al., 2005; Wu et al., 2008). These processes result in plant growth and productivity being reduced. For example, it has been reported that yield in amaranths species decreased due to water stress (Mng'omba et al., 2003; Olufolaji et al., 2010). However, the major issue is increasing food production and nutrient density under limited resource availability particularly under drought conditions.

Amaranths are also known as the crop that has high adaptability to tolerate adverse climatic conditions such as drought, high temperature, and light intensities but yield is negatively influenced. Furthermore, it also performs well in poor soils (Jacobsen et al., 2003; Pulvento et al., 2015). Although Amaranths is considered as a drought-tolerant crop, there is little known about the effect of drought on growth, physiological parameters (stomatal conductance and chlorophyll content), and yield responses based on seed position of the parent plant. Additionally, mechanisms of tolerance used by amaranth landraces to respond to drought are also limited. In this study, it was hypothesized that drought stress will not have any effect on the growth of amaranths landraces-based position of the parent plant. Therefore, the study was conducted to evaluate the effect of drought stress on morphological, physiological, and yield responses of amaranths landraces based on seed position.

4.2. Method and materials

4.2.1. Site description

The experiment was conducted in pots (5 L) in a temperature-controlled tunnel located at the Controlled Environment Facility (CEF), University of KwaZulu-Natal, South Africa Pietermaritzburg Campus (29° 35' S, 30° 25' E).

4.2.2. Plant material

The plant material used in this study is described in detail in chapter 3 in Figure 3.1.

4.2.3. Experimental design, potting procedure, and water stress treatments

The experiment was laid out using factorial design arranged in a randomised completely block design (RCBD) with the treatment structure of (3 x 2 x 3 x 3). Sites (Umbumbulu (UMB), Kokstad (KOK), and Swayimani (SWA)), landraces (landrace A, landrace B); position (top third, middle third and bottom third) and water stress (three levels- 30% FC (severe), 50% FC (moderate) and 100% FC (control)) were the factors replicated 3 times.

The soil used in the experiment was collected from the University of KwaZulu-Natal Research Farm (Ukulinga). Seeds were firstly grown in trays for 3 weeks and later transplanted into pots based on their positions. Water stress treatments were done according to Muchero et al., (2008). To measure field capacity, pots were saturated and allowed to drain freely for 12 hours, until a constant weight was reached. Thereafter, the mass of pots was weighed again, hence, it was measured using the gravimetric method (Kramer, 1983) (Equation 3.2). One week after transplanting, all pots were well watered (100% FC) then after 7 days, water was withheld to reach 50% FC and 30% FC. The soil moisture was monitored using theta probe connected to an HH2 handheld moisture meter (Delta-T, UK), at weekly interval. Stressed plants were then re-watered to the level of the well-watered. Water use was measured by recording the amount of water added when watering pots.

$$\theta m = \left(\frac{\theta wm - \theta dm}{\theta dm}\right) x\ 100 \qquad \text{(Equation 3.2)}$$

Where: θm = gravimetric field capacity,

θwm = wet mass of soil (g) and

θdm = dry mass of soil (g)

4.3. Crop Management

Avi-Gard insecticide® was sprayed at recommended rates (1 mL L -1) to control insects 3 weeks after planting. Fertilizer application was based on the results of soil analysis.

4.4. Data collection

4.4.1 Determination of growth parameters

Plant height and leaf number, stomatal conductance, chlorophyll content and leaf area index/PAR assessed weekly until flowering. Plant height was measured using a ruler from the base of the stem to the apical bud. Leaf number determined by counting the number of leaves that have at least 50% green leaf area. Chlorophyll content was measured using CCM-200 *Plus* (Optisciences, USA). While stomatal conductance was measured using a steady-state leaf porometer (Model SC-1, Decagon Devices, USA). Leaf area was measured using the methods as described by Pearcy et al., (1989). Above fresh biomass (g plant^{-1}) was measured at harvest.

$$Leaf\ Area = 0.5\ (length\ x\ width\ (of\ leaf)) \qquad \text{(Equation 3.3)}$$

4.5. Description of statistical analysis

Data collected in the study were subjected to analysis of variance (ANOVA), GenStat 14th edition. Means of significant different variables were separated using LSD at 5% probability levels (Steel, Torrie, and Dickey, 1980).

4.5. Results

Morphological parameter

4.5.1. Plant height

Plant height showed a significant ($p<0.05$) effect on site, position, water regimes and time (weeks). The tallest landraces recorded was (~15 cm) from the bottom position and the shortest landrace recorded was (~12 cm) (Figure 4. 1)Figure 4. 1: Plant height based on seed position (B-Bottom, M-Middle, and T-Top). The interaction between site, landraces, and position and water regimes had also a significant ($p<0.001$) effect on plant height. The pattern of plant height was consistently higher in landraces watered at 100%FC (unstressed treatments) compared to landraces watered with 50%FC (moderate stressed) and 30%FC (severe stressed (Figure 4. 2). Tallest plant height (~25 cm) and (~15 cm) in both 100%FC (unstressed treatments) and

50%FC (moderate stress) water regimes were observed from the seeds collected at UMB from the middle in Landrace A, respectively, while the lowest plant height was observed from the seeds collected at KOK from the top position (~10 cm) (unstressed treatments) and SWA from the middle position (~9 cm) in both landrace B. However, under severe stress, tallest plant height was observed from the seeds collected at UMB from the bottom (~15 cm) in landrace A while the lowest was observed from the seeds collected at KOK from the bottom position (~8 cm) in landrace A (Figure 4. 3).

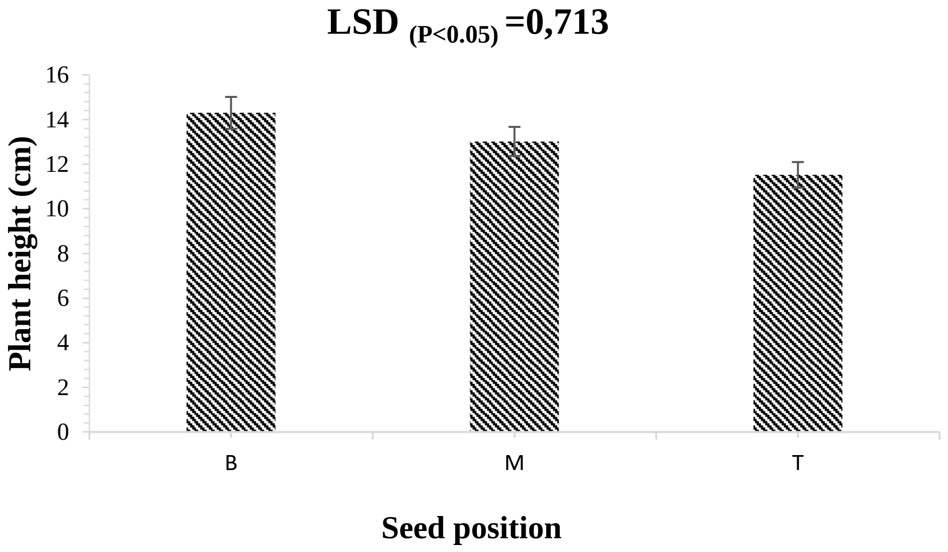

Figure 4. 1: Plant height based on seed position (B-Bottom, M-Middle, and T-Top).

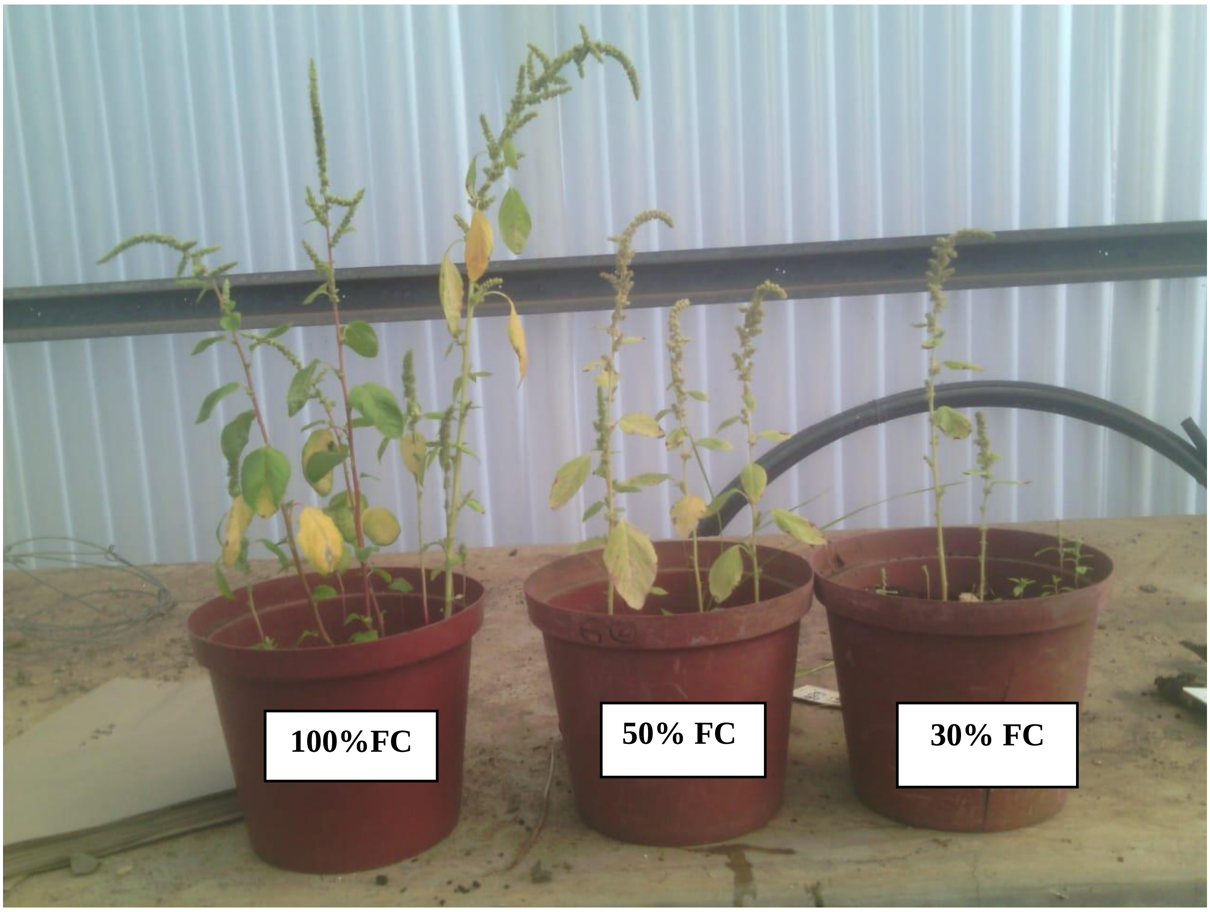

Figure 4. 2: Amaranths landraces under different water regimes.

LSD $_{(P<0.05)}$ **=1.746**

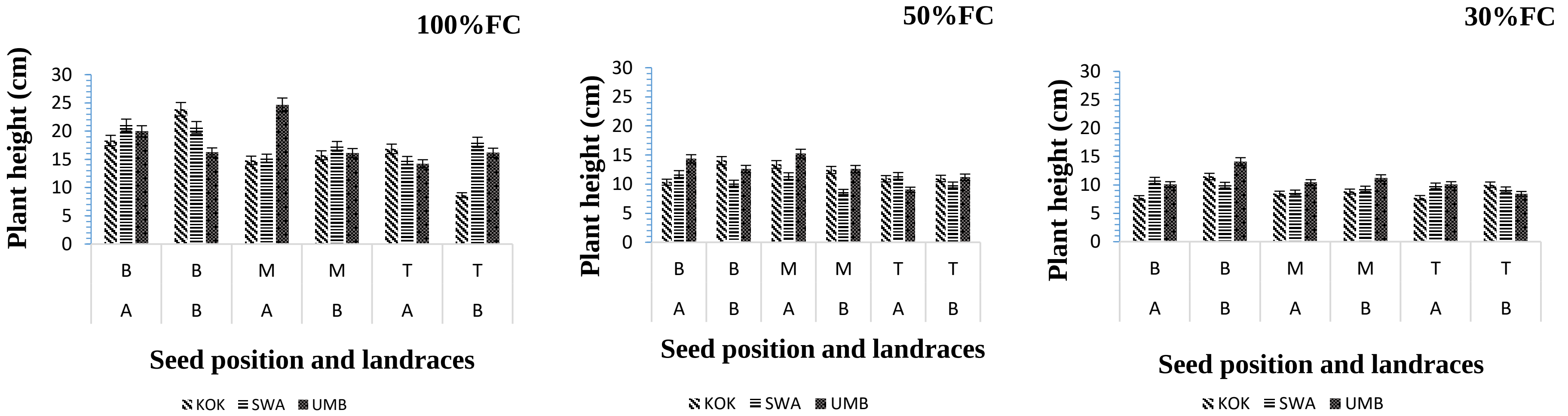

Figure 4. 3: Plant height of amaranths landraces (landrace A and B) from different sites (KOK-Kokstad, SWA-Swayimane, and UMB-Umbumbulu) based on seed position (B-Bottom, M-Middle, and T-Top) under different water regimes (100%FC, 50%FC, and 30%FC).

4.5.2. Leaf number

The results of leaf number showed that there is a significant (p<0.05) difference among site, landraces, position, and water regimes and time. The interactions between site, landraces, and position and water regimes had no significant (p>0.05) effect on leaf number. However, the interactions between (water regimes x position), (water regimes x landraces x position) and (site x landrace x position) had significant (p<0.05) effect on leaf number. The pattern of leaf number was such that treatments watered at 100%FC had the highest leaf number compared to landraces watered at 50%FC and 30%FC, 100%FC>50%FC>30%FC (Figure 4. 4 and Figure 4. 5). Based on the interaction between seed position and water regimes, as expected seed from the 100%FC (unstressed treatments) had the highest leaf number compared to moderate and severe stress plant. Middle and bottom positions seeds had relatively similar leaf number which is high compared to top position seeds (Figure 4. 4)Figure 4. 6.

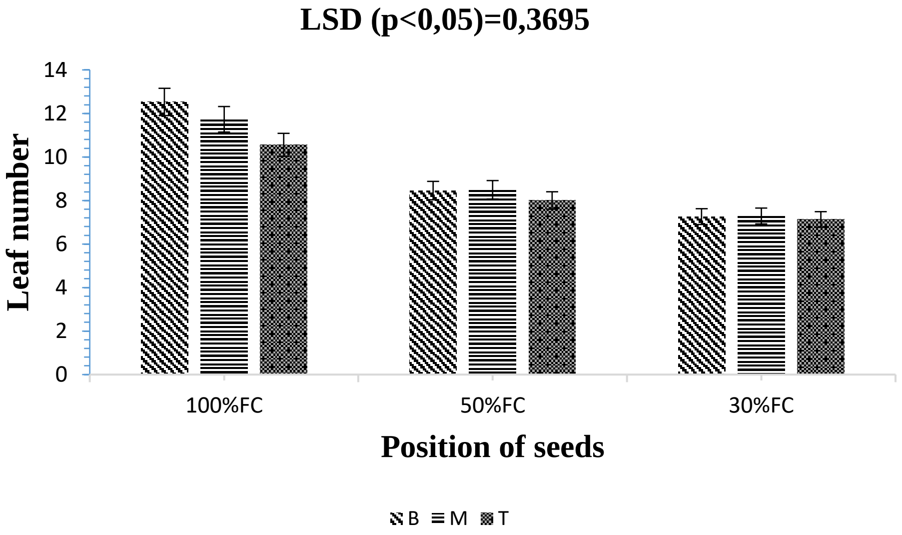

Figure 4. 4: Leaf number based on seed position (B-Bottom, M-Middle, and T-Top) under different water regimes (100%FC, 50%FC, and 30%FC).

Based on the interaction between the water regimes, landrace, and position, under both moderate and severe stress, middle position seeds from landrace A had the highest leaf number (9) and (8), however, the lowest leaf number was observed from the middle position (7) and top position (6) both from landrace B, respectively (Figure 4. 5). Moreover, the results of the interaction between site, landraces, and position, showed that the seeds collected at UMB from the middle in Landrace A had the highest leaf number while the lowest leaf number was observed from the seeds collected at KOK from the top position in landrace B (Figure 4. 6).

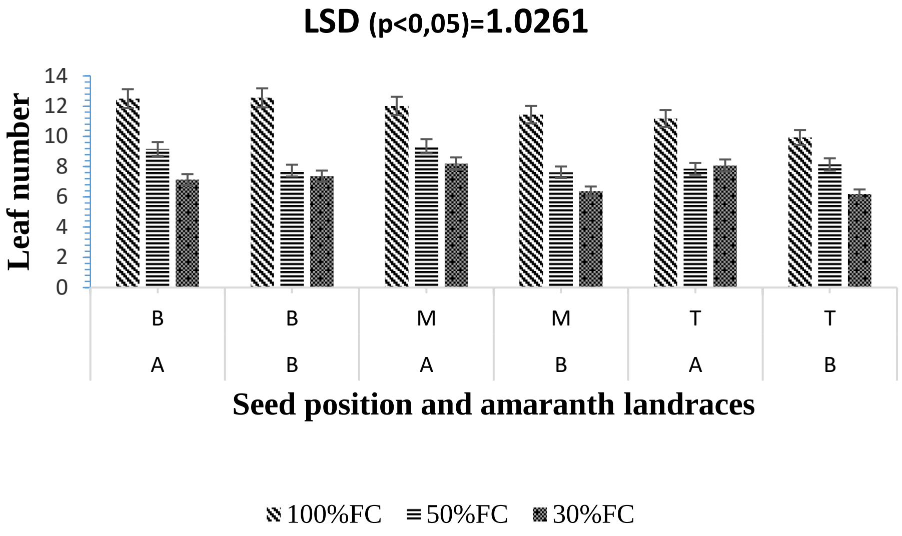

Figure 4. 5: Leaf number of amaranths landraces (landrace A and B) based on seed position (B-Bottom, M-Middle, and T-Top) under different water regimes (100%FC, 50%FC, and 30%FC).

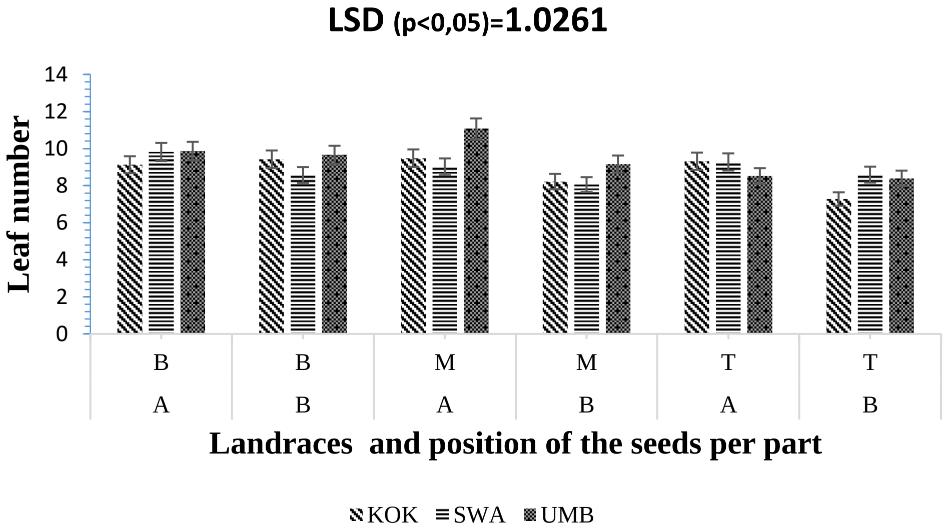

Figure 4. 6: Leaf number of amaranths landrace (Landrace A and B) based on seed position (B-Bottom, M-Middle, and T-Top).and site (KOK-Kokstad, SWA-Swayimane, and UMB-Umbumbulu).

4.5.3. Leaf area

There were significant ($P<0.05$) effect of sites, water regimes, position and time (weeks) with respect to leaf area. The biggest leaf area (~65 mm^2) was observed from plants that originated from seed at the bottom while the smallest leaf area (48.69~65 mm^2) was observed in the top position. The interaction between site, landraces, and position and water regimes had also significant ($P<0.05$) effect on leaf area. The trend in water regimes was consistent, 100%FC had higher leaf area compared to both 50%FC and 30%FC (Figure 4. 7). Seeds collected at SWA and UMB from the bottom position in landrace A had the highest leaf area (~60 mm^2) under moderate stress, while the lowest leaf area was observed from the seeds collected at SWA from the middle position (~33 mm^2) in landrace B. However, under severe stress conditions, seeds collected at UMB from the middle position (~54 mm^2) in landrace A had the highest leaf area while the lowest was observed from the seeds collected at UMB from the bottom position (~29 mm^2) in landrace A (Figure 4. 8).

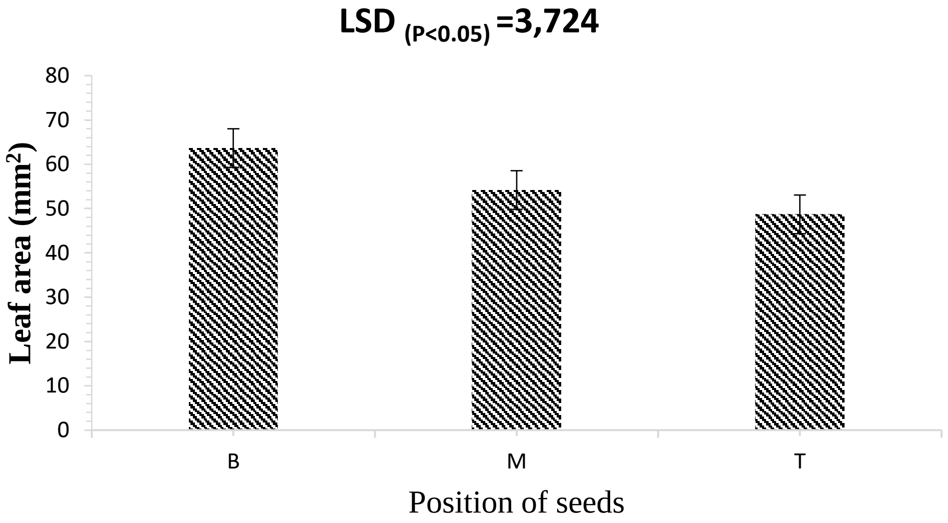

Figure 4. 7: Leaf area based on seed position (B-Bottom, M-Middle, and T-Top).

LSD $_{(P<0.05)}$ = 9.122

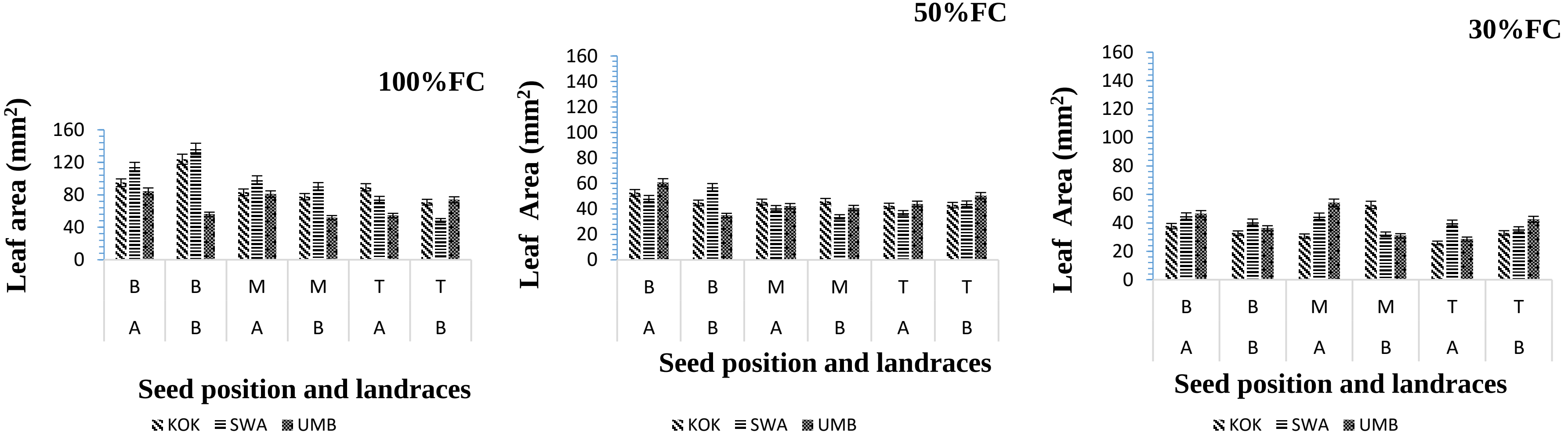

Figure 4. 8: Leaf area of amaranths landraces (landrace A and B) from different sites (KOK-Kokstad, SWA-Swayimane, and UMB-Umbumbulu) based on seed position (B-Bottom, M-Middle, and T-Top) under different water regimes (100%FC, 50%FC, and 30%FC).

4.5.4. Chlorophyll content

Results show that water regimes, time (weeks) and the interaction between the two, were the only one to have a significant effect ($p<0.05$) on chlorophyll content index. The chlorophyll content index was fluctuating over time, the maximum chlorophyll content recorded was (~35 ccl) during week 3 and the minimum was (~30 ccl) during week 1 (Figure 4. 9). With respect to water regimes, chlorophyll content was maximum at 100%FC with the value of (~40 ccl) compared to 50%FC and 30%FC with the values of (~30 ccl) and (~26 ccl), respectively (Figure 4. 10).

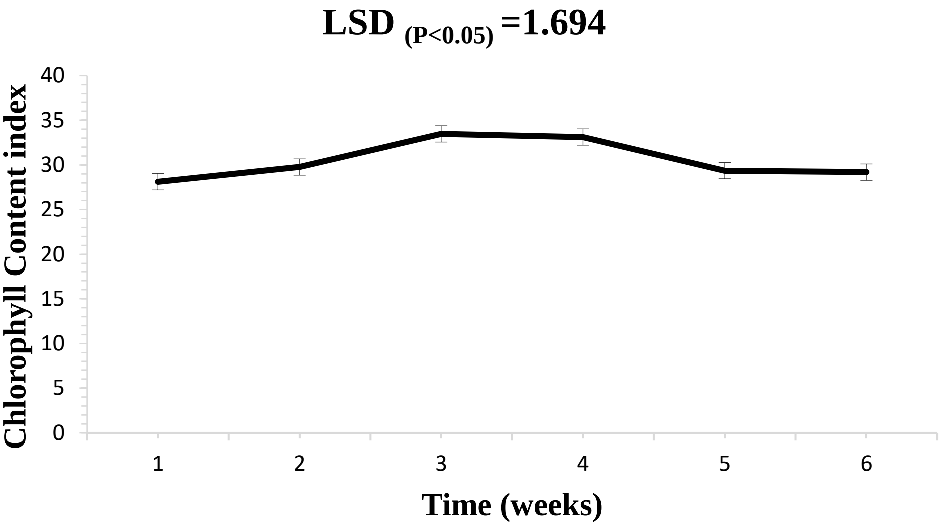

Figure 4. 9: Chlorophyll content index (CCI) over time (weeks).

Sites, landraces, the position of seeds and their interactions were statistically similar ($p>0.05$) on chlorophyll content index. However, based on the observed results, with respect to stress conditions, In 100%FC (unstressed treatments), highest ccl was observed from seeds collected at SWA and UMB from the top position (41 ccl) in landrace B while lowest (31 ccl) was recorded from the seeds collected at UMB in the bottom position in landrace B. however, in both moderate and severe stress conditions, highest ccl was observed from seeds collected SWA and UMB from the bottom position (32 ccl) in landrace B and middle position (34 ccl) in landrace A, while the lowest ccl was recorded from the seeds collected at KOK and SWA from the top position (28 ccl)in landrace B and from the bottom position (21 ccl) in landrace A, respectively (Figure 4. 11).

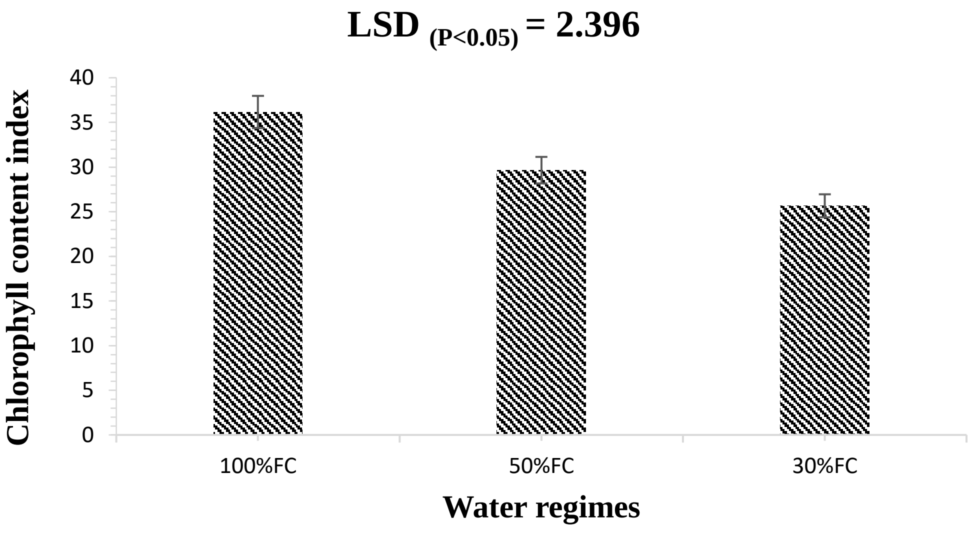

Figure 4. 10: Chlorophyll content index under different water regime (100%FC, 50%FC, and 30%FC).

LSD $_{(P<0.05)}$ = 4.149

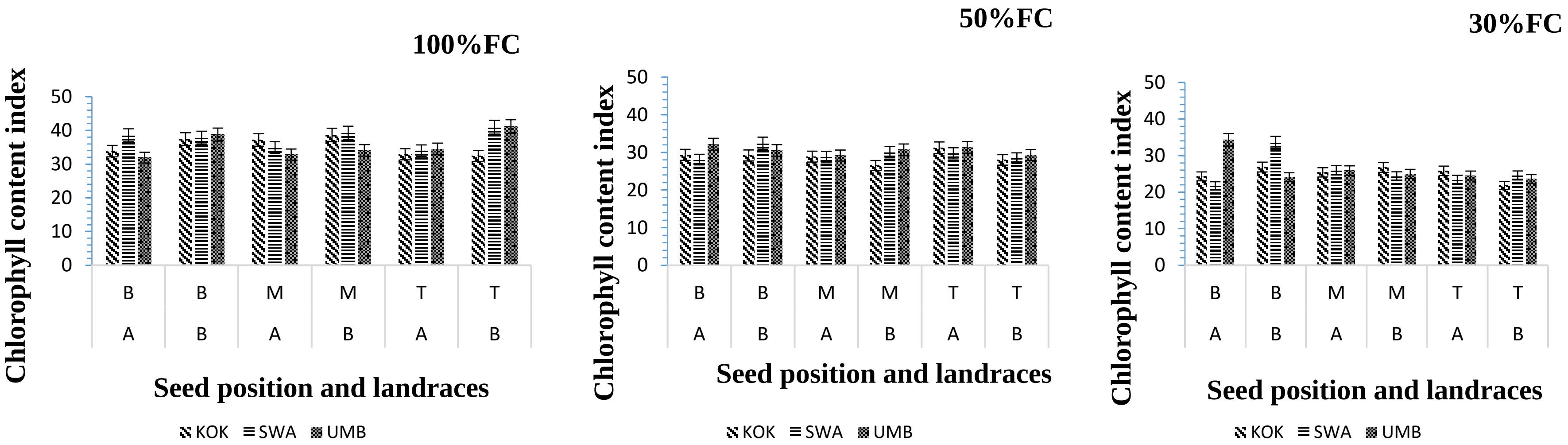

Figure 4. 11: Chlorophyll content index of amaranths landraces (landrace A and B) from different sites (KOK-Kokstad, SWA-Swayimane, and UMB-Umbumbulu) based on seed position (B-Bottom, M-Middle, and T-Top) under different water regimes (100%FC, 50%FC, and 30%FC).

4.5.5. Stomatal conductance

Results of stomatal conductance were not significantly ($p>0.05$) affected by sites, landraces, position, time (weeks) and their interactions. However, stomatal conductance was significantly ($p< 0.05$) affected by water regimes. Stomatal conductance was higher 55 mmol^{-2}s^{-1} at plants irrigated at 100% FC treatment compared to landraces irrigated at 50%FC and 30%FC treatments, (25 mmol^{-2}s^{-1}) and (20 mmol^{-2}s^{-1}), respectively (Figure 4. 12). Regarding water stress treatment, in 100%FC (unstressed treatments), highest SC was observed from seeds collected at KOK from the middle position (50 mmol^{-2}s^{-1}) in landrace B while lowest (30 mmol^{-2}s^{-1}) was recorded from the seeds collected at KOK in the middle position in landrace B. In both moderate and severe stress conditions, highest SC was observed from seeds collected KOK and UMB from the top position (29 mmol^{-2}s^{-1}) in landrace B and top position (28 mmol^{-2}s^{-1}) in landrace A while the lowest SC was recorded from the seeds collected at SWA and UMB from the bottom position (18 mmol^{-2}s^{-1}) and (19 mmol^{-2}s^{-1}) in landrace B, respectively (Figure 4. 13).

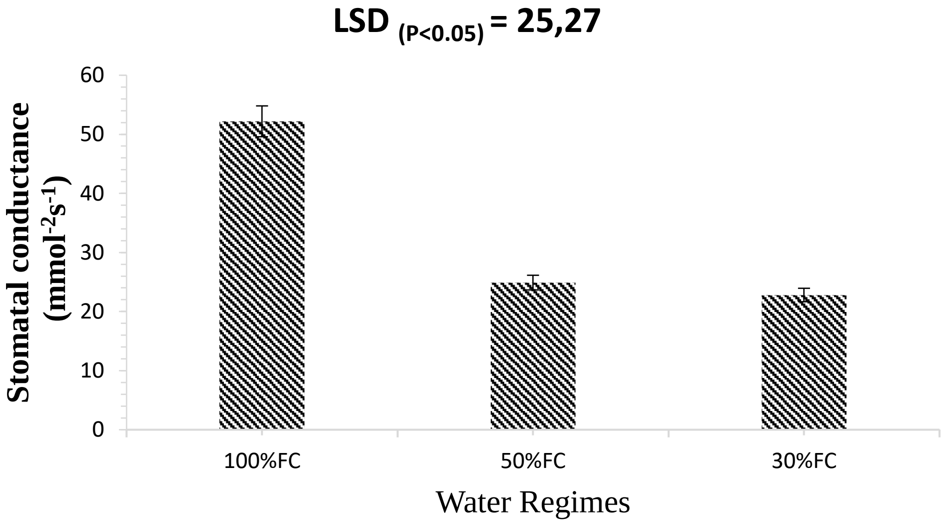

Figure 4. 12: Stomatal conductance under different water regimes ((100%FC, 50%FC, and 30%FC).

LSD $_{(P<0.05)}$ = 61.90

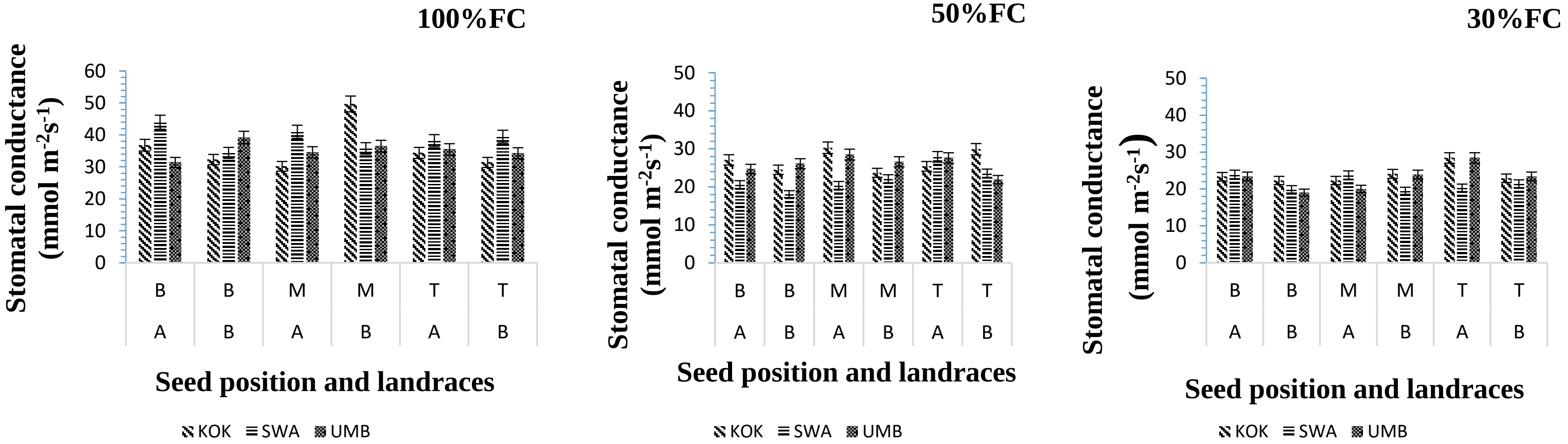

Figure 4. 13: Stomatal conductance of amaranths landraces (landrace A and B) from different sites (KOK-Kokstad, SWA-Swayimane, and UMB-Umbumbulu) based on seed position (B-Bottom, M-Middle, and T-Top) under different water regimes (100%FC, 50%FC, and 30%FC).

4.5.6. Leaf yield

Results showed that there were no significant differences (P > 0.05) in sites, landraces, and position based on leaf yield. However, Water regimes had a highly significant (P < 0.001) effect on leaves yield. Treatments irrigated with 100% FC had highest leave yield (6.54 g) compared to treatment irrigated with 50% FC (moderate) and 30% FC (severed stressed) which had (3.32 g) and (3.10 g) leaves yield, respectively (Figure 4. 14). Although the interaction between sites, landrace, position, and water regimes was not significant (P > 0.05), however, the interaction between sites and water regimes was significant (P < 0.05). As expected, the highest leave yield was observed under 100%FC (unstressed treatments) from seeds collected at SWA (7.54 g). While, in both 50%FC (moderately stressed treatments) and30% FC (severely stressed treatments), the highest leaves yield was observed in seeds collected from UMB (3.8 g) and (3.59 g) and the lowest was observed from seeds collected SWA (2.81 g) and (2.8 g), respectively (Figure 4. 15).

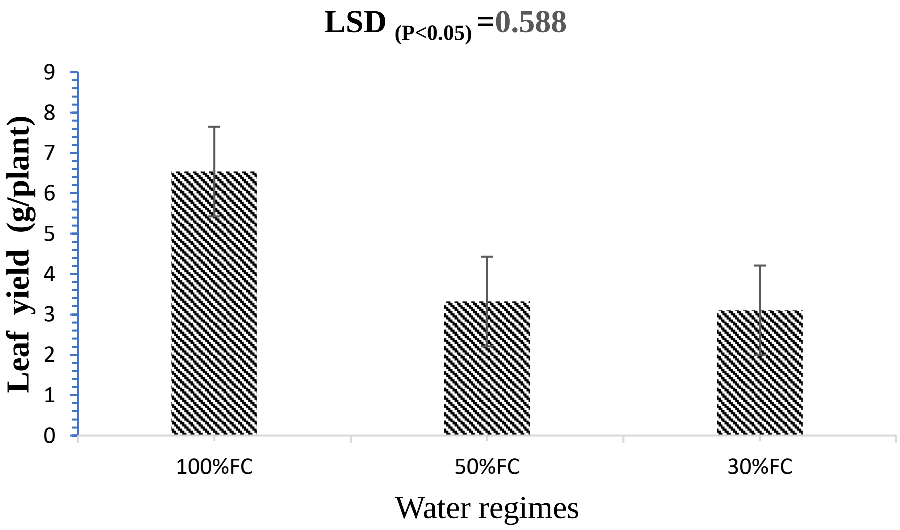

Figure 4. 14: Leaf yield under different water regimes ((100%FC, 50%FC, and 30%FC)

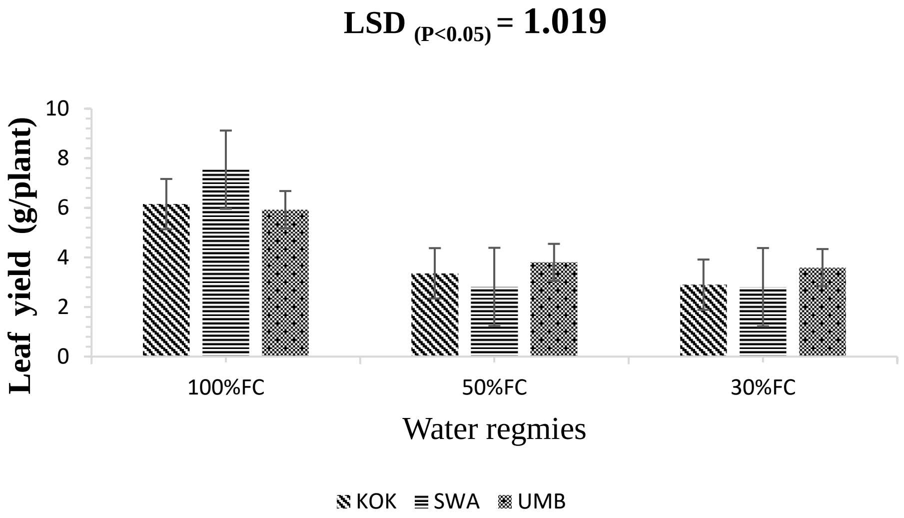

Figure 4. 15: Leaf yield under different water regimes ((100%FC, 50%FC, and 30%FC) and site (KOK-Kokstad, SWA-Swayimane, and UMB-Umbumbulu).

4.6. Discussion

This study was set out with the aim to examine the effect of water stress on morphological, physiological, and nutritional and yield responses of amaranths landraces based on seed position. That is, checking if the seed position has an influence on the growth of the plant under drought conditions.

Morphological growth parameters such as plant height (PH), leaf number (LN) and leaf area (LA) were significantly ($p<0.05$) affected by water stress. With the increase in stress severity, PH, LN and LA became reduced. Several researchers have reported morphological, physiological, molecular and biochemical processes in plants are extremely affected by water stress and this adversely affects their growth and productivity (Duque et al., 2013; Jan et al., 2017; Kaufmann and Hensel, 2018).

Plant height

The source of seeds (seed position) affect plant height and the results indicated that the middle and bottom position seeds had a higher plant height compared to top position seeds under drought stress conditions (Figure 4. 3). This may be attributed to the fact that resource allocation (e.g from the source (leaves) to sinks (tissues)) to the top position was poorly translocated compared to middle and bottom due to water stress (Lemoine et al., 2013). Thus, causing the landraces to reduce the rate of cell expansion and differentiation (Nonami, 1998). Studies done by other researchers have revealed that water stress affects the flow of assimilates (i.e carbohydrates) from the leaves to the sink organs (Deng et al., 1990; Li et al., 2003).

Leaf number and leaf area

There was a decrease in both LN and LA with increasing stress in water treatments (Figure 4. 4,Figure 4. 5 and Figure 4. 8). Serang et al., (2015) have also reported similar results in amaranths. According to Chaves et al., (2003), the plants reduce their LA, under water stress conditions to limits transpiration losses. The difference in LA and LN irrespective to whether seeds come from the top, middle and bottom position under water-stress may also be due to inhibition of cell division, leading to reduced leaf development (Rucker et al., 1995; Anjum et al., 2003; Anjum et al., 2011). Which causes the plant to absorb less water from the soil as the leaf area is reduced. Jaleel et al. (2009) also reported that reduced leaf expansion and cell growth under water stress may be caused by low turgor pressure. The current result showed that seed collected at UMB from the middle and bottom position in landrace A under drought stress regimes had higher LN and LA compared to top position seeds and landrace B (Figure 4. 6 and Figure 4. 8). This suggests that middle and bottom seeds had a higher rate of cell division, leading to higher LN and LA under drought-stressed conditions. Moreover, environmental factors experienced by individual mother plant and genetic makeup (Sestak et al., 1971), had an influence on the behavior crops

Chlorophyll content index

Water regimes, time and their interaction had a significant ($p<0.05$) effect chlorophyll content (Figure 4. 9). Such results concurred with the study done on amaranths species (Jomo and Netondo, 2016), wheat (Moaveni, 2011), cowpea (Ntombela, 2012). The result further showed that chlorophyll content was not significantly ($p>0.05$) affected by the interaction between the site, landraces, seed position, and water regimes (Figure 4. 11), this suggests chlorophyll content index was not sensitive to water stress as it was able to recover rapidly, after watering. Hence, drought avoidance mechanisms were responsible for the adaptation to drought over time.

Stomatal conductance

Generally, the first mechanism used by the plants to respond to water stress is the closure of stomata (reduced stomatal conductance) to limits water loss through transpiration as mention above (Mansfield and Atkinson, 1990; Cornic et al., 1996). This, in turn, have a negative effect on carbon assimilation in the leaves (Fariduddin et al., 2009; Vurayai et al., 2011). Hence, it decreases the processes of photosynthetic and it limits the production of high yield (Chaves et al., 2003). Current results showed that SC was significantly ($p>0.05$) not affected by an interaction between site, landraces, seed position and water regimes (**Error! Reference source not found.**). Such results suggest that seed position, landrace and site had no influence on SC. However, SC under stressed regimes, it was significantly ($p<0.05$) lower compared to 100%FC (unstressed regime), suggesting that the stomatal control played a role in a plant to reduce water loss. This stomatal regulation is also recognized as the mechanism used by amaranths to avoid drought stress. Similar results have also been reported in cowpea (Ilunga, 2014), bean (Miyashita et al., 2005), amaranths (Liu and Stützel, 2002).

Reduction in LN and LA under drought stress condition is recognized as the adaptive mechanism plants used to cope with drought (Nonami, 1998) and maintain high water potential (Mitchell et al., 1998; Blum, 2005) as it's been mentioned earlier in the literature that amaranths use avoid. Others mechanism that amaranths use to adapt to water stress other than regulation of stomata, is the osmotic adjustment. Under water stress conditions, the osmotic adjustment helps the plants to maintain the metabolic and physiological function to contribute to higher expansion in growth and photosynthetic rate (Serraj and Sinclair, 2002; Bolla et al., 2009). Liu and stutzel (2002a, b) also reported that under extreme drought stress, Amaranths sustain a high capacity of osmotic adjustment to allow it to function normally.

Leaf yield

Current results showed leave yield was reduced under moderate and severe stress (Figure 4. 14). However, this implies that all landraces were able to tolerate drought stress conditions as they were able to survive with low water uptake. Similar reports have also been reported in A. *tricolor* (Sarker and Oba, 2018). This may be attributed to physiological and biochemical process being inhibited or affected (Farooq et al., 2009). Which ultimate can cause growth and yield to reduce (Wahid et al., 2007; Fathi and Tari, 2016). In the current study, processes that were affected or reduced were stomatal conductance, chlorophyll content, cell division, cell elongation and expansion, and reduced turgor pressure.

4.7 Conclusion

The study showed that water availability during growth and development affects amaranths crop quality. Crop quality is affected both in the context of plant morphology and plant physiology. For crop utilization, both these measures of crop quality are important. Plant morphology is an indicator of plant size and relates directly to biomass. Biomass is important for leafy vegetables. Plant physiology was measured to determine how dry mass accumulation is affected by water availability. The results showed that water deficit is directly associated with a reduction in the photosynthesis area and in turn, this is related to chlorophyll content index. Reduction in water stress caused limitations associated with photosynthesis mainly due to lower stomatal conductance, which means both carbon dioxide access and transpiration were reduced. These differences were less significantly associated with a source of seed in terms of position on the plant than it was the case with respect to location. This response requires further investigation to determine whether or not the environmental conditions under which the previous crop was grown genetically or nutritionally affects seed quality of the next generation

Chapter 5

General conclusion and study limitations

The aim of the study was to determine the possible effect of sites (locations) and position on the plant on seed quality, growth and development parameters of amaranths. Amaranths were selected for its dominance as a serious commercial agriculture endemic weed species, which has otherwise been shown as an important vegetable in the traditional indigenous agriculture system of South Africa, Africa, and the world. Seed formation and development follow pollination and fertilization, which is expected to take place at significantly different times in seed species characterized by the location of the flowers in different branches and different positions of the branch, such as amaranths. It is also assumed that seed quality, due to size, metabolite and nutrient content at the time of maturation will differ with location on the plant.

The study indicated that although both source of seed (i.e. location) and position of the seed on the plant affected seed characteristics of seed quantity (mass), size and moisture content at harvest, this trend was not clear and consistent. Further investigation into some seed quality parameters showed that (i) seeds water content was mainly affected by landraces and (ii) seed water activity was mainly affected by source (site or location).the most critical seed quality parameters is germination. This is due to the fact that germination is linked to crop establishment and that one of the characteristics of amaranths is that it is endemic as a commercial agriculture weed due to long-lasting seeds. In this study, seed germination, was significantly different between landraces, as expected. Also, both position on the plant and location (source of the seed) influence germination. What is interesting is that the trend of germination differences was not the same per landraces and per source of location of the plants. In some cases, the bottom seeds were better than those of the above. In other cases, the middle or top position seeds were better. This made it difficult to make a conclusive decision that can be true all the time about seed quality parameters. Also, landraces collected at different localities were not checked or confirmed that all are similar or not. Therefore, the conclusion of this study has limitations.

There was a need to determine crop performance of amaranths landraces coming from different sources, i.e. harvest location and position on the plant. The findings of the study showed that crop quality to different regimes of water availability was affected during growth. Unlike the seed quality aspect of the study, the findings were clearer and allowed a more confident generalization about the differences between landraces, seed position, and the seed source location. It was clear that crop was affected by water stress in that the lower water availability, the less plant growth, and development as determined by size, rate of growth, chlorophyll content and final yield. The trend was generally the same across landraces and location. To this extent, it can be concluded that the trend of seed quality is generally better from the bottom to the top location. Therefore, it can be assumed that crop establishment and yield will be derived more from the bottom seeds.

Future research

This study has the following limitation, which can be addressed in future research.

i. Seed quality parameters did not include viability, seed germination rate and final seedling size. A future study should determine the level of seed viability using the tetrazolium chloride test that is recognized by the international seed testing association. It is important to link this to the nutritional content of the seeds because seed performance is directly linked to the availability of mineral and other metabolite nutrients from the cotyledons to the embryo in the early stages of imbibition and growth-related metabolic activities.
ii. The environmental conditions of the seed source location should include soil and bioresource information, in addition to geographic location.
iii. Landraces collected at different localities was not confirmed to be genetically similar. Another important aspect is the pollinators of the seed collected (could have been some cross-pollination from other nearby genotypes- with a significant effect of the seed/seedling quality and performance). Therefore a future study should confirm first that the landraces collected are genetically similar or not.
iv. The nutritional value of the harvested crop is important to conclude whether or not growing amaranths seeds from a different position on the plant and under water stress conditions is an indicator of crop nutritional value.

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Appendices

Appendix 1: List of ANOVA for Chapter 3

Variate: Seed mass per species (g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
site	2	127986.11	63993.06	2133.10	<.001
landrace	1	159236.06	159236.06	5307.87	<.001
site.landrace	2	461217.44	230608.72	7686.96	<.001
Residual	12	360.00	30.00		
Total	17	748799.61			

Variate: Length per part (cm)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	22.584	11.292	1.33	
replication.*Units* stratum					
site	2	0.161	0.081	0.01	0.991
landraces	1	49.307	49.307	5.81	0.021
Position	2	258.139	129.070	15.22	<.001
site.landraces	2	47.658	23.829	2.81	0.074
site.Position	4	45.567	11.392	1.34	0.274
landraces.Position	2	9.458	4.729	0.56	0.578
site.landraces.Position	4	24.338	6.084	0.72	0.586
Residual	34	288.323	8.480		
Total	53	745.535			

Variate: Seed mass per part (g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	6.48	3.24	0.06	
replication.*Units* stratum					
site	2	40981.59	20490.80	363.20	<.001

landraces	1	89874.24	89874.24	1593.03	<.001
Position	2	4076.70	2038.35	36.13	<.001
site.landraces	2	115851.15	57925.57	1026.74	<.001
site.Position	4	31678.96	7919.74	140.38	<.001
landraces.Position	2	27202.26	13601.13	241.08	<.001
site.landraces.Position	4	10611.19	2652.80	47.02	<.001
Residual	34	1918.19	56.42		
Total	53	322200.76			

Variate: Thousand seed weight (mass) (g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	0.34700	0.17350	5.68	
Replication.*Units* stratum					
site	2	0.50667	0.25334	8.29	0.001
landraces	1	1.21200	1.21200	39.67	<.001
Position	2	0.17623	0.08811	2.88	0.070
site.landraces	2	0.56663	0.28331	9.27	<.001
site.Position	4	0.44950	0.11237	3.68	0.014
landraces.Position	2	0.04594	0.02297	0.75	0.479
site.landraces.Position	4	0.24665	0.06166	2.02	0.114
Residual	34	1.03873	0.03055		
Total	53	4.58934			

Variate: Seed water content (g)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	854.474	427.237	89.89	
replication. *Units* stratum					
site	2	11.025	5.513	1.16	0.326
landraces	1	29.315	29.315	6.17	0.018
Position	2	14.600	7.300	1.54	0.230
site.landraces	2	9.887	4.943	1.04	0.364
site.Position	4	20.279	5.070	1.07	0.388
landraces.Position	2	25.268	12.634	2.66	0.085
site.landraces.Position	4	88.429	22.107	4.65	0.004
Residual	34	161.599	4.753		
Total	53	1214.875			

Variate: Water activity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	0.766046	0.383023	41.56	
replication.*Units* stratum					
site	2	0.185291	0.092645	10.05	<.001
Position	2	0.009409	0.004705	0.51	0.605
landraces	1	0.026312	0.026312	2.86	0.100
site.Position	4	0.022788	0.005697	0.62	0.653
site.landraces	2	0.031205	0.015602	1.69	0.199
Position.landraces	2	0.014801	0.007400	0.80	0.456
site.Position.landraces	4	0.028588	0.007147	0.78	0.549
Residual	34	0.313316	0.009215		
Total	53	1.397755			

Variate: % Germination

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Position	2	3900.98	1950.49	29.26	<.001
day	9	27480.89	3053.43	45.81	<.001
landrace	1	6771.20	6771.20	101.58	<.001
site	2	10215.64	5107.82	76.63	<.001
Position.day	18	966.58	53.70	0.81	0.682
Position.landrace	2	9755.20	4877.60	73.17	<.001
day.landrace	9	1422.58	158.06	2.37	0.032
Position.site	4	7532.09	1883.02	28.25	<.001
day.site	18	523.91	29.11	0.44	0.968
landrace.site	2	1456.53	728.27	10.93	<.001
Position.day.landrace	18	1363.02	75.72	1.14	0.361
Position.day.site	36	1323.02	36.75	0.55	0.961
Position.landrace.site	4	2775.47	693.87	10.41	<.001
day.landrace.site	18	1432.36	79.58	1.19	0.316
Residual	36	2399.64	66.66		
Total	179	79319.11	443.12		

Appendix 2: Lists of ANOVA for Chapter 4

Variate: plant_height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	360.96	180.48	8.46	
Replication.*Units* stratum					
site	2	278.87	139.44	6.54	0.002
landraces	1	4.51	4.51	0.21	0.646
Position	2	1259.12	629.56	29.51	<.001
water_regimes	2	10091.34	5045.67	236.49	<.001
weeks	5	42819.59	8563.92	401.39	<.001
site.landraces	2	140.73	70.36	3.30	0.038
site.Position	4	501.38	125.34	5.87	<.001
landraces.Position	2	176.96	88.48	4.15	0.016
site.water_regimes	4	257.29	64.32	3.01	0.018
landraces.water_regimes	2	150.87	75.43	3.54	0.030
Position.water_regimes	4	560.54	140.13	6.57	<.001
site.weeks	10	117.85	11.78	0.55	0.853
landraces.weeks	5	14.34	2.87	0.13	0.984
Position.weeks	10	429.63	42.96	2.01	0.030
water_regimes.weeks	10	5252.43	525.24	24.62	<.001
site.landraces.Position	4	660.93	165.23	7.74	<.001
site.landraces.water_regimes	4	402.97	100.74	4.72	<.001
site.Position.water_regimes	8	583.16	72.90	3.42	<.001
landraces.Position.water_regimes	4	67.79	16.95	0.79	0.529
site.landraces.weeks	10	63.16	6.32	0.30	0.982
site.Position.weeks	20	302.09	15.10	0.71	0.820
landraces.Position.weeks	10	118.02	11.80	0.55	0.852
site.water_regimes.weeks	20	351.35	17.57	0.82	0.686
landraces.water_regimes.weeks	10	153.99	15.40	0.72	0.704
Position.water_regimes.weeks	20	649.36	32.47	1.52	0.067
site.landraces.Position.water_regimes	8	976.57	122.07	5.72	<.001
site.landraces.Position.weeks	20	384.59	19.23	0.90	0.586
site.landraces.water_regimes.weeks	20	351.07	17.55	0.82	0.687
site.Position.water_regimes.weeks	40	711.52	17.79	0.83	0.758
landraces.Position.water_regimes.weeks	20	139.56	6.98	0.33	0.998
site.landraces.Position.water_regimes.weeks	40	597.27	14.93	0.70	0.919

Residual	646	13782.87	21.34		
Total	971	82712.67			

Variate: leaf number

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Replication stratum	2	238.113	119.057	16.15	
Replication.*Units* stratum					
site	2	78.484	39.242	5.32	0.005
landraces	1	198.273	198.273	26.89	<.001
Position	2	124.718	62.359	8.46	<.001
water_regimes	2	3368.854	1684.427	228.48	<.001
week_1	5	14062.413	2812.483	381.49	<.001
site.landraces	2	3.027	1.513	0.21	0.814
site.Position	4	117.979	29.495	4.00	0.003
landraces.Position	2	40.274	20.137	2.73	0.066
site.water_regimes	4	80.140	20.035	2.72	0.029
landraces.water_regimes	2	13.619	6.810	0.92	0.398
Position.water_regimes	4	105.572	26.393	3.58	0.007
site.week_1	10	19.628	1.963	0.27	0.988
landraces.week_1	5	66.931	13.386	1.82	0.108
Position.week_1	10	16.208	1.621	0.22	0.994
water_regimes.week_1	10	1217.591	121.759	16.52	<.001
site.landraces.Position	4	94.733	23.683	3.21	0.013
site.landraces.water_regimes	4	64.239	16.060	2.18	0.070
site.Position.water_regimes	8	40.842	5.105	0.69	0.698
landraces.Position.water_regimes	4	125.399	31.350	4.25	0.002
site.landraces.week_1	10	19.084	1.908	0.26	0.989
site.Position.week_1	20	57.576	2.879	0.39	0.993
landraces.Position.week_1	10	20.356	2.036	0.28	0.986
site.water_regimes.week_1	20	97.564	4.878	0.66	0.865
landraces.water_regimes.week_1	10	11.047	1.105	0.15	0.999
Position.water_regimes.week_1	20	37.317	1.866	0.25	1.000
site.landraces.Position.water_regimes	8	58.965	7.371	1.00	0.435
site.landraces.Position.week_1	20	43.934	2.197	0.30	0.999
site.landraces.water_regimes.week_1	20	47.613	2.381	0.32	0.998
site.Position.water_regimes.week_1	40	52.010	1.300	0.18	1.000

```
landraces.Position.water_regimes.week_1
                                20        65.045          3.252       0.44    0.984
site.landraces.Position.water_regimes.week_1
                                40        70.628          1.766       0.24    1.000
Residual                       646      4762.553          7.372

Total                          971     25420.727
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Variate: LEAF_area_mm2

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Source of variation           d.f.          s.s.           m.s.       v.r.    F pr.

Replication stratum              2       10890.0         5445.0       9.34

Replication.*Units* stratum
site                             2       13357.7         6678.8      11.46    <.001
landraces                        1        2103.7         2103.7       3.61    0.058
Position                         2       37042.2        18521.1      31.78    <.001
water_regimes                    2      387092.5       193546.3     332.15    <.001
week_1                           5      552252.9       110450.6     189.54    <.001
site.landraces                   2        7070.1         3535.1       6.07    0.002
site.Position                    4       12759.2         3189.8       5.47    <.001
landraces.Position               2        2469.1         1234.5       2.12    0.121
site.water_regimes               4       34080.0         8520.0      14.62    <.001
landraces.water_regimes          2         472.0          236.0       0.41    0.667
Position.water_regimes           4       30299.0         7574.7      13.00    <.001
site.week_1                     10       19822.4         1982.2       3.40    <.001
landraces.week_1                 5        5158.4         1031.7       1.77    0.117
Position.week_1                 10        6879.0          687.9       1.18    0.301
water_regimes.week_1            10      119618.2        11961.8      20.53    <.001
site.landraces.Position          4       21947.1         5486.8       9.42    <.001
site.landraces.water_regimes
                                 4        1075.2          268.8       0.46    0.764
site.Position.water_regimes
                                 8       25337.5         3167.2       5.44    <.001
landraces.Position.water_regimes
                                 4        8913.3         2228.3       3.82    0.004
site.landraces.week_1           10        3454.5          345.5       0.59    0.820
site.Position.week_1            20       14284.4          714.2       1.23    0.226
landraces.Position.week_1
                                10        3592.3          359.2       0.62    0.800
site.water_regimes.week_1
                                20       18417.9          920.9       1.58    0.052
landraces.water_regimes.week_1
                                10        5720.3          572.0       0.98    0.458
Position.water_regimes.week_1
                                20       10919.5          546.0       0.94    0.540
site.landraces.Position.water_regimes
                                 8       19035.1         2379.4       4.08    <.001
site.landraces.Position.week_1
                                20        6763.1          338.2       0.58    0.927
site.landraces.water_regimes.week_1
                                20        4842.3          242.1       0.42    0.989
```

site.Position.water_regimes.week_1	40	17834.0	445.9	0.77	0.853
landraces.Position.water_regimes.week_1	20	17150.9	857.5	1.47	0.084
site.landraces.Position.water_regimes.week_1	40	15323.9	383.1	0.66	0.950
Residual	646	376433.6	582.7		
Total	971	1812411.3			

Variate: stomatal_conductance

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Replication stratum	2	48465.	24232.	0.90	
Replication.*Units* stratum					
site	2	52100.	26050.	0.97	0.379
landraces	1	31323.	31323.	1.17	0.280
Position	2	44113.	22056.	0.82	0.440
water_regimes	2	174024.	87012.	3.24	0.040
Weeks	5	175102.	35020.	1.31	0.260
site.landraces	2	49596.	24798.	0.92	0.397
site.Position	4	105653.	26413.	0.98	0.415
landraces.Position	2	58816.	29408.	1.10	0.335
site.water_regimes	4	93556.	23389.	0.87	0.481
landraces.water_regimes	2	43809.	21904.	0.82	0.443
Position.water_regimes	4	110747.	27687.	1.03	0.390
site.Weeks	10	253369.	25337.	0.94	0.492
landraces.Weeks	5	115547.	23109.	0.86	0.507
Position.Weeks	10	239061.	23906.	0.89	0.541
water_regimes.Weeks	10	281238.	28124.	1.05	0.401
site.landraces.Position	4	89886.	22471.	0.84	0.502
site.landraces.water_regimes	4	97934.	24483.	0.91	0.456
site.Position.water_regimes	8	212974.	26622.	0.99	0.441
landraces.Position.water_regimes	4	113651.	28413.	1.06	0.376
site.landraces.Weeks	10	285078.	28508.	1.06	0.389
site.Position.Weeks	20	562070.	28103.	1.05	0.403
landraces.Position.Weeks	10	282444.	28244.	1.05	0.397
site.water_regimes.Weeks	20	495226.	24761.	0.92	0.558
landraces.water_regimes.Weeks	10	243310.	24331.	0.91	0.526
Position.water_regimes.Weeks	20	524150.	26207.	0.98	0.489
site.landraces.Position.water_regimes	8	189093.	23637.	0.88	0.532
site.landraces.Position.Weeks	20	516161.	25808.	0.96	0.508

site.landraces.water_regimes.Weeks	20	545123.	27256.	1.02	0.441
site.Position.water_regimes.Weeks	40	1077829.	26946.	1.00	0.466
landraces.Position.water_regimes.Weeks	20	547430.	27371.	1.02	0.435
site.landraces.Position.water_regimes.Weeks	40	992861.	24822.	0.93	0.605
Residual	646	17334708.	26834.		
Total	971	25986443			

Variate: CCl

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Replication stratum	2	6803.2	3401.6	28.22	
Replication.*Units* stratum					
site	2	236.5	118.2	0.98	0.376
landraces	1	217.9	217.9	1.81	0.179
Position	2	402.8	201.4	1.67	0.189
water_regimes	2	18192.9	9096.5	75.45	<.001
Weeks	5	4038.5	807.7	6.70	<.001
site.landraces	2	469.1	234.6	1.95	0.144
site.Position	4	309.3	77.3	0.64	0.633
landraces.Position	2	100.7	50.3	0.42	0.659
site.water_regimes	4	296.9	74.2	0.62	0.651
landraces.water_regimes	2	693.8	346.9	2.88	0.057
Position.water_regimes	4	360.7	90.2	0.75	0.560
site.Weeks	10	1691.4	169.1	1.40	0.175
landraces.Weeks	5	371.1	74.2	0.62	0.688
Position.Weeks	10	1690.3	169.0	1.40	0.175
water_regimes.Weeks	10	2826.1	282.6	2.34	0.010
site.landraces.Position	4	526.9	131.7	1.09	0.359
site.landraces.water_regimes	4	655.1	163.8	1.36	0.247
site.Position.water_regimes	8	793.6	99.2	0.82	0.583
landraces.Position.water_regimes	4	184.7	46.2	0.38	0.821
site.landraces.Weeks	10	2187.6	218.8	1.81	0.055
site.Position.Weeks	20	1484.2	74.2	0.62	0.903
landraces.Position.Weeks	10	340.4	34.0	0.28	0.985
site.water_regimes.Weeks	20	1991.9	99.6	0.83	0.683
landraces.water_regimes.Weeks	10	772.4	77.2	0.64	0.779
Position.water_regimes.Weeks	20	1788.3	89.4	0.74	0.784
site.landraces.Position.water_regimes	8	1615.5	201.9	1.68	0.101

site.landraces.Position.Weeks	20	1957.2	97.9	0.81	0.701
site.landraces.water_regimes.Weeks	20	1974.3	98.7	0.82	0.692
site.Position.water_regimes.Weeks	40	2439.4	61.0	0.51	0.995
landraces.Position.water_regimes.Weeks	20	1934.4	96.7	0.80	0.712
site.landraces.Position.water_regimes.Weeks	40	3597.6	89.9	0.75	0.875
Residual	646	77880.4	120.6		
Total	971	140825.0			

Variate: leaves_mass

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	77.754	38.877	16.35	
Replication.*Units* stratum					
sites	2	2.840	1.420	0.60	0.552
landraces	1	5.837	5.837	2.45	0.120
position	2	13.912	6.956	2.92	0.058
water_regimes	2	400.687	200.344	84.24	<.001
sites.landraces	2	10.596	5.298	2.23	0.113
sites.position	4	4.743	1.186	0.50	0.737
landraces.position	2	4.111	2.056	0.86	0.424
sites.water_regimes	4	40.101	10.025	4.22	0.003
landraces.water_regimes	2	4.794	2.397	1.01	0.369
position.water_regimes	4	4.328	1.082	0.45	0.769
sites.landraces.position	4	3.874	0.968	0.41	0.803
sites.landraces.water_regimes	4	5.580	1.395	0.59	0.673
sites.position.water_regimes	8	6.105	0.763	0.32	0.957
landraces.position.water_regimes	4	6.113	1.528	0.64	0.633
sites.landraces.position.water_regimes	8	18.861	2.358	0.99	0.447
Residual	106	252.102	2.378		
Total	161	862.337			