

**GROWTH AND PHYSIOLOGICAL RESPONSE OF AMARANTH
SEEDLINGS TO TEMPERATURE AND DROUGHT STRESS**

By

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DECLARATION

I declare that this dissertation, hereby submitted for the qualification Magister Scientiae degree at the University of Free State, is my own work and that I have not previously submitted the same work for a qualification at/in another University/faculty.

I also agree that the University of the Free State has the sole right to the publication of this dissertation.

Signed: _____ Date: _____

DEDICATION

I would love to dedicate the work of this thesis to my beloved and caring parents Kuku Wilhelmina “Noa” Nuugulu and Tate David Nuugulu as well as the entire Nuugulu family.

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LIST OF ABBREVIATIONS

AC = *Amaranthus cruentus*
ADP = Adenosine-5-diphosphate
AH = *Amaranthus hybridus*
ARC = Agricultural Research Council
ATP = Adenosine-5-triphosphate
DW = Dry Weight
FGP = Final Germination Percentage
FW = Fresh Weight
GEI = Genes Environment Interaction
GI = Germination Index
HCl = Hydrochloric acid
HK = Hexokinase
HSR = Heat Shock Response
ISTA = International Seed Testing Association
LSD = Least Significant Difference
M = Moisture
MG = Mean Germination
MRL = Mean Root Length
MTG = Mean Time for Germination
NADH = Nicotine amide adenine dinucleotide reduced
NADPH = Nicotine amide adenine dinucleotide phosphate reduced
ns = non-significant
OASA = Association of official seed analysis
OPP = Oxidative Pentose Phosphate pathway
PEG = Polyethylene glycol
PFK = Phosphofructokinase
PGI = Phospho-gluco-isomerase
PGP = Phospho-enol pyruvic acid
PS = Photosystem
RNA = Ribonucleic acid
RUBISCO = Ribulose-1,5-bisphosphate dehydrogenase
S = Species
SVI = Seed Vigour Index
T = Temperature
VOPI = Vegetable and Ornamental Plant Institute

Chapter 1

Introduction and Rationale

Malnutrition and food security remain vital issues in the world today, but especially for the developing world including the whole of Africa (Labuschagne *et al.*, 2008). Almost a decade ago about 800 million people around the world were subjected to malnutrition (FAO, 2003) and this number has increased since. Moreover, starvation in many rural communities around the world is associated with drought and unfavourable temperature conditions (Steckel *et al.*, 2004). These are the two main environmental factors currently limiting crop productivity and causing significant yield losses in many agricultural crops worldwide (Amisi & Doohan, 2010).

Crop production on arable land is limited in about one third of the world due to frequent and unpredictable high temperature and drought stress conditions. In recent years many southern African countries, including the rather developed Namibia and South Africa, occasionally suffered major water shortage and extreme temperature conditions (Jansen Van Rensburg *et al.*, 2007). These changes are attributed to global climatic adjustment. Recently Sun *et al.* (2011) projected that the mean annual and global surface temperature will increase by 1.7 and 3.8 °C, respectively, by 2100. Such temperature changes might have a radical effect on crop production and negatively influence food security.

The adverse effects of global warming show considerable regional variation and developing countries are likely to be affected to a much greater degree (Moran & Showeler, 2005). This is not just because many of these countries are classified as arid or semi-arid regions, but in many cases agricultural land in these developing countries are already marginal and vulnerable to annual climatic fluctuations (Bavec & Mlakar, 2002). Increased food insecurity will be the end result unless man can adapt in terms of (i) identifying alternative tolerant crops, (ii) understanding the mechanisms of tolerance on a physiological, biochemical and genetic level and (iii) transforming crops in an attempt to assist staple food plants in adapting to a changing environment. Of these

three, understanding the action mechanisms that lead to resistance is the first step. Further, due to the relationship between elevated temperature conditions and available moisture for crop plants, agriculturalists are challenged to identify quality and high yielding alternative crops and/or cultivars of existing crops that can be cultivated under limited water supply and extreme high temperature (Modi, 2006). Amaranth (*Amaranthus* spp.) is seen by many as a new dicotyledonous pseudo-cereal and vegetable crop of high nutritional value and its development as alternative crop has attracted the attention of several researchers over the past decade (Aufhammer *et al.*, 1998; Coastea & Damason, 2001; Leon *et al.*, 2004). Amaranth can be used as animal feed and its leaves and seeds are suitable for human consumption. As a leaf vegetable, amaranth has been rated equivalent or higher than spinach in terms of taste and higher than cabbage in terms of calcium, iron and phosphorous content (Dale & Egley, 1971; Game *et al.*, 2006; Choudhury *et al.*, 2008). Further, amaranth is regarded as more valuable than all other spring and summer leaf vegetables to which it was compared to by Oyodele (2000). For example, leaf amaranth produces 4686 units of vitamin per 100 g of edible portion, compared to 600 g by Swiss chard and 280 g by cabbage (Oyodele, 2000). Important in this study is to come to grips with the ability of amaranth to endure both high temperature and drought conditions, as well as its physiological response as an indication of the possible mechanism of action involved.

Generally seed germination represents the primary stage at which the plant competes for an environmental niche while subsequent seedling growth under these environmental field conditions is vital for successful crop production (Ghorbani *et al.*, 1999). It has been established that relatively high temperature and moderate moisture are key factors that enhance amaranth seed germination and seedling growth (Leon *et al.*, 2004). Above and beyond, indications are that amaranth has the ability to adapt well in arid and semi arid regions and grow on marginal land (Blazick *et al.*, 2005).

Under both arid and semi-arid environmental conditions drought and extreme temperature may lead to a series of morphological, physiological, biochemical and genetic changes that can adversely affect seed germination, seedling growth, plant development and the final yield. Drought and heat are often interconnected and may

cause similar cellular damages to plants (Hurro & Cees, 1991). Although amaranth seems to be capable of surviving several environmental stress conditions, little is known about its physiological response that makes this crop rather tolerant to high temperature and reduced water level conditions (Ghorbani *et al.*, 1999; Chayan *et al.*, 2003).

In this study seeds of two amaranth species, viz. *A. hybridis* and *A. cruentus*, will be compared in terms of the optimum temperature for germination as well as the tolerance limits of subsequent seedling growth to both drought and heat stress. Polyethylene glycol (PEG 8000), frequently used to simulate drought stress and study the response of higher plant species (Michael, 1983), will be employed to follow the physiological response of seedlings to heat and drought stress by using selected parameters.

1.1 References

- AMISI, K.J. & DOOHAN, D. 2010.** Redroot pigweed (*Amaranthus retroflexus*) seedling emergency and growth in soil amended with composted dairy cattle manure and fresh dairy cattle manure under greenhouse conditions. *Weed Technology* 24, 71-75.
- AUFHAMMER, W., CHUCZOROVA, H.P.K. & KRUSE, M. 1998.** Germination of grain amaranth (*A. hypochondriacus* x *A. hybridus*): Effects of seed quality, temperature, light, and pesticides. *European Journal of the Agronomic Society* 8,127-135.
- BAVEC, T. & MLAKAR, S.G. 2002.** Effects of soil and climate condition on emergence of grain amaranths. *Journal of Agronomy* 17, 93-103.
- BLAZICK, F.A., WARREN, S.L., NASH, D.L. & REECE, W.M. 2005.** Seed germination of Seabeach Amaranth (*Amaranthus pumilus*) as influenced by stratification, temperature and light. *Environmental Horticulture* 23, 33-36.
- CHAYAN, A.A.I.M., RAHMAN, H.M., ROZENA, S. & ISLAM., M.R. 2003.** Initial moisture content and different storage container potentiality on vigourity of stem

amaranth (*Amaranthus oleranceus*) seed. Cambridge University, Department of Horticulture 4, 1197-1203.

CHOUDHURY, M.R.Q., ISLAM, S.T., ALAM, R., AHMAD, I., ZAMAN, W., SEN, R. & ALAM.,M.N. 2008. Effects of arsenic on red amaranth (*Amaranthus retroflexus* L). *Acta Horticulture* 3, 48-52.

COASTEY, M. & DEMASON, D.A. 2001. Stem morphology and anatomy in *Amaranthus* L. (Amaranthaceae), Taxonomic Significance. *Terry Botany* 128(3), 254-281.

DALE, J.E. & EGGLEY, G.H. 1971. Stimulation of Witchweed Germination by Run-off Water and Plant Tissues. *Weed Science* 19, 678-681.

FAO. 2003. Improving bioavailability of iron in Indian diet through food-based approaches for the control of iron deficiency anaemia. *FAO*, 32, 51-61.

GAME, H.T., LINSSEN, J.P., MESALLAM, A.S., DAMIR, A.A. & SHEKIB. L.A. 2006. Seed treatments effects and antinutritional properties of amaranth flours. *Food Science* 86, 1095-1102.

GHORBANI, R., SEEL, W. & LEIFERT, C. 1999. Effects of environmental factors on germination and emergency of *Amaranthus retroflexus*. *Weed Science* 48, 505-510.

HURRO, J.B. & CEES, M.K. 1991. The dual role of temperature in the regulation of the seasonal changes in dormancy and germination of seeds of *Polygonum*. *Oecologia* 90, 88-94.

JANSEN VAN RENSBURG, W.S., AVERBEKE, V.W., SLABBERT, R., FABER, M., VAN JAARVELD, V.P., VAN HEERDEN, V.I., WENHOLD, F. & OELOFSE, A. 2007. African leaf vegetables in South Africa. ARC, Roodeplaat, Pretoria.

LABUSCHAGNE, M.T, ELAGO, O. & KOEN, E. 2008. The influence of temperature extremes on some quality and starch characteristics in bread, biscuit and durum wheat. *Cereal science* 49, 184-189.

- LEON, R.G., KNAPP, A.D. & OWEN, M.D.K. 2004.** Effect of temperature on the germination of common waterhemp (*Amaranthus tuberculatus*), Giant Foxtail (*Setaria faberi*), and Velvetleaf (*Abutilon theophrasti*). *Weed Science* 52, 67-73.
- MICHAEL, B.E. 1983.** Evaluation of the water potentials of solutions of polyethylene glycol 8000 both in absence and presence of other substances. *Plant Physiology* 72, 66-70.
- MODI, A.T. 2006.** Growth temperature and plant age influence on nutritional quality of amaranthus leaves and seed germination capacity. *Water SA* 33, 0378-4738.
- MORAN, P.J. & SHOWELER, A.T. 2005.** Plant response to water deficit and shade stresses in pigweed and their influences on feeding and oviposition by waterhemp. *Environmental Entomology* 34, 929-937.
- OYODELE, V.I. 2000.** Influence of soil water stress at different physiological stages on drought and seed yield of amaranth species. *Acta Horticulture* 357, 114-121.
- STECKEL, L., CRISTY, L.S., EDWARD, W.S. & LOYD, M.W. 2004.** Temperature effects on germination of nine *Amaranthus* species. *Weed Science* 52, 217-221.
- SUN, Y., DU, X., ZHANG, W & LI, R. 2011.** Seed germination and physiological characteristics of *Amaranthus L.* under drought stress. *Advanced Material Research* 1071, 183-185.

Chapter 2

Literature Review

2.1 Introduction

Many conventional crop farmers propagate their plants from seeds. The degree of success is largely determined by the quality or germination capacity of the seed (Aufhammer *et al.*, 1998; Hartmann *et al.*, 2011). Germination capacity depends on seed viability that is mainly determined by the growth capacity of the embryo and mobilization of stored food supply during germination (Ehleringer, 1983). Generally, germination is regarded as complete when the radicle protrudes through the testa (ISTA, 2010). However, seedling establishment that follows is not guaranteed, but is depending on environmental conditions including water availability, an appropriate temperature range, oxygen supply and in some cases light.

Plotting seed germination data over time often results in a sigmoidal curve indicating how a seed population behaves (Hartmann *et al.*, 2011). Germination of a certain seed lot can be measured by gauging three important germination parameters as highlighted by Brainard *et al.* (2006). The first parameter, according to the authors, is germination percentage which is the number of seed that produce seedlings from a seed population expressed as percentage. The second parameter is the germination speed (rate) which is the measure of how rapid a seed lot germinates or the time required for a seed lot to reach a predetermined germination percentage. Lastly germination can be measured by means of germination uniformity which measures how close in time seeds germinate or seedlings emerge. Several germination media is used in the laboratory to measure seed germination and this includes paper, sand or nutrient agar.

According to Aufhammer *et al.* (1998), seed purity and germination ability are the most important parameters that describe seed quality, but this has some shortcomings. These two parameters only distinguish between normal, abnormal and dead seed of a seed lot and do not reflect seedling vigour or viability. Seedling vigour can be described as the ability of the seedling to penetrate the soil surface and grow vigorously in the early growing season (Ghorbani *et al.*, 1999). In the case of leafy vegetables, research

has indicated that highly vigorous seedlings are likely to contribute to high harvestable yields because faster growing plants complete the vegetative growth stage faster and produce large plants that will produce more leaves (Modi, 2006). Reports on grain crops similarly concluded that rapid growing seedlings enter the pollination stage faster providing a longer period for grain filling, generating higher yields (Dieleman *et al.*, 1996).

In this thesis a comprehensive study on seed germination and seedling growth of two amaranth species under temperature and drought stress was undertaken. By using selected physiological parameters an attempt was made to follow the physiological impact of water and temperature stress on amaranth seedlings in order to assess drought tolerance or susceptibility of the two species.

2.2 Background to amaranth

2.2.1 General

The family Amaranthaceae, and more specifically the genus *Amaranthus*, consist of about 70 species of which 40 are native to the Americas. Other species originated from Australia, Africa, Asia and Europe (Coastea & Demason, 2001). Amaranth has been grown as a crop in East Africa, Asia and Southern Mexico as long ago as 6700 BC (Akanbi & Togun, 2002). It is an erect, annual herb with average maturity height ranging between 60 and 120 cm and has been regarded as a weed (Muyonga *et al.*, 2008). The plant's dark-green leaves are oval with average length of two to four centimetres that often contain dark ring spots (Pedro *et al.*, 1995). The abaxial leaf epidermis of young plants is also often purple-spotted, which makes the entire seedling to appear red in colour. *Amaranthus* species bear small flowers that are placed close to the stem.

Leaves are consumed as a vegetable and the small grains (0.6-0.8 mg) can be utilized as cereal (Muyonga *et al.*, 2008). Harvesting of leaves and tender shoots from cultivated plants starts about a month after sowing, or two to three weeks after the first rains, and stop as soon as the crop starts flowering (Aynehband, 2008). Dieleman *et al.* (1997) reported that harvesting amaranth leaves and tender shoot stimulates the crop's

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

Amaranth is propagated through seeds that can be planted by direct sowing in the soil where it takes four to six days to emerge (Dieleman *et al.*, 1996). The authors suggested that, since amaranth seeds are too small to be sown alone, they can be mixed thoroughly with dry sand to obtain a homogenate mixture that can be broadcasted at the rate of one and half to two kg ha⁻¹. Amaranth seeds can alternatively be germinated in nursery trays and transplanted as seedlings approximately four weeks after germination when the seedlings are about four to eight centimeter tall (Oyedele, 2002). Thinning may be done at about two weeks where needed. Once established, amaranth can effectively smother most grass weeds, and is remarkably drought-tolerant. Even though the crop is grown on a marginal land, amaranth leaf and grain yield increase with fertility of the soil (Leon *et al.*, 2004).

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

Abiotic stress conditions such as extreme temperatures and insufficient water commonly limit the development and productivity of major crop species, and this is also expected to be true for amaranth. However, vegetable amaranth has been considered

as a prospective crop for marginal lands and semi-arid regions due to its tolerance towards high temperature and low soil water. Together with its nutritional value, these properties qualify amaranth to be described as a drought tolerant crop (Steckel *et al.*, 2004). In this regard, a study by Myers (1974) who compared eight different crops with respect to drought tolerance, including their physiological responses, indicated that amaranth plants have an astonishing capacity to recover after a period of severe drought stress. Oyedele *et al.* (2002) later reported that drought tolerance in amaranth might be due to the ability of the crop to shut down transpiration through wilting while recovering easily when moisture is made available.

To cope with high-temperature stress, generally, plants have developed mechanisms that include both heat avoidance and tolerance (Tucker, 1986; Paland & Chang, 2003). Heat avoidance may result from specific morphological characteristics such as altered leaf shape. Tolerance, on the other hand, results from altered physiological processes. In many cases, heat stress is due to a brief exposure to sub lethal temperatures, which results in reversible damage to cellular and sub cellular structures and functions (Kigel *et al.*, 1977). Amaranth is also described as a heat-tolerant crop due to its capability of repairing damaged tissues and resuming normal metabolic functions faster than other leaf vegetables (Tucker, 1986; Paland & Chang, 2003; Moran & Showeler, 2005). Amaranth plants have also been reported to possess a competitive advantage because they can resume normal cellular functions, such as photosynthesis, sooner after heat stress than non-heat tolerant plants (Gou & Al-Khatib, 2003).

Since this study will concentrate mainly on the response of amaranth seedlings to temperature and drought stress, a short review on previously acquired information follows.

2.2.2 Amaranth seed germination and subsequent seedling growth

In many scientific publications, the term 'germination' is often used loosely and in many cases wrongly (Gou & Al-Khatib, 2003). It is, therefore, important to clarify its meaning. The germination process begins with water uptake (imbibition) and ends when the

radicula protrudes the testa after elongation of the embryonic axis (Gou & Al-Khatib, 2003; Sun *et al.*, 2011). The germination process is rather complex and includes numerous events including protein hydration, reserve mobilization from the endosperm to the axis, sub-cellular structural changes, anaerobic respiration, macromolecular synthesis and cell elongation (Liu & Stutzel, 2002). According to the authors none of these events is unique to germination, but their combined effect is to convert a dehydrated, resting embryo with barely detectable metabolic activity into one that has a vigorous metabolism culminating in growth. It is, therefore, crucial to recognize that germination does not include seedling growth which only commences when germination finishes. A grey area still exists in terms of the demarcation between the two processes, germination and seedling growth (Blazick *et al.*, 2005). Hence, it is scientifically wrong to equate germination with seedling emergence from soil because germination always ends sometime before the seedling emerges from the soil or even the seed coat.

Generally, a seed in which none of the mentioned activities associated with the germination process is taking place is said to be quiescent or dormant. Quiescent seeds are actually resting organs, with low moisture content (5-15%), in which metabolic activity is almost totally at rest (Aufhammer *et al.*, 1998). The unique property of seeds is that they are capable of surviving in a quiescent state, often for many years, and subsequently resume their normal metabolic activity when a suitable environment is provided. For germination to take place, quiescent seeds need to be hydrated under conditions that encourage metabolism, for instance suitable temperature in the presence of oxygen (Baskin & Baskin, 1988).

Baskin & Baskin (1988) further added that germination events may not necessarily lead to emergence of the primary root (radicula) and germination. When conditions are apparently favourable for germination events such as imbibition, respiration, nucleic acid and protein synthesis as well as a host of other metabolic events may proceed while cell elongation does not occur. This failure in cell elongation and radicle protrusion is due to germination inhibitors within the seed that prevent seed from germinating (Hurro & Cees, 1991). When young amaranth seeds are dispersed from the mother plant, germination inhibitors prevent them from germinating immediately after their

dispersal and this is referred to as primary dormancy. Sometimes inhibitors develop in hydrated, mature seeds when they experience a certain unfavourable environmental condition, and this is referred to as induced or secondary dormancy. Dormancy in amaranth seeds can easily be broken by pre-germination priming treatments such as a light stimulus or a period of low alternating temperature. Pre-germination priming conditions undo the role of germination inhibitors allowing the seed to germinate successfully (Baskin & Baskin, 1988, Bewley & Black, 1994).

2.3 Seed germination and seedling growth research

2.3.1 Germination mediums: General

For *in vitro* seed germination testing it is important to note that seeds from various horticultural crops prefer germination mediums that provide enough apertures or spaces for air and water. The ideal medium should at least have high water retention characteristics and provide sufficient aeration needed for normal seed germination (Steckel *et al.*, 2004). However, even by mixing, the water holding capacity of the germination media can be adjusted to fit the water requirements of seeds from a specific plant species. Generally a series of germination media can be employed including paper, pure sand, nutrient agar or mixtures of organic compounds supplemented with minerals (ISTA, 2010).

2.3.2 Paper as germination medium

The paper to be used as a germination material should be the product of wood, cotton or other purified vegetable fibres. Filter paper, paper blotters and paper towels also serve as good germination media. Good paper media allows seedling roots to grow on and not through them and should be strong enough to resist tearing when handled throughout the test (Kendrick & Frankland, 1969; ISTA, 2010). Paper germination medium has been used more often in germination tests of small and medium sized seeds than any other medium (Steckel *et al.*, 2004; Hartmann *et al.*, 2011).

2.3.3 Sand as germination medium

Sand should be uniform and should not contain too large or small particles. Round sand particles are often recommended (ISTA, 2010). It is also recommended that 90% of the sand should be sieved with 0.8 mm mesh. This aids the medium to retain water for the seedling and at the same time provides aeration necessary for further development of the seedlings (Hurro & Cees, 1991).

2.3.4 Nutrient agar as germination medium

Nutrient agar is a germination and growth medium commonly used for the routine cultivation of non-fastidious bacteria and some seed germination testing experiments. It is useful because it remains solid even at relatively high temperatures (Botha *et al*, 1992). Soluble treatment effectors can also be infused in the agar to enable researchers to literally study their effects on seed bacterial growth, seed germination or seedling development. Nutrient agar germination medium provides a favourable germination/growth environment and all nutrients needed for seedling development that represents the seed requirements in natural habitats (Hartmann *et al.*, 2011). Generally, the agar medium is composed of water, a carbon and energy source, a nitrogen source, trace elements and some growth factors. Ingredients may include peptone, casein hydrolysates, meat extract, yeast extract and malt extract (Thomas *et al*, 2006). Besides these elements, the pH of the medium must be set according to the requirements of specific seeds.

2.3.5 Measuring seed germination

The extent to which germination has progressed can be determined roughly, say by measuring water uptake or respiration rate, but these measurements only supply a very narrow identification protocol of the stage at which germination has progressed. It is generally accepted that no universally useful biochemical marker for the progressive events of germination has been found and the only stage of germination that we can easily and precisely time is its termination (Ghorbani *et al.*, 1999).

However, this aspect is not without controversy. The protrusion of the primary root (radicula) through the seed testa is often used to indicate the completion of germination. But, this is probably not totally accurate as the axis could have grown rather vigorously in the adjoining seed tissue (endosperm) before protruding the seed coat. As a result, some researchers prefer to measure the completion of seed germination gravimetrically as soon as a sustained increase in seed fresh weight is observed (Sun *et al.*, 2011). Nevertheless, the protrusion of the testa by the primary root, when oxygen freely enters the seed and anaerobic respiration is replaced by aerobic respiration, was taken as the termination of germination in this study.

The degree to which germination has been completed in a population is usually expressed as a percentage of the number of seeds chosen as spot check and is normally determined at time intervals over the course of the germination period (Bewley & Black, 1994). This data is then used to draw germination curves with germination % over time which is usually sigmoid. The latter means that the minority of seed in the population germinates early, followed by a more or less rapid increase in germination while, finally, relatively few late germinators emerge (Bewley & Black, 1994). The curves are often positively slanted because a greater percentage germinates in the first half of the germination period than in the second. Although the curves have the same general shape, differences in behaviour between seed populations are always visible. For example, if seeds are non-viable then the germination behaviour of the seed population can be related to either dormancy or environmental conditions such as temperature or light which do not favour germination of most of the seeds (Hartmann *et al.*, 2011).

Specifically, in many amaranth seed germination experiments, it has been found that seed lots may be alike as far as germination capacity is concerned, but there still might be a difference in their germination rates (Ghorbani *et al.*, 1999). The rate of germination can be defined as the reciprocal of the time taken for the process to be completed, starting from the time of sowing. Therefore, seed germination rate was recently well-defined as the inverse of the time to radicle emergence or the initiation of embryo growth (Aufhammer *et al.*, 1998). Hartman *at al.*, (2011) defined seed

germination speed as the measure of how rapid a seed lot germinates. Germination rate has also been referred to as the time required for the seed lot to reach a predetermined germination percentage (ISTA, 2010).

Since all the seeds in a population do not experience simultaneous germination, the estimation of germination rate is restrained to single points on the germination progressive curve. For instance, the time required for seeds to reach 10, 30, 50 or 70% germination is based on the final germination percentage. Researchers have developed equations to quantify the germination speed of the seed lots. Aryun & Baywa (2005) used the following equation to estimate seed germination rate: (Germination rate) $G_R = (N_1/1) + (N_2-N_1) \times 1/2 + (N_3-N_2) \times 1/3$, where N is the proportion of the germinated seeds obtained during the first (N₁), second (N₂), and the third (N₃) days of the experiment. In addition, Covell *et al.* (1986) calculated germination rate using the following formula, $1/t(G) = (T - T_b(G))/0t(G)$ where $t(G)$ is the time taken in days for cumulative germination to reach the value G, T is temperature (°C), $T_b(G)$ is the base temperature for the given sub-set (G) of the seed population at which temperature $1/t(G)$ is zero and $0t(G)$ is thermal time (number of day-degrees above $T_b(G)$ required by the seed fraction G to germinate). However, an additional increase in temperature above an optimal $T_o(G)$ was reported to result in a decline of the rate of progress of cumulative germination to G until a maximum temperature is reached where $1/t(G)$ is again zero (Covell *et al.*, 1986).

Apart from the rate of seed germination indicated above, the following parameters have been widely used in measuring germination of several ornamental and vegetable amaranth species. These parameters are: final germination percentage (FGP), germination index (GI), mean time germination (MTG) and mean daily germination (MDG). According to ISTA (2010), the final germination percentage (FGP) can be quantified as follow: $FGP = N_g / N_t \times 100$, where N_g = total number of germinated seeds, N_t = total number of seeds evaluated. OASA (1983) further indicated that germination index (GI) can be estimated as the number of germinated seed/Days of first count+.....+ number of germinated seed / Days of final count. MTG can be predicted according to the formula: $MTG = \sum(n_i \times d_i)/N$ where n_i is the number of germinated seed

at day i , d_i is the incubation periods in days and N is the total number of germinated seeds in the treatment (Sadeghi *et al.*, 2011) and finally, the Mean Daily Germination (MDG) which is an index of daily germination was reported to be calculated from the following equation: $MDG = FGP / d$, where **FGP** is the final germination percentage and **d** is days to the maximum of final germination (Scott *et al.*, 1984).

The calculations of PEG 8000 (polyethylene glycol) concentrations that were used to provide different water potential in the seedling growth media in this study was done based on the standard set by Michael (1983). The seedlings which were germinated under non-PEG concentrated germination media served as a control of the experiment.

2.4 Factors affecting amaranth seed germination

As mentioned earlier, germination of all seed is affected by the viability of the seeds, seed dormancy and adequate environmental factors. If one of the three aspects is not sufficiently considered, germination can be hugely delayed or inhibited, leading to secondary dormancy (Tucker, 1986).

2.4.1 Seed viability

Seed viability represents the ability of non-dormant seeds to germinate and viability testing is essential in determining seed quality. For this purpose it is advisable to use at least 400 seeds sampled at random and divided into lots of 100 each. If any two of these lots vary by more than 10%, a retest should be performed. The official germination percentage is the average of the four tests. Seeds need to be subjected to optimum temperature and light conditions in order to induce germination (Hartmann *et al.*, 2011; ISTA, 2010).

There are many techniques for performing seed viability tests in laboratories that can be chosen from. Amaranth seed tests, for example, are commonly carried out on germination trays, plastic boxes and paraffinic cardboard boxes or covered glass Petri dishes (Tucker, 1986). Blue blotter- and washed paper towels are also regularly used by commercial seed laboratories for seed viability testing. Other mediums include

absorbed cotton, filter paper and sand for larger seeds. Containers are placed in a germinating chamber where environmental factors can be regulated (Shukla *et al.*, 2003).

To avoid growth of micro-organisms in the growing medium, all materials and equipment should be cleaned thoroughly, sterilized if possible, and the water quantity in the germination container cautiously regulated. Neither should the germination medium be so wet that a film of water appears around the seeds (Robert & Robert, 1982). Relative humidity in the germination chamber should be maintained at 90% or higher in order to avoid drying. There is a need to add water during the testing period (ISTA, 2010).

Baskin and Baskin (1988) reported that, generally, viability tests should run from one to four weeks, but could continue for three months in the case of slow germinating and dormant tree seeds. The duration of viability tests for amaranth seeds is 14 days maximum (ISTA, 2010). Amaranth seeds that fail to germinate after 2 weeks can be regarded as either dead or unfilled. The first counting of germinated amaranth seeds is carried out 48 hours after planting and, subsequently, every 24 hours. Steckel *et al.* (2004) reported that viable seedlings are those with well-developed shoots and roots although Ghorbani *et al.* (1999) argued that the criteria for normal viable seedlings differ from species to species. Amaranth seed is considered viable when its radicle has grown longer than the 3 mm (Aufhammer *et al.*, 1998). Importantly, if any fungal growth is observed on even only one seed it should be recorded and the seed removed immediately to avoid fungi growing uncontrollably and jeopardise the experiment (Steckel *et al.*, 2004).

2.4.2 Seed dormancy

Seed dormancy is the condition whereby seeds do not germinate even if they are subjected to favourable conditions that are normally beneficial for germination (Mayer & Mayber, 1995). Seed dormancy prevents germination and manages the time, condition and place that germination will take place (Hartmann *et al.*, 2011). Essentially,

dormancy is an adaptation mechanism that prevents seeds from germinating after it has been dispersed by mother plants and that only permits germination when environmental conditions are favourable (Baskin & Baskin, 1988).

However, seed dormancy differs from plant species to plant species. Those with hard coated seeds, for instance, prevent imbibition to occur and this, in return, prevents germination. Other plant species have seeds of whereof germination is controlled by a phenomenon referred to as physiological seed dormancy. Physiological dormancy is mainly controlled by factors within the embryo that must be altered first before the seed can germinate (Hurro & Cees, 1991). Baskin and Baskin (1988) furthermore that concluded physiologically dormant seeds of many species, especially herbaceous plants, germinate well if the embryo is separated from the seed coat. Obvious reasons are the limiting physical roles of the endosperm and seed coat in preventing the axis from growing. The physical strength of the endosperm and seed covering has been reported to restrict germination in both herbaceous and woody plants. Therefore dormancy in these plant species can be overcome by i) weakening the seed covering, ii) by increasing the growth potential in the embryo or iii) by combining the seed covering and embryo effects (Hartman *et al.*, 2011).

Dormancy in amaranth seed is reported to be high at the time seeds are detached from mother plants, but decline as seed water content decreases (Baskin & Baskin, 1988). Generally, dormancy is at its peak within two to three months after seed harvesting. In amaranth seed dormancy after ripening is associated with natural occurring compounds present in the seed at maturity and oven drying or naturally air drying can help to reduce amaranth seed dormancy (Hartmann *et al.*, 2011).

2.4.3 Environmental factors

Temperature (Thomas *et al.*, 2006), water (Bewley & Black, 1994), gasses and light (Dupriez & Leener, 1989) are the most important environmental factors that influence seed germination.

2.4.3.1 Effect of temperature on seed germination

Temperature is believed to be one of the key environmental factors that control germination initially, simply due to its role in breaking dormancy as well as climatic alteration, and because it affects both germination percentage and the rate of germination (Thomas *et al.*, 2006). Generally, germination rate is consistently low at a low temperature, but elevates progressively as temperature increases similar to a chemical rate reaction curve. Above the most favourable level, where germination rate is most rapid, a decline occurs as the temperature reaches a lethal limit where the seed is injured. Germination percentage, unlike germination rate, may remain relatively constant, at least over the middle part of the temperature range if enough time is allowed for germination to take place (Aufhammer *et al.*, 1998; Hartmann *et al.*, 2011).

In essence, there are three ways whereby temperature acts to regulate germination. Firstly, it determines the capacity and the rate of germination. Secondly, temperature removes primary and secondary dormancy and lastly it induces secondary dormancy (Bewley & Black, 1994). Therefore, a specific crop's sensitivity to temperature limits its germination at a particular time of the year. For instance, *Amaranthus retroflexus* prefer temperatures above 25°C for optimum germination while *Chenopodium album* and *Ambrosia artemisiifolia* both prefer a temperature below 11°C. Out of the three genera *Amaranthus* germinate only in the late spring and early summer while the other two can only germinate during winter (Liu & Stutztel, 2002). Seeds that fail to germinate during early spring may have entered into a state of secondary dormancy and need a chilling period for dormancy to be broken. This control is very important as it helps to regulate germination so that seeds can only germinate when the environmental conditions is favourable for seedling development. The combination effect of low temperature requirements for germination and induced secondary dormancy helps to avoid germination of amaranth seed species during winter and early spring (Bewley & Black, 1994). Amaranth and other seed species whose dormancy is broken by chilling, germinate when the temperature begins to increase in early spring.

Apart from dormancy control, temperature has an effect on the rate of water absorption by seeds during germination. A study conducted on barley and *Amarantus caudatus*

seed germination almost six decades ago indicated that the velocity at which seeds absorbed water was the sole function of temperature. The rate of moisture absorption was also shown to increase with elevated temperature and vice versa (Crocker & Barton, 1953).

Bavec and Mlakar (2002) reported that minimum, optimum and maximum temperatures are the levels regularly selected for seed germination tests and this varies in plant species. The term 'minimum' represents the lowest temperature for effective germination while 'maximum' is the highest temperature at which germination still occurs. Above the maximum, seeds are either injured or enter a dormant state. The term 'optimum' represents the ideal temperature where the largest percentage of seedlings is produced and at the highest rate. Amaranth species can germinate in a temperature range between 15°C and 40°C, although the optimum germination temperature ranges between 24°C and 35°C (Steckel *et al.*, 2004; ISTA, 2010).

Baskin and Baskin (1988) reported further added that native and cultivated seed plant species can be categorized into temperature requirement groups based on their climatic origin. Cool temperature tolerant species are mostly native to temperate zones. Seed from these species can germinate over a wide temperature range from about 4.5°C to the lethal limit of about 45°C. The optimum temperature for cool temperature tolerant plants is usually between 24°C and 30°C. Broccoli, cabbage and carrots are some examples. On the other hand, cool temperature requiring plants are adapted to low temperature regions and seeds of these plants fail to germinate at a temperature higher than 25°C. Species in this category are known as winter crops and their seeds can only germinate in winter while germination is inhibited in late spring or summer. Onions, celery and lettuce are some examples. To the contrary, seeds from warm temperature requiring plants fail to germinate at a temperature below 10°C. The latter crops originated in tropic or sub-tropic regions and include beans, amaranth species, eggplant, pepper and cucumber (Aufhammer *et al.*, 1998; Ghorbani *et al.*, 1999; Hartmann *et al.*, 2011).

Some controversy still exists with regard to preferred temperature by amaranth seeds. More than a decade ago Ghorbani *et al.* (1999) examined the germination of

Amaranthus palmeri seed under different temperatures ranging from 5 to 35°C. The highest germination percentage (85%) was observed at 35°C. More recently, according to Steckel *et al.* (2004) the optimum germination temperature range for amaranth is between 24-30°C while Thomas *et al.* (2006) maintained that amaranth seeds can optimally germinate between 20 and 40°C. Therefore, a need for further research exists in order to find the exact temperature range that best support amaranth seed germination. Most probably species differences in this regard have not been sorted out completely.

2.4.3.2 Seed water up-take

The uptake of water by seed is an important initial step towards its germination. The total amount of water taken up during imbibition is generally fairly small and may not exceed two or three times the dry weight of the seed (Oyedele, 2002). However, to sustain subsequent seedling growth, a larger supply of water is required for establishing the root and shoot systems (Bewley & Black, 1994).

Several factors govern the movement of water from the soil to the seed, but of these the water relationship between seed and soil is particularly central. The term 'water potential' (Ψ) is an expression of the water energy status where the net diffusion of water occurs down an energy gradient from high to low Ψ . Pure water normally has the highest potential and, by convention, it is assigned a zero value. In seed three components or factors determine the Ψ , namely the osmotic potential (Ψ_{π}), pressure potential (Ψ_p) and matrix potential (Ψ_c) (Crocker & Barton, 1953). The sum ($\Psi_{\pi} + \Psi_c + \Psi_p$) of the three terms determines the Ψ .

In short, Ψ_{π} is determined by the concentration of dissolved solutes in water or the cell, Ψ_c , by soil particles or cell wall, starch and protein bodies and their ability to adsorb water and Ψ_p , by internal pressure build up in a cell which exert a force on the cell wall. The sum of the three terms (water potential) is normally negative except in a fully turgid cell where it approaches zero (James, 1973).

The difference in water potentials between seeds and the soil is one of the key factors that determine availability and the rate of water flow into the seed (Liu & Stutzel, 2002).

The authors explained that, at first, the difference in water potential between the dry seed and the moist germination medium is rather large because of the higher matrix potential of the dry coats, cell wall and storage reserves. However, as seed moisture content increases during imbibition and the tissue becomes hydrated, the water potential of the seed increases (becoming less negative) and that of the seed bed surroundings decreases as the water has moved into the seed. Hence the rate of water movement into the seed decline with time (Bewley & Black, 1994).

It is therefore crucial to note that the movement of water uptake into the seed is largely influenced by the properties of the seed as well as by the environment in which the seed is situated. The water potential gradient between the seeds and its surroundings is a driving force for water uptake, but the permeability of the seed to water is more important in determining its rate of uptake. Seed permeability is influenced by morphology, structure, composition, initial moisture content and temperature at imbibition. The rate of water uptake is not necessarily influenced by only one of the above mentioned events, but by their complex interactions (Bryant, 1985; Ghorbani *et al.*, 1999).

2.4.3.3 Gases

Exchange of gas between the germination medium and seed embryos is essential for fast and consistent germination. Nonwithstanding the fact that anaerobic respiration takes place during early germination, oxygen is required by the sprouting seed to switch to aerobic respiration (Pretorius *et al.*, 1998) while the rate of oxygen uptake by the seed eventually dictates the germination progress. Broadly, oxygen uptake by the seed is proportional to the metabolic activities taking place in the seed. Amaranth seeds sprout well in the presence of atmospheric gases because it contains 21% oxygen and the germination rate has been reported to rise when seeds germinate in a high oxygen containing medium (Ghorbani *et al.*, 1999).

The presence of excessive water in the germination medium suppresses the amount of available oxygen inhibiting the minimum respiration rate required for the germination process (Aufhammer *et al.*, 1998; Pretorius *et al.*, 1998). Under poorly aerated field conditions, carbon dioxide can accumulate in the soil as a by-product of respiration and

germination of amaranth seed can be inhibited if the carbon dioxide level exceeds that of oxygen in the medium (Dupriez & Leener, 1989; Ghorbani *et al.*, 1999). It is believed that high carbon dioxide accumulation can slow down germination by maintaining seed dormancy (Steckel *et al.*, 2004).

2.4.3.4 Light

Hartmann *et al.* (2011) reported that light can be involved in both dormancy stimulation and breaking mechanisms and this, together with the role of temperature in this regard, facilitate the adaption of plants to definite niches in their environments. In terms of the effect of light on seed germination, both quality (wavelength) and duration (photoperiod) may play a role. The basic mechanism of sensitivity in seeds involves a photochemically reactive pigment called phytochrome extensively present in plants with small seeds (Hartmann *et al.*, 2011) that is the photo receptor allowing plants to perceive light. Ghorbani *et al.* (1999) reported that the exposure of imbibed seed to red light causes the photo receptor to change to its far-red form (Pfr) which stimulates germination.

The effect of light on amaranth seed germination was extensively studied by Aufhammer *et al.* (1998). Germination of seed from 274 amaranth cultivars was evaluated in both light and darkness. The study revealed that 67% seed of the 274 cultivars germinated well in the presence of light. High germination response of amaranth cultivars to light is due to the ability of light to break dormancy of small seeds like those of amaranth (Dupriez & Leener, 1989).

2.4.3.5 Seed storage

Seeds from different crops can be stored for different periods of time after harvesting. Seed viability at the end of the storage period depends on the initial viability at harvest and the rate of deterioration during storage. Deterioration differs with seed species as well as the storage conditions including temperature and relative humidity (Muyonga *et al.*, 2008).

Seeds can be classified as either recalcitrant or orthodox based on their genetic potential to tolerate storage. Recalcitrant seed are those that do not tolerate seed

moisture below 25% after seed maturation. Then again, orthodox seeds can tolerate drying from 10% down to 4% moisture content after seed development and these differ in the length of time they can tolerate storage (Barker & Duarte, 1998). According to Hartmann *et al.* (2011), orthodox seeds can be further divided into medium-lived and long-lived categories. Medium-lived seeds can remain viable for periods of two to five years provided that seeds are stored at relative low humidity and temperature. Seeds of most vegetables, flowers and grain crops belong to this group. It is important for seed storage to be designed in such a way that it should not create conditions that will negatively affect seed and /or seedling vigour.

2.5 Storage factors affecting germination

During seed deterioration the seed first loose vigour or the ability to germinate when environmental conditions are not favourable. Loss of vigour due to poor storage can result in reduced capacity for normal seed germination and finally low seed viability. Seed deterioration during storage is stimulated by high respiration and other metabolic rates which injure the embryo (Hartmann *et al.*, 2011)

2.5.1 Moisture content

Moisture content in seeds is the most important factor in seed longevity and, therefore, important to consider during storage (Baskin & Baskin, 1988). For example, seed having orthodox characteristics can be best stored at a non-fluctuating low moisture level as they can tolerate low moisture content. Seed moisture content of about 4–6% is suitable for prolonged storage of seed from many vegetable species. However, many storage problems may arise when seed moisture content is elevated during storage (Baskin & Baskin, 1988): i) At about 8-10% moisture content several insects are active and can reproduce, ii) above 12% seed moisture content fungi are active and can multiply to produce spores and iii) at the higher seed moisture content levels respiration, germination and disease activity are stimulated leading to reduced seed viability (Baker & Duarte, 1998).

On the other hand, too low water content in some seeds can have a reducing effect on seed viability and germination rate (Abdullah *et al.*, 2011). For this reason, hydration is necessary for seeds stored at a humidity atmosphere below 2%, to avoid seed injury, as this can influence the moisture content of the stored seed. Conversely, in the case of some species, dry climate increases seed longevity while high relative humidity (RH) results in shorter seed life (Barker & Duarte, 1998). The authors suggested that seed should be stored in sealed moisture resistant containers.

Amaranth seeds can be stored safely for up to three years at a temperature below 8°C and at 10% RH in a tightly closed moisture resistant container (Hartmann *et al.*, 2011). Ideal containers are air-tight such as a sealed glass jars, metal cans or foil envelopes as they maintain seed water content best. Seed in containers should be stored in a cool, shady and dry place to extend seed shelf life (Hartmann *et al.*, 2011).

2.5.2 Storing temperature

The common perception of temperature's effect on stored seed durability is extended as storage temperature decreases and this is especially true for 'orthodox' seeds (McCormack, 2004). According to the author, the relationship between temperature and seed longevity is that for each 5.6°C decrease in temperature, longevity doubles. This law applies to seeds stored between 0 and 50°C assuming that the moisture content is constant.

However, this is merely a general guideline. The actual longevity of some vegetable species decreases faster than recommended by the rule, while the longevity of others declines more slowly in relation to storage temperature. The longevity of seeds is usually not affected by subfreezing temperatures provided the moisture content is less than 11% because high water content forms ice crystals which will result in injury of the seed embryo (Leon *et al.*, 2004). Excellent germination can be still obtained for approximately twenty years from seed stored at -7°C and below if initially dried to about five percent moisture content. This is the ideal way to store seed, especially small seed that does not require much freezer space (Oryokot *et al.*, 1997; Ghorbani *et al.*, 1999;

McCormack, 2004). The bottom-line is that storage conditions should preserve seed vitality and, subsequently, seedling vigour.

2.6. Seedling vigour

Seedling potency or vigour is a complex issue, governed by many physiological parameters that are, *inter alia*, an important attribute of seed vigour. In other words, seed vigour not only plays a crucial role during germination, but also during early seedling establishment (Myers, 1974). Several definitions have been offered to explain the term “seed vigour”. Considering the complexity of the topic, the International Seed Testing Association (ISTA, 2010) has described seed vigour as the “sum total of those properties of the seed that determine the level of activity and performance of the seed during germination and seedling emergence”. From this it is clear that seed vigour and seedling potency is often regarded as one and the same phenomenon. Bretagnolle *et al.* (1995) links seed and seedling vigour by defining seed vigour as “the aspect of seed quality that controls the potential for speedy, uniform emergence and development of normal seedlings under a wide range of field”.

Since germination tests are commonly carried out in laboratories where the conditions can be made optimal for specific plant species, it is not always possible to get an exact indication of the seed lot performance under field conditions on the basis of seed vigour tests. This is because field conditions fluctuates and differ much from that in the laboratory (Shah *et al.*, 1985). It follows that, even though seed quality is believed to be the most important factor that dictates the success or failure of crop establishment, the elements that make up seed quality are still not well understood (Abdul-Baki & Anderson, 1973).

Seed vigour is reported to be compromised during seed harvesting, drying, shipping and most importantly during storage (Abdul-Baki & Anderson, 1973). According to the authors, many researchers have used laboratory germination and seedling growth measurements in the past to predict seed vigour, but time and again these measurement criteria failed. Failure is believed to be caused by an array of

physiological and biochemical events that contribute to a reduction in seed vigour (Shah *et al.*, 1985). Already almost four decades ago Abdul-Baki and Anderson (1973) identified three common biochemical or physiological events that occur during the reduction of seed vigour namely:

- i) reduction of respiratory metabolism,
- ii) increased permeability of cellular membranes as reflected by electron microscope imaging as well as leaching of metabolites from the seeds and
- iii) reduction in polysaccharide and protein content.

Recently Hartmann *et al.* (2011) confirmed that all of these events are correlated with seed vigour, and more specifically the loss thereof. According to the authors, most of the physiological and biochemical events which lead to the loss of seed vigour are rather the result of either temperature or moisture effects.

Despite the factors described above for seed vigour, and the secondary effect it may have on seedling growth, seedling vigour is controlled by specific genes that may or may not be related to stress tolerance (Bretagnolle *et al.*, 1995). However, the authors investigated the genetic control of drought stress tolerance in *Dactylus glomerata* (orchard grass) and concluded that genes contributing to vigour might be different from genes responsible for drought and extreme temperature tolerance. Ten years later the findings of Zhang *et al.* (2005) were more or less in concert with that of Bretagnolle *et al.* (1995) except that the former categorized genes associated with vigour into two types: i) those that only contribute to vigour and ii) those that contribute to both vigour and low temperature or drought tolerance. Hence, exposing seed species to low temperature or drought stress may influence the expression of vigour genes. These genes appear to differ in response to stresses and their relationship has been described as gene-environment (GE) interactions for seedling vigour (Singh *et al.*, 2000). Seedling vigour can be quantified by calculating the seedling vigour index (SVI; Abdul-Baki and Anderson, 1973) by means of the following formula:

$$SVI = \frac{(MRL + MSL) \times MG\%}{100}$$

Where: MRL = mean root length, MSL = mean shoot length and MG% = mean germination %.

2.7 Physiology of seed germination

Demarcation between seed germination and seedling development is rather problematic since it is difficult to determine the end of germination and the exact commencement of the seedling development stage. However, both seed germination and early seedling growth are regulated by distinct physiological processes following water imbibition by the seed that are almost certainly not fully understood. Of these the respiration process is probably the most important initial physiological activity (Hurro & Cees, 1991). The process is crucial simply because it fulfils two essential, but competitive, key roles namely the breakdown of substrates to supply energy and the alteration of substrates to intermediates required for biosynthesis (Botha *et al.*, 1992).

In essence respiration is an energy supplying process and entails a series of events, including glycolysis, the Krebs cycle and the oxidative pentose phosphate (OPP) pathway, depending on the availability of oxygen (Oryokot *et al.*, 1997). Initially a germinating seed experiences a lack of oxygen, due to the impermeability of the seed coat, and anaerobic respiration proceeds where only glycolysis is involved and energy supply is rather limited (Bewley & Black, 1994). However, the availability of O₂ appears to be a critical factor for germination and successful seedling establishment (Crawford, 1978).

Whatever the criterion used by seed technologists to define seed germination, it is only regarded as successful by farmers when seedlings emerge from the soil. In this study, and for this reason, amaranth seeds will be germinated under aerobic conditions and the physiological aspects involved in early seedling growth quantified, rather than that during seed germination.

2.8 Physiology of seedling development

It is common knowledge that abiotic stresses such as drought and extreme temperatures are serious threats to crop production and food security in general. Although there are many abiotic stresses that affect crop production, temperature and water deficit stress are said to be the primary cause of crop losses worldwide. Drought and extreme temperatures were reported to decrease the average yield of several major crops by more than 50% (Bray *et al.*, 2000). Moreover, drought and temperature stresses are believed to be responsible for many physiological, morphological, biochemical and molecular changes that greatly affect plant growth and productivity (Wang *et al.*, 2003).

These stress conditions affect plant species in many ways. As defensive strategies, crops have developed different mechanisms to cope with these stresses. Usually plant species shut their stomata as soon as they experience water shortage in order to prevent further loss of moisture through transpiration (Mould & Rutherford, 1980). Many scientific studies have been conducted on many plant species in an attempt to understand the physiological and biochemical effects of water deficit, but little has been done on amaranth species and more specifically on *A. cruentus* and *A. hybridis*. Special attention will be given to these aspects in this study.

2.8.1 Drought stress and protein synthesis

For plants to adapt to a warm or low moisture environment they require the capacity to survive moderate to extreme drought and heat stress conditions (Wang *et al.*, 2003). The mechanism to achieve this may include either water and heat stress avoidance or tolerance. In this change of gene expression in tolerant plants may result in alteration of nucleic acid structures (point mutation) and/or proline metabolism or both (Valluri *et al.*, 1988). Changes in proline (free amino acid) content in plants under stress have often been used as marker for one or other activated tolerance mechanism (Shao *et al.*, 2006). For this reason, understanding fluctuations in protein profiles during abiotic stress may improve our grasping of drought tolerance and crop responsiveness to

stress. In this regard the effect of drought stress on protein content or profile was reported in soya bean (Dasgupta & Bewley, 1984), maize (Berver & Talvis, 1990) and amaranth (*A. caudatus*) (Liu & Stutzel, 2002).

Furthermore, a strong correlation exists between the synthesis of so-called heat shock proteins (HSPs) and thermo-tolerance (Queitsch *et al.*, 2000). It has been widely assumed that the purpose of the heat shock response (HSR) is to protect organisms from the detrimental effects of heat and/or drought stress (Thomas *et al.*, 2006). Thermo treatment is often described as “the capacity of an organism to tolerate an otherwise lethal heat treatment when pre-heated with some appropriate non-lethal heat shock or an agent that induces a heat shock response, such as arsenate” (Key *et al.*, 1987). Thermo tolerance was reported to be a universal characteristic in many plant species, including amaranth (Lindquist & Craig, 1988). Interestingly, a common exhibition of HSPs was reported in amaranth, maize and potato plants under stress (Vierling *et al.*, 1989).

2.8.2 Changes in free proline levels during drought stress

Accumulation of proline in various plant species subjected to hyperosmotic stress conditions has been studied for more than four decades (Kishor *et al.*, 2005). Proline is one of the most water soluble amino acids that most of the time exists in a zwitterion state comprising both weak negative and positive charges at the carboxylic acid and nitrogen groups, respectively. It possesses this property in common with other compounds which are jointly referred to as “compatible solutes” that are accumulated by a wide range of organisms to adjust cellular osmolality (Verslues & Sharma, 2010). Although proline can also be synthesized under normal conditions, the glutamate shunt is predominant in plants subjected to a water shortage or nitrogen deficit (Hong-Bo *et al.*, 2008).

It is generally believed that proline accumulation plays a crucial role in the adaptation of plant cells to osmotic stress. This role is said to include the stabilization of proteins, membranes and subcellular structures as well as protecting cellular functions by scavenging reactive oxygen species (Russell *et al.*, 1998). Other aspects, such as

cellular redox buffering, are also reported to be important in the overall functioning and/or regulation of proline metabolism. However, some physiologists (Kishor *et al.*, 2005) are still uncertain about whether the accumulation of proline furnishes some adaptive advantages or whether it is simply produced as a result of changes in metabolism due to stress. Proline metabolism, therefore, remains of great research interest to individual scholars who are seeking to understand the physiology and metabolic regulation of plants under stress (Verslues & Sharma, 2010).

2.8.3 Photosynthesis and chlorophyll fluorescence in drought stressed plants

Photosynthesis is a crucial process that supports crop growth and development. It has been reported that photosynthesis is sensitive to drought stress in many higher plants species (Maksymiec & Baszynski, 1996). It is, therefore, not surprising that scores of research projects on plant photosynthesis under stress conditions have been undertaken in the past. However, this has not been free of criticism due to conflicting results. The latter depended heavily on the plant material used and the experimental procedures followed for investigating this parameter (Shangguan *et al.*, 2000). Further, it is still not clear how and where the chloroplast suffers damage under drought stress and how the injury can be detected and easily assessed (Rong-hua *et al.*, 2006).

The initial response of a plant to drought stress is an increase in abscissic acid (ABA) levels that, in turn, regulate the closure of stomata in an attempt to reduce water loss (Bowler *et al.*, 1992). However, this also has an effect on gas exchange, and especially CO₂ absorption, that adversely affect the photosynthesis rate (Orgen, 1990). Moreover, the reported decline in photosynthesis rate due to drought stress is also ascribed to reduced chloroplast activities and the weakening of carbon assimilation (Slabbert & Van den Heever, 2007). According to the authors chloroplasts are known to be severely affected by drought stress.

More than a decade ago Maksymiec and Baszynski (1996) reported that, in general, the photosynthesis rate declines as leaf water potential decreases. Although the decrease in photosynthetic activities has been attributed to stomatal closure in the past, some

evidence has shown that the loss in chloroplast activity may be more limiting than stomatal closure at a low water potential (Gou & Al-Katib, 2003). Stomatal behaviour at low water potential is likely to be under some form of metabolic control while a decrease in chloroplast activity, with an accompanying decline in photosynthesis rate, involves inhibited electron transport and photophosphorylation (Rong-hau et al., 2006). According to the authors the latter is associated with alteration of the thylakoid membranes under stress.

However, translocation of photosynthate (sucrose) from the source (leaves) to sinks was reported to be continuing at a low water potential despite the loss of photosynthetic activity in leaves (Zlatev & Yordanov, 2004). Experiments conducted by the authors both in the laboratory and under field conditions on beetroot revealed that leaf water potential, which is low enough to result in the cessation of dry matter accumulation by the whole plant, nevertheless allowed the accumulation of dry matter in other parts of the plant, including developing leaves. Additionally, the authors observed that losses in photosynthetic activity at a low water potential accelerated leaf senescence. Already three decades ago Paleg & Aspihall (1981) observed that leaf senescence occurs more rapidly at low than high water potential conditions and this is believed to be the result of partially wilted leaves (photosynthetic surface) from the crop canopy.

Chlorophyll fluorescence is a useful technique to quantify photosynthesis capacity (Anderson *et al.*, 1997) and can be used to detect plant stress in crops (Earl & Tollenaar, 1999). Under water stress conditions they observed that PSII quantum efficiency and electron transport rates were 25% lower for stressed plants versus non-stressed plants. The so-called JIP test is used that quantifies several fluxes, for example the absorbance of photons by the reaction centre at PSII, the trapping of excited electrons as well as the transfer of electrons through the photosynthetic electron transport chain to eventually produce an energy rich molecule, NADPH (Hura *et al.*, 2007).

By means of chlorophyll fluorescence measurements Havaux (1992) showed that within the photosynthetic apparatus, photosystem II seems to be thermo sensitive, while photosystem I activity, stomatal enzymes and the chloroplast envelope are

comparatively more thermo stable. Changes in leaf water potential affect the thermal tolerance of the photosynthesis process as a whole (Seemen *et al.*, 1986; De Ronde *et al.*, 1999). Dannehl *et al.* (1995) reported that high temperature regimes or moderate drought conditions can diminish the activity of photosystem II reaction centres, probably via degradation of enzyme proteins, leading to a reduction in photosynthesis capacity (Dannehl *et al.*, 1995). In this study the photosynthetic response of amaranth to heat and drought stress was, *inter alia*, followed by measuring chlorophyll levels and the total photosynthesis rate.

2.8.4 Respiration

While photosynthesis is the primary metabolic process that produces carbohydrates in all green vegetation, respiration is the follow-up process that releases the chemical energy required for growth and development. Soluble sugars, lipids, organic acids and proteins all serve as respiratory substrates (Graham *et al.*, 2007). Importantly, a relationship between plant growth and respiration exist which may assist in understanding the resistance or susceptibility of a crop to drought (Ren *et al.*, 2011). This implies that respiration, or at least the energy supplied via the process, may be part of the resistance mechanism towards stress in plants. The latter supplied the rationale for following respiratory activities in amaranth seedlings under stress in this study.

A textbook description of the overall respiration process involves a series of oxidation-reduction reactions whereby compounds are completely broken down to CO₂ and water while energy is released in the form of ATP and NADH (Pinheiro & Chaves, 2011). Especially carbohydrates such as starch, sucrose, glucose and fructose, but also lipids and protein can serve as respiratory substrate. However, the respiration rate in plant tissues is related to both substrate supply and energy demand (Dwivedi, 2000). Rakhmankulova *et al.* (2001) maintained that respiration plays a role in plant adaptation to adverse conditions. It is, therefore, no surprise that one of the major physiological changes during both seed germination and early seedling development is a rapid increase in the rates of energy supplying respiratory pathways such as glycolysis, the oxidative pentose phosphate (OPP) pathway and the tri-carboxylic or Krebs cycle

(Muscolo *et al.*, 2001). All three these metabolic pathways are finely regulated within a living cell (Côme & Corbineau, 1989).

Glycolysis is the predominant pathway during seed germination due to the lack of oxygen (Nardi *et al.*, 2007). It is a metabolic pathway that involves a series of reactions leading to the breakdown of glucose to two molecules of pyruvic acid while energy is transferred to high energy bio-molecules such as ATP and NADH that act as cellular energy sources. More specifically phosphofructokinase (PFK; EC 2.7.1.11), involved in the phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphate, is regarded as the main regulatory enzyme of glycolysis (Wong *et al.*, 1987). It acts as the main control point in the glycolytic pathway as it is immediately downstream of the entry points for hexose sugars (Pretorius & Small, 1992). The activity of PFK is inhibited when citric acid, phospho-enol pyruvic acid (PEP) and ATP levels are high and activated when ADP and AMP levels are high (Salisbury & Ross, 1991). In this way the rate of glycolysis as well as the reverse cycle, gluconeogenesis, is regulated depending on the energy needs of the crop.

Subsequently, pyruvate is completely broken down during the Krebs cycle if sufficient O₂ is available. The Krebs cycle is the second step of aerobic respiration that takes over from the glycolysis pathway by completely breaking down pyruvic acid to CO₂, H₂O and energy (Alisdair *et al.* 2004). Citrate synthase and isocitrate dehydrogenase are the main regulatory enzymes of the pathway (Ophardt, 2003) that are allosterically inhibited by high ATP and NADH levels (Nardi *et al.*, 2007).

The oxidative pentose phosphate pathway (OPP) is seen by many physiologists as an alternative respiratory pathway (Pretorius and Small, 1992). Despite its energy providing function, especially the high energy bio-molecule NADPH, it also supplies ribose-5-phosphate necessary for DNA and RNA synthesis as well as erythrose-4-phosphate necessary for the synthesis of essential amino acids (Salisbury & Ross, 1991). The most important regulatory enzyme of the OPP pathway is glucose-6-phosphate dehydrogenase involved in the first oxidative reaction where glucose-6-phosphate is

converted to 6-phosphogluconate (Hauschild & Von Schaewen, 2003). This enzyme is regulated by NADPH levels within the cell.

In summary, the supply of substrate as well as the demand for energy dictates the rate of respiration in seedlings, while sugar serves as the major source of energy and carbon flux (Dwivedi, 2000). The oxidative pentose phosphate (OPP) pathway and the first step of aerobic respiration, glycolysis, are the two major biochemical pathways that are regulated during seed germination and early seedling development (Côme & Corbineau, 1989). In active growing seedlings regulation of the second step of aerobic respiration, the Krebs cycle becomes equally important. Regulation is via key enzymatic events that control the rate of the entire respiration process depending on the demand for energy (Rakhmankulova *et al.*, 2001). Undoubtedly energy supplying pathways and fine regulation thereof play a pivotal role in resistance mechanisms towards abiotic and biotic stress factors (Muscolo *et al.*, 2001; Fernie *et al.*, 2004).

In this study the respiration rates of two amaranth species subjected to heat and drought stress were measured and compared. Additionally, *in vitro* activity of one regulatory enzyme from each of the OPP-pathway and glycolysis in the two amaranth species under stress was compared.

2.8.5 Effect of drought stress on sugar accumulation

Water soluble sugars such as sucrose, glucose and fructose play a pivotal role in plant structure and metabolism at the cellular and whole plant levels (Couée *et al.*, 2006). Measuring sugar levels in plants can therefore be a handy indication of the potential energy status of a crop as well as the potential substrate availability for normal growth and development at a specific time interval (Pretorius & Small, 1992).

Further, accumulation of sugars is believed to, *inter alia*, promote drought tolerance in plants by contributing towards maintaining cell turgor through osmotic adjustment (Couée *et al.*, 2006).

Drought stress remains the main limiting factor for sustainable crop production in arid and semi-arid regions around the globe (Bianco *et al.*, 2000). Understanding physiological responses of the plant under water deficit conditions may, therefore, aid in comprehending mechanisms involved in tolerating water shortages (Barnabas *et al.*, 2008). Accumulation of carbohydrates is at least believed to be part of such a tolerance mechanism or adaptation of a crop to drought stress (Kerepesi & Galiba, 2000). According to the authors, sucrose is believed to replace water and maintain membrane phospholipids in a liquid-crystalline state and avoid structural membrane changes during drought. The monosaccharides glucose and fructose are well known respiratory substrates that are probably mostly responsible for maintaining a healthy energy state during both normal and abnormal growth conditions (Roover *et al.*, 2000).

However, although in an indirect fashion, fructose might be more implicitly involved in a tolerance mechanism towards drought stress via the production of fructans. Fructans act as a carbohydrate reserve in seedlings and has been considered to be involved in inducing tolerance towards drought stress in many plant species including amaranth (Roover *et al.*, 2000). Fructans are polymers of fructose molecules linked via sucrose molecules believed to protect membranes of cell organelles against the adverse effects of drought (Anonymous, 2012). Pilon-Smits *et al.* (1995) believed that fructans have a direct influence on the plant's growth process.

2.9 Summary and the way forward

In Africa amaranth has been an important food source for many years although its cultivation in terms of hectare numbers probably still falls in the lower category. Both leaves and grains are consumed making it an ideal alternative crop. Additionally, some medicinal uses of amaranth plants have been documented. Technically amaranth is not a new crop. However, due to its nutritional potential from a food security perspective, a rationale exists for studying amaranth as an alternative crop in an attempt to learn more about the plant.

Although abiotic stress conditions commonly limit the development and productivity of major crop species, vegetable amaranth has been shown to be rather tolerant. However, this general statement has not been verified for all 70 known amaranth species. This study will concentrate mainly on the growth and physiological response of seedlings from two amaranth species, *A. cruentus* and *A. hybridis*, to temperature and drought stress.

In chapter three, the study examined the effects of drought and temperature stress on seed germination and early growth of amaranth seedlings. Seedling roots and shoots hypocotyl growth (length and dry matter production) as well as the total seedlings dry mass of the seedling was quantified. All these efforts were conducted to establish how water deficits and high temperature effects germination and early seedling growth of the two amaranth species (*A. cruentus* and *A. hybridis*). In chapter four, the researcher examined the physiological effects of water and temperature stress on total soluble water protein and sugars (glucose, fructose and sucrose). The study further observed water shortage and temperature effects on photosynthesis and respiration rates as well as on the two main enzymes of glycolysis and OPP-pathway (phosphofructokinase and glucose-6-phosphate dehydrogenase). A general discussion or integration of results follows in chapter five.

2.10 References

- ABDUL-BAKI, A. & ANDERSON, J.D. 1973.** Vigour determination in Soybean seed by multiple criteria. *Crop Science* 13, 630-633.
- ABDULLAH, M. A., ABDULLAH, A. A., SAFWAT, O. K., MAHMOUD, A., W., MAHRAN, E.N. & ABDULLAH, A. I. 2011.** Influence of storage conditions on seed quality and longevity of four vegetable crops. *Agriculture & Environmental Science* 11 (3), 353-359.
- AKANBI, W.B. & TOGUN, A.O. 2002.** The influence of maize-Stover compost and nitrogen fertilizer on growth, yield and nitrogen uptake of Amaranths. *Scientia Horticulturae* 93, 1-8.
- ALISDAIR, F., CARRARI, F. & SWEETLOVE, L.J. 2004.** Respiratory metabolism: Glycolysis, the TCA cycle and mitochondrial electron transport. *Plant Biology* 7, 254-261.
- ANDERSON, J.M., PARK, Y.I. & CHOW, W.S. 1997.** Photo-inactivation and photoprotection of photosystem II in nature. *Plant Physiology* 100, 214-223.
- ANONYMOUS, 2012.** www.croplangenetics.com/FINDSEED/CORN/ECMDO14190. (Accessed June 2012).
- AOSA (ASSOCIATION OF OFFICIAL SEED ANALYSTS). 1983.** Testing handbook: contribution. Zurich. pp 88.
- ARYUN, C.P. & BAYWA, P.K. 2005.** Effects of soil water shortage and low temperature on seed germination of maize (*Zea mays*) cultivars under glasshouse conditions. *Oecologia* 30, 234-243.
- AUFHAMMER, W., CHUCZOROVA, H.P.K. & KRUSE, M. 1998.** Germination of grain amaranth (*A. hypochondriacus* x *A. hybridus*): Effects of seed quality, temperature, light, and pesticides. *Journal of Agronomy* 8, 127-135.

- AYNEHBAND, A. 2008.** Cultivar and nitrogen Splitting Effects on Amaranth forage yield and weed Community. *Pakistani Journal of Biological Sciences* 11(1), 80-85.
- BARKER, L. A. & DUARTE, P. R. 1998.** Retrogradation of amaranth starch at different storage temperatures and the effects of salt and sugars. *Cereal Chemistry* 75(3), 308-314.
- BARNABAS, B., JAGER, K. & FEHER, A. 2008.** The effect of drought and heat stress on the reproductive process in cereals. *Plant Cell and Environment* 31, 11-38.
- BASKIN, C.C. & BASKIN, J.M. 1988.** Germination ecophysiology of herbaceous plant species in a temperate region. *Botanical Society* 72(2), 286-305.
- BAVEC, T. & MLAKAR, S.G., 2002.** Effects of soil and climate condition on emergence of grain amaranths. *Journal of Agronomy* 17, 93-103.
- BERVER, A. & TARVIS, R.L. 1990.** Effects of NaCl and mannitol on plasma membrane proteins in corn roots. *Plant Physiology* 155, 76-84.
- BEWLEY, J.D. & BLACK, M. 1994.** **Seeds:** Physiology of Developments and Germination. Prenum Press, New York, USA. 147-153.
- BIANCO, R.L., RIEGER, M. & SUNG, S.J. 2000.** Effect of drought on sorbitol and sucrose metabolism in sinks and sources of peach. *Physiologia Plantarum* 108, 71-78.
- BLAZICK, F.A., WARREN, S.L., NASH, D.L. & REECE, W.M. 2005.** Seed germination of Seabeach Amaranth (*Amaranthus pumilus*) as influenced by stratification, temperature and light. *Environmental Horticulture* 23, 33-36.
- BOTHA, F.C., POTGIETER, G.P. & BOTHA, A.M. 1992.** Respiratory metabolism and gene expression during seed germination. *Plant Growth Regulation* 11, 211-224.
- BOWLER, C., VAN MONTANGU, M. & INZE, D. 1992.** Superoxide dismutase and stress tolerance. *Plant Molecular Biology* 43, 83-116.

- BRAINARD, D. C., A. DITOMMASO, AND C. L. MOHLER. 2006.** Intraspecific variation in germination response to ammonium nitrate of Powell amaranth (*Amaranthus powellii*) seeds originating from organic vs. conventional vegetable farms. *Weed Science* 54, 435–442.
- BRAY, E.A., BAILEY-SERRES, J. & WERETILNYK, E. 2000.** Responses to abiotic stresses. In: *Biochemistry and Molecular Biology of Plants* (Eds. **B.B. BUCHANAN, W. GRUISSEM & R.L. JONES**), pp.1158–1203. American Society of Plant Physiologists, Rockville, MD, USA.
- BRETAGNOLLE, F., THOMPSON, J.D. & LUMARET, R. 1995.** The influence of size variation on seed germination and seedling vigour in diploid and tetraploid *Dactylis glomerata* L. *Annals of Botany* 76, 607-615.
- BRYANT, J.A. 1985.** Seed Physiology. The Institute of Biology, Edward Arnold, London. pp 257-280.
- COASTEA, M. & DEMASON, D.A. 2001.** Stem morphology and anatomy in *Amaranthus* L. (Amaranthaceae), Taxonomic Significance. *Terry Botany* 128(3), 254-281.
- CÔME, D. & CORBINEAU, F. 1989.** Some aspects of metabolic regulation of seed germination and dormancy. In: R. B. Taylorson (Ed.). *Recent advances in the development and germination of seeds*. Plenum Press, New York, USA. pp 165-179.
- COUÉE, I., SULMON, C., GOUESBET, G. & EL-AMRANI, A. 2006.** Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. *Experimental Botany* 57(3), 449-459.
- COVELL, S., ELLIS, R.H., ROBERTS, E.H. & SUMMERFIELD, R.J. 1986.** The influence of temperature on seed germination rate in grain legumes. *Experimental Botany* 37(178), 705-715.

- CRAWFORD, R.M.M. 1978.** Metabolic adaptations to anoxia. In: Plant life in anaerobic environments. D.D. Hook & R.M.M. Crawford (Eds.). Ann Arbor Science Publishers, Michigan. pp. 119-136.
- CROCKER, W. & BARTON, L.V. 1953.** The Physiology of Seeds: an introduction to the experimental study and germination problems 2nd ed., Chronica Botanica Co., Waltham. pp 1-4.
- DANNEHL, H.A., HERBIK, A. & GODDE, D. 1995.** Stress- induced degradation of the photosynthetic apparatus is accompanied by changes in thylakoid protein turnover and phosphorylation. *Plant Physiology* 93,179–86.
- DASGUPTA, J. & BEWLEY, J.P. 1984.** Variations in protein synthesis in different regions of greening leaves of barley seedlings and effects of imposed water stress. *Experimental Botany* 35, 1450-1459.
- DE RONDE, J.A., VAN DER MESCHT, A., LAURIE, R.N., SPREETH, M.H. & CRESS, W.H. 1999.** Molecular and physiological approach to drought tolerance for selected crops. ARC-Roodeplaat, Pretoria.
- DIELEMAN, A. HAMILL, A.S., FOX, G.C. & SWANTON, C.J. 1997.** Decision rules for post emergence control of pigweed (*Amaranthus* spp) in soybean (*Glycine max*). *Weed Science* 44, 126-132.
- DUPRIEZ, H. & P. LEENER, D.E. 1989.** African Gardens and Orchards: Growing Vegetables and Fruits. Macmillians, London., 282-287.
- DWIVEDI, P. 2000.** Regulation of root respiration and sugar mediated gene expression in plants. *Current Science* 78 (10), 1196-1202.
- EARL, H.J. & TOLLENAAR, M. 1999.** Using chlorophyll fluorescence to compare photosynthetic performance of commercial maize (*Zea Mays* L.) hybrids in the field. *Field Crops Research* 61: 201-210.
- EHLELINGER, J.R. 1983.** Characterization of *Amaranthus caudatus*: Morphology, ecophysiology, and field observations. *Oecologia* 57, 303-310.

- EHLERINGER, J.R. & CERLING, T.E. 2002.** C3 and C4 Photosynthesis. *Encyclopedia of Global Environmental Change* 5, 186–190.
- FERNIE, A.R., CARRARI, F. & SWEETLOVE, L. 2004.** Respiratory metabolism: Glycolysis, the TCA cycle and mitochondrial electron transport. *Current Opinions in Plant Biology* 7, 254-261.
- GHORBANI, R., SEEL, W. & LEIFERT, C. 1999.** Effects of environmental factors on germination and emergency of *Amaranthus retroflexus*. *Weed Science* 48, 505-510.
- GOU, P. & AL-KHATIB, K.A. 2003.** Temperature effects on germination and growth of redroot pigweed (*Amaranthus retroflexus*), Palmer Amaranth (*A. palmeri*), and common waterhemp (*A. rudis*). *Weed Science* 51, 869-879.
- GRAHAM, J.W.A., WILLIAMS, T.C.R., MORGAN, M., FERNIE, A.R., RATCLIFFE, R.G. & SWEETLOVE, L.J. 2007.** Glycolytic enzymes associate dynamically with mitochondria in response to respiratory demand and support substrate channeling. *Plant Cell* 19: 3723–3738.
- HARTMANN, H.T., KESTER, D.E., JR, F.T.D. & GENEVE, R.L. 2011.** Plant propagation principles and practices, 7th Edition. Prentice Hall Publishers, New Jersey. pp 281-340.
- HAUSCHILD, R. & VON SCHAEWEN, A. 2003.** Differential regulation of glucose-6-phosphate dehydrogenase isozyme activities in potato. *Plant Physiology* 133, 47-62.
- HAVAUX, M. 1992.** Stress tolerance of photosystem II *in vivo*: antagonistic effects of water, heat, and photoinhibition stresses. *Plant Physiology* 100, 424-432
- HONG-BO, S., LI-YE, C., MING-AN, S., SHI-QING, L. & JI-CHENG, Y. 2008.** Bioengineering plant resistance to abiotic stresses by the global calcium signal system. *Biotechnology Advances* 26, 503–510.

- HURA, K., KAJITA, R., TORII, K.U., BERGMANN, D.C. & KAKIMOTO, T. 2007.** The secretory peptide gene EPF1 enforces the stomatal one cell-spacing rule. *Genes Developments* 21, 1720–1725.
- HURRO, J.B. & CEES, M.K. 1991.** The dual role of temperature in the regulation of the seasonal changes in dormancy and germination of seeds of *Polygonum*. *Oecologia* 90, 88-94.
- ISTA (INTERNATIONAL RULES FOR SEED TESTING ASSOCIATION). 2010.** Bassersdorf, Switzerland.
- JAMES, W.O. 1973.** An introduction to plant physiology. Oxford University Press, Great Britain. pp 57-69.
- KENDRICK, R.E. & FRANKLAND, B. 1969.** Photo control of germination in *Amaranthus caudatus*. *Planta (Bern)* 85, 326-339.
- KEREPESI, I. & GALIBA, G. 2000.** Osmotic and salt stress induced alteration in soluble carbohydrate content in wheat seedlings. *Crop science* 40, 482-487.
- KEY, J.L., NAGAO, R.T., CZARNECKA, E. & GURLEY, W.E. 1987.** Heat stress expression and structure of heat shock protein genes. *In: Plant Molecular Biology. D. VON WETTESTEIN & N-H. CHUA, (Eds.).* Plenum Publishing Corporation. pp 385 – 397.
- KIGEL, J., OFIR, M. & KOLLER, D. 1977.** Control of germination responses of *Amaranthus retroflexus* L. seed by their parental photo thermal environment. *Experimental Botany* 106, 1125-1136.
- KISHOR, P.B.K., SANGAM, S., AMRUTHA, R.N., LAXMI, P.S., NAIDU, K.R., RAO, K.S. 2005.** Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. *Current Science* 2005; 88:424–38.

- LEON, R.G., KNAPP, A.D. & OWEN, M.D.K. 2004.** Effect of temperature on the germination of common waterhemp (*Amaranthus tuberculatus*), Giant Foxtail (*Setaria faberi*), and Velvetleaf (*Abutilon theophrasti*). *Weed Science* 52, 67-73.
- LINDGUIST, S. & CRAIG, E.H. 1988.** The heat shock proteins. *Plant Genetics* 22, 631-642.
- LIU, F. & STUTZEL, H. 2002.** Leaf water relations of vegetable amaranth (*Amaranthus* spp.) in response to soil drying. *Journal of Agronomy* 16, 137-150.
- MAKSYMIEC, W. & BASZYNSKI, T. 1996.** Chlorophyll fluorescence in primary leaves of excess Cu treated runner bean plants depends on their growth stages and the duration of Cu-action. *Plant Physiology* 149, 196-200.
- MAYER, A.M. & MAYBER, A.P. 1995.** The germination of seeds, 2nd Edition. Pergamon Press, United Kingdom. pp 237-247.
- McCORMACK, J.H. 2004.** Principles and practices of seed harvesting, processing and storage: an organic seed production manual for seed growers in the Mid-Atlantic and Southern U.S. (www.carorinafarmstewards.org). (Accessed 17/09/2010).
- MICHAEL, B.E. 1983.** Evaluation of the water potentials of solutions of polyethylene glycol 8000 both in absence and presence of other substances. *Plant Physiology* 72, 66-70.
- MODI, A.T. 2006.** Growth temperature and plant age influence on nutritional quality of amaranthus leaves and seed germination capacity. *Water SA* 33, 0378-4738.
- MORAN, P.J. & SHOWELER, A.T. 2005.** Plant response to water deficit and shade stresses in pigweed and their influences on feeding and oviposition by waterhemp. *Environmental Entomology* 34, 929-937.
- MOULD, R.D. & RUTHERFOLD, R.J. 1980.** The effects of moisture stress during consecutive growth stages on tuber yield and quality of BPI potatoes (*Solanum tuberosum* L) *Crop Productions* 9, 89-95.

MUSCOLO, A., PANUCCIO, M.R. & SIDARI, M. 2001. The effect of phenols on respiratory enzymes in seed germination respiratory enzyme activities during germination of *Pinus laricio* seeds treated with phenols extracted from different forest soils. *Plant Growth Regulation* 35, 31-35.

MUYONGA, J.H., NABAKABYA, D., NAKIMBUNGWE, D.N. & MASINDE, D. 2008. Effects to promote Amaranth production and consumption in Uganda to fight malnutrition. *In: G.L. Robertson & J.R. Lubien* (Eds.). *Food Sciences & Technology*. London, United Kingdom. pp 34-48.

MYERS. M.J. 1974. The physiology of eight selected field crops. Standard publishers, London, United Kingdom. pp 264-271.

NARDI, S., MUSCOLO, A., VACCARO, S., BAIANO,, S., SPACCINI, R. & PICCOLO, A. 2007. Relationship between molecular characteristics of soil fractions and glycolytic pathway and Krebs cycle in maize seedling. *Soil Biology and Biochemistry* 39 (12), 3138-3146.

OPHARDT, C.E. 2003. Virtual Chembook, Elmhurst College, Elmhurst, IL, USA.

ORGEN, E. 1990. Evaluation of chlorophyll fluorescence as a probe for drought stress in willow leaves. *Plant Physiology* 93, 1280-1285.

ORYOKOT, J.O.E., MURPHY, S.D., THOMAS, A.G. & SWANTON, C.J. 1997. Temperature and moisture dependent models of seed germination and shoot elongation in green and redroot pigweed (*Amaranthus powellii*, *Amaranthus retroflexus*). *Weed Science* 45, 488-496.

OYEDELE, V.I. 2002. Influence of nitrogen on yield of *Amaranthus* species. *Agronomy Research* 56, 118-121.

PALAND, M.C. & CHANG, L.C. 2003. Suggested cultural practices for vegetable amaranth. *Food Chemistry* 34, 234-245.

PALEG, L.G. & ASPIALL, D., 1981. The physiology and biology of drought resistance in plants. Academic press, Sydney, Australia. pp 57-62.

- PEDRO, W.E., RATIKANTA, M., GRACIELA, C.D., DIANA, I.G. & FERNANDO, S.A. 1995.** Contribution to the botany and nutritional value of some wild *Amaranthus* species (Amaranthaceae) of Nuevo Leon, Mexico. *Economic Botany* 49(4), 423-430.
- PILON-SMIT, E.A.H., EBSKAMP, M.J.M., PAUL, M.J., JEUKEN, M.J.W., WEISBEEK, P.J. & SMEEKENS, S.C.M. 1995.** Improved performance of transgenic fructan-accumulating tobacco under drought stress. *Plant Physiology* 107, 125-130.
- PINHEIRO, C. & CHAVES, M.M. 2011.** Photosynthesis and drought: Can we make metabolic connections from available data? *Experimental Botany* 62(3), 869–882.
- PRETORIUS, J.C., & SMALL, J.G.C. 1992.** The effect of soaking injury in bean seeds on aspects of the oxidative pentose phosphate pathway in embryonic axes. *Seed Science Research* 2, 33-39.
- PRETORIUS, J.C., SMALL, J.G.C. & FAGERSTEDT, K.V. 1998.** The effect of soaking injury in seeds of *Phaseolus vulgaris* L. on germination, respiration and adenylate energy charge. *Seed Science Research* 8, 17-28.
- QUEITSCH, C., HONG, S.W., VIERLING, E. & LINDQUIS, S. 2000.** Heat shock protein 101 plays a crucial role in thermotolerance in Arabidopsis. *American Society of Plant Physiologists* 12, 479–492.
- RAKHMANKULOVA, Z.F. FEDYAEV, V.V, PODASHEVKA, O.A. & YU.USMANOV, I. 2001.** Alternative respiration pathways and secondary metabolism in plants with different adaptive storage under mineral deficiency. *Plant Physiology* 50 (2), 206-212.
- REN, S., WEEDA, S., AKANDE1, O., GUO, Y., RUTTO, L. & MEBRAHTU, T. 2011.** Drought tolerance and AFLP-based genetic diversity in purslane (*Portulaca oleracea* L.). *Biological Research* 3:51-61.

- ROBERT, D.W. & ROBERT, E.H. 1982.** The effects of naturally occurring phenolic compounds on seed germination. *Weed Science* 20, 206-212.
- RONG-HAU, L., PIE-GUO, G., BAUM, M., GRANDO, S. & CECCARELLI, S. 2006.** Evaluation of chlorophyll content and fluorescence parameters of drought tolerance in barley. *Science Direct* 5(10), 751-757.
- ROOVER, J.D., VANDENBRANDEN, K., LAERE, A.V. & VAN DEN ENDE, W. 2000.** Drought induces fructan synthesis and 1-SST (Sucrose: sucrose fructosyltransferase) in roots and leaves of chicory seedling (*Cichorium intybus* L.). *Planta* 210, 808-814.
- RUSSELL, B.L., RATHINASABAPATHI, B. & HANSO, A.D. 1998.** Osmotic stress induces expression of choline monooxygenase in sugar beet and amaranth. *Plant Physiology* 116, 859-865.
- SADEGHI, H., FARDIN, K., LIELA, Y. & SAMAN, S. 2011.** Effect of seed osmopriming on seed germination behaviour and vigour of soybean (*Glycine max* L.). *Agricultural and Biological Science* 6(1), 39-43.
- SALISBURY, F.B. & ROSS, C.W. 1991.** *Plant Physiology*. 4th Edition, Wadsworth Publishing Company, Belmont, California. pp 254-263.
- SCOTT, S.J., JONES, R.A. & WILLIAMS, W.A. 1984.** Review of data analysis methods for seed germination. *Crop Science* 24, 1192-1199.
- SEEMEN, J.R., DOWNSTON, W.J.S. & BERRY, J.A. 1986.** Temperature and leaf osmotic potential as factors in the acclimation of photosynthesis to high temperature in desert plants. *Plant Physiology* 80, 926-930.
- SHAH, M., KHATTAK, J.K. & RIZVI, S.A. 1985.** Effect of boron and zinc fertilization on the yield of maize. *Sarhad Journal of Agriculture* 1, 205–209.
- SHANGGUAN, Z., SHAO, M. & DYCHMANS, J. 2000.** Effects of nitrogen and water deficit on net photosynthetic rate and chlorophyll fluorescence in winter wheat. *Plant Physiology* 156, 46-52.

- SHAO, H.B., CHEN, X.Y., CHU, L.Y., ZHAO, X.N., WU, G., YUAN, Y.B., ZHAO, C.X. & HU, Z.M. 2006.** Investigation on the relationship of proline with wheat anti-drought under soil water deficits. *Biointerfaces* 53, 113–119.
- SHUKLA, S., PANDEY, V., PACHAURI, G., DIXIT, B.S., BANERJI, R. & SINGH, S.P. 2003.** Nutritional contents of different foliage cuttings of vegetable amaranth. *Plant food for human Nutrition*, 85, 1-8.
- SINGH, A., SHARMA, J., REXER, K.H. & VARMA, A. 2000.** Plant productivity determinants beyond minerals, water and light. *Piriformospora indica: a revolutionary plant growth promoting fungus. Current Science* 79, 101-106.
- SLABBERT, R. & VAN DEN HEEVER, E. 2007.** Selection of traditional crops for improved drought tolerance in leafy amaranth: Moving toward sustainable food supply. *Acta Horticulture* 752, 281-286.
- STECKEL, L., CRISTY, L.S., EDWARD, W.S. & LOYD, M.W. 2004.** Temperature effects on germination of nine *Amaranthus* species. *Weed Science* 52, 217-221.
- SUN, Y., DU, X., ZHANG, W & LI, R. 2011.** Seed germination and physiological characteristics of *Amaranthus* L. under drought stress. *Advanced Material Research* 1071, 183-185.
- THOMAS, W.E., BURKE, I.C., SPEARS, J.F. & WILCUCT, J.W. 2006.** Influences of environmental factors on slender amaranth (*Amaranth viridis*) germination. *Weed Science*. 45, 316-320.
- TROLINDER, N.L. & SHANG, X. 1991.** Selection and regeneration of amaranth resistant to heat temperature stress. *Plant Cell Reproduction* 10, 448-452.
- TUCKER, J.B. 1986.** Amaranth: the once and future crop. *BioScience* 36, 9-13, 59-60.
- VALLURI, J.V., TREAT, W. J., NEWTON, R.J., COBB, B.G. & SOLTES, E.J. 1988.** Protein synthesis in slash pine callus cultures exposed to water stress. *Tree Physiology* 4, 181-186.

- VERSLUES, P.E. & SHARMA, S. 2010.** Proline metabolism and its implications for plant-environment interaction. *The Arabidopsis Book 2010*; 8: e0140.
- VIERLING, E., HARRIS, LM. & CHEN, Q. 1989.** The major LMW HSP in chloroplasts shows antigenic conservation among diverse higher plant species. *Molecular Cell Biology* 9, 461-468
- WANG, W., VINOCUR, B. & ALTAN, A. 2003.** Plant response to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta* 218, 1-14.
- WONG, J.H., YEE. B.C. & BUCCHANAN, B.B. 1987.** A novel type of phosphofructokinase from plants. *Biological Chemistry* 262 (7), 3185-3191.
- ZHANG, J., SIMMONS, C., YALPANI, N., CRANE, V., WIKINSON, H. & KOLOMIETS, M. 2005.** Genomic analysis of the 12-oxo-phytodienoic acid reductase gene family of *Zea mays*. *Plant Molecular Biology* 59, 323–343.
- ZLATEV, Z.S. & YORDANOV, I.T. 2004.** Effects of soil drought on photosynthesis and chlorophyll fluorescence in bean plants. Bulg. *Plant Physiology* 30(1-4), 3-18.

Chapter 3

Amaranth seed germination and early seedling growth

3.1 Introduction

Amaranth species are seasonal herbs that belong to the family Amaranthaceae. These plant species are grown mainly for their leaves, which are consumed as a vegetable (Muyonga *et al.*, 2008), but the grain is also used as cereal (Shukla *et al.*, 2003). Therefore, from an economic perspective, amaranth must be considered an important new crop worthy of investigation.

As is the case with all crops, amaranth also experiences harsh abiotic and biotic stress conditions when planted in the field. E.g. drought or water deficiency stress affects germination as well as normal growth and production of many plant species (Dale & Egley, 1971; Moran & Showler, 2006). Despite water deficit stress and other environmental factors, unfavorable temperatures have also been shown to reduce seed germination, seedling development and other crop developmental stages (Angelo & Tielborger, 2006; Chayan *et al.*, 2010). There are some plant species that have undergone natural selection as weeds, so enabling them to complete their life cycle with limited water in extreme temperatures (Hartmann *et al.*, 2011).

Vegetable amaranth species are reported to be tolerant to adverse environmental effects (Dieleman *et al.*, 1996; Ghorbani *et al.*, 1999). They have been growing wild in arid and semi-arid ecological regions, which means that they could be more tolerant to low water and high temperature conditions (Hurro & Cees, 1991; Modi, 2006). Many African communities believe that because amaranth is found in the wild, there is no need to cultivate these plants (Jansen van Rensburg *et al.*, 2007). However, amaranth can be propagated from seed in the early summer (Muyonga *et al.*, 2008). High evaporation rates due to increased temperature may occur during planting time, resulting in drying of the soil and causing a noticeable decrease in soil water potential (Pettersson, 1995; Wang *et al.*, 2003; Guo & Al-Katib, 2003). Although this new crop has been reported to tolerate the adverse effect of water deficits, germination can be

delayed or reduced when the water potential of the surrounding medium decreases (Oyodele, 2000).

Amaranth seeds are very small in size, the weight of 1000 seeds varying from 0.7 to 0.9 grams. Using grain sieves as a measuring device, it has been noted that the largest amaranth seeds are only 1/16 inches and some varieties have seed as small as 1/23 inches in diameter. Seeding rates of 1.2 to 3.5 kg seed ha⁻¹ planted to an average depth of 1.3 cm is the recommended sowing rate for amaranth (Webb *et al.*, 1987). According to the authors, planting depth needs to be monitored carefully as the deeper range might delay and decrease seedling emergence. On the other hand, shallower planting depth is also believed to decrease seed-soil contact, exposes the seeds to pests and increases the risk of being washed away by water (Stallknecht & Schulz-Schaeffer, 1993). Further, amaranth seed requires a firm moist seed bed with a soil temperature above 15°C to ensure proper seedling emergence and good plant establishment. Consequently, drying of the top soil during the germination process can reduce seed germination and subsequently seedling establishment. Therefore, amaranth seed germination depends mostly on moisture and temperature, with an optimal temperature range between 16°C and 35°C (Muthomi & Musyimi, 2009). This view is shared by other authors (Aufhammer *et al.*, 1998; Olufolaji & Ojo, 2010).

Thomas *et al.* (2006) candidly stated that the effects of unsuitable weather conditions are probably more critical during germination and early seedling development than at any other stage of vegetative growth. Studies on other plant species have indicated that the optimum temperature range for seedling growth is fairly broad (Hartmann *et al.*, 2011). However, seedling establishment may be hindered by rapid drying of the growth medium surface after being wet (Al-Karaki *et al.*, 2007; Frank *et al.*, 2007). Final germination percentages of many plant species are reported to be sensitive to water and temperature stress (Goodchild & Walker, 1970; Boyer & Westgate, 2004). Plants growing in arid and semi-arid environments should therefore have the ability to produce roots rapidly once germination has occurred to ensure a continuous water supply for transpiration and growth (Kigel *et al.*, 1994).

There is little information in the literature about the combined effect of temperature and moisture stress on germination and early root growth of *Amaranthus cruentus* and *Amaranthus hybridus*. The objective of this study was, therefore, to quantify the influence of temperature and drought stress on amaranth seed germination and early seedling growth. A second objective was to attempt identifying the optimal temperature and water potential for germination and early seedling development of the two species in a comparative study.

3.2 Materials and methods

3.2.1 Trial procedure

Seed germination and subsequent seedling growth of two amaranth species (*A. cruentus* and *A. hybridus*), were investigated under temperature and water stress conditions at the University of the Free State, Bloemfontein (29° 07' S; 26° 11' N), South Africa. The effects of four temperatures (25, 30, 35 and 40°C) together with six water potentials (0, -250, -500, -750, -1000 and -1250 kPa) were tested in a fully randomized factorial experiment with four replicates. Amaranth species were selected based on their use as food (vegetable and grain) in many areas of the arid and semi-arid ecological regions of the world (Tucker, 1986; Leon *et al.*, 2004). Seed was obtained from the Agricultural Research Council-Roodeplaat Vegetable and Ornamental Plant Institute.

The growth medium was prepared by dissolving 8.4g of agar in 1ℓ of distilled water and autoclaving at 120°C for 25 min, after which 10 ml of the medium was poured into a disposable plastic Petri dish (90 x 15 mm) and allowed to solidify. Polyethylene glycol (PEG8000) was dissolved in distilled water at a rate of 0, 130, 180, 240, 280, and 350 g ℓ⁻¹ to produce solutions with osmotic potentials of 0, -250, -500, -750, -1000 and -1250 kPa (Michael, 1983). Twenty milliliters of the appropriate PEG solution was added to the solidifying agar in each Petri dish and placed in a laminar flow cabinet under sterile conditions for 24 hours to permit the PEG molecules to infuse into the agar. Thirty amaranth seeds were planted in each Petri dish on top of the agar medium. The Petri dishes were then covered with a parafilm to retain moisture. Replicates of Petri dishes infused with different osmotic potentials (0, -250, -500, -750, -1000, and 1250) were incubated at each of the five temperatures (25, 30, 35 and 40°C). After a period of four

days, the Petri dishes were removed and various growth parameters (section 3.2.2) were determined.

3.2.2 Data collection

3.2.2.1 Seed germination

The number of seeds that had germinated was counted. Germination was regarded complete when the radicle protruded the seed testa (ISTA, 2010). Germination percentage was then calculated for each replicate (Steckel *et al.*, 2004).

3.2.2.2 Seedling length growth

Seedlings were divided into hypocotyl and root sections by cutting at the joint between these sections. The length of the hypocotyl and root sections of 15 randomly selected seedlings from each Petri dish was determined with a digital Caliper. Hypocotyl length was measured from the joint between the root and the tip of the newly formed leaves. Root length was measured from this joint to the tip of the longest root.

3.2.2.3 Seedling fresh and dry mass

Separated hypocotyls and roots of all seedlings from a Petri dish were then weighed to obtain the total hypocotyl and root fresh mass. Subsequently, hypocotyls and roots were dried separately at 40°C for 48 hours in a drying oven (Steckel *et al.*, 2004) and the dry masses determined. The total seedling mass for each replicate was then calculated by adding the hypocotyl and root masses. Fresh mass of leafy vegetable was reported by many researchers to be more fluctuating (Dieleman *et al.*, 1996; Muyonga *et al.*, 2008) making the reporting inaccurate. Therefore, fresh mass was not considered in this study.

3.2.3 Experimental design and statistical analysis

Only statistically significant results were reported in this study. All data was converted to percentage of the control treatment prior to statistical analysis in order to negate the inherent growth differences between species. Statistical analysis was conducted using the SAS Version 9.1.3 for personal computers. Means of treatments that were statistically significant at the 5% level of significance were separated using Tukey's

Least Significant Difference (LSD) analysis procedure as described by Steel & Torrie (1981). Special attention was given to temperature x water potential x species interactions.

3.3 Results

3.3.1 Germination percentage

The germination percentage of amaranth seed was significantly influenced by the temperature x water potential (Table 3.1), temperature x species (Fig 3.1) and water potential x species (Fig 3.2) interactions. The second order interaction between temperature, water potential and species had no significant effect on the germination percentage.

The significant temperature by moisture potential interaction shows that there is interdependence between these two factors for the germination of amaranth seed (Table 3.1). From Table 3.1, it can be seen that both increasing temperature and decreasing water potential significantly decrease the germination percentage of amaranth seed. The effect of water potential is particularly evident at 35°C and 40°C where significant decreases in germination percentage occur with each incremental decrease in water potential from -500 to -1250 kPa. At 25°C there are far fewer significant decreases in the germination percentage as water potential decreases than at 30, 35 or 40°C. It can also be seen that the germination percentage at each water potential decreases as the temperature increases, with the lowest germination percentage always being found at the highest temperature. The high germination percentage obtained at 30°C – 750 kPa, as well as -1250 kPa at 25 and 35° cannot be explained.

These results indicate that amaranth generally germinates best at a temperature between 25 and 35°C and a water potential of 0 to -250 kPa, although the highest (0) water potential is affected by temperature.

Table 3.1: Combined effect of decreasing moisture potential and increasing temperature on the germination percentage of amaranth seeds.

Moisture potential	Temperature (°C)				Mean
	25	30	35	40	
0	90.63 ^a	90 ^{ab}	87.7 ^{abc}	80 ^{ef}	87.08
-250	88.13 ^{abc}	88.75 ^{abc}	82.5 ^{de}	76.25 ^{fg}	83.91
-500	86.25 ^{bcd}	83.75 ^{de}	80 ^{ef}	73.75 ^g	80.94
-750	85.63 ^{cd}	91.25 ^a	72.5 ^g	61.25 ⁱ	77.66
-1000	83.13 ^{de}	80.63 ^e	66.25 ^h	54.38 ^j	71.10
-1250	91.25 ^a	91.25 ^a	59.63 ⁱ	42.5 ^k	71.16
Mean	87.50	87.61	74.76	64.69	

LSD_{(T)(0.05)}M=6.5 T=6.28 MXT=6.1

Figures followed by the same letter do not differ significantly at the 5% level of significance

The interaction effect of temperature and species showed no significant difference in germination percentage between the species at 25, 30 and 40°C (Figure 3.1). At 35°C, however, seeds of *A. hybridus* germinated significantly better than those of *A. cruentus*. From Figure 3.1 it follows that no significant differences in germination percentage of *A. hybridus* seed between the lower range temperatures occurred until a significant decrease at 40°C. *Amaranthus cruentus*, on the other hand, already showed a significant decrease in germination percentage at 35°C. These results indicate that *A. hybridus* seed germinate optimally at a higher temperature than those of *A. cruentus*.

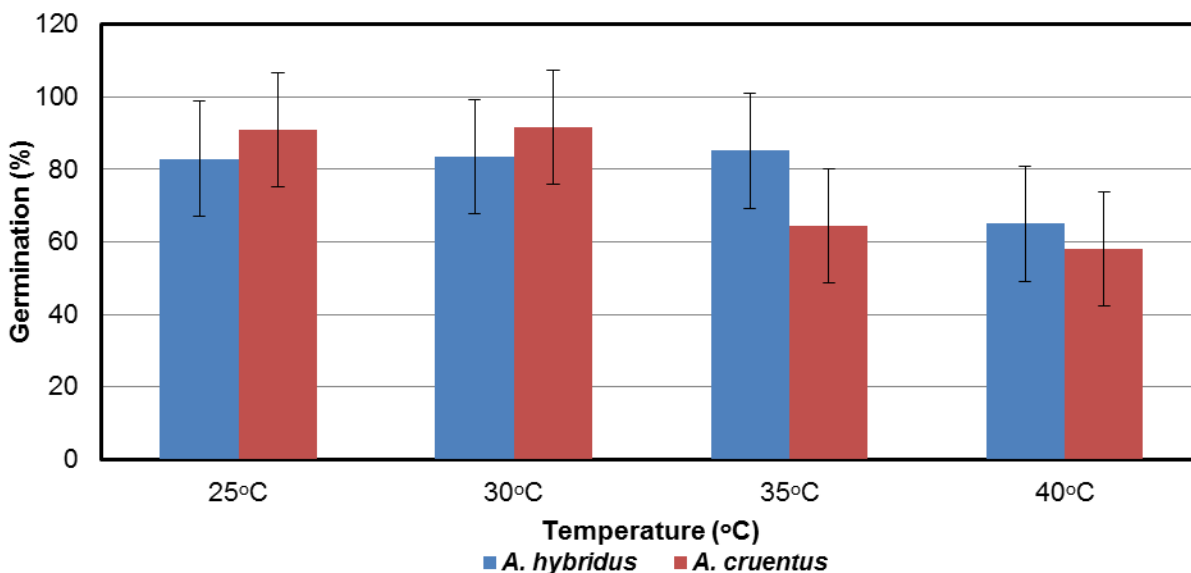


Figure 3.1 Differences in germination percentage of two amaranth species at different temperatures ($LSD_{T(0.05)} = 15.80$)

The interaction effects between amaranth species and water potential was significant at the 5% level of significance (Figure 3.2). However, there are no differences in the way in which these two species reacted to decreasing water potential up to -1250 kPa, where the germination percentage of *A. cruentus* seed is lower than that of *A. hybridus*. Germination of *A. cruentus*, however, does appear to be more sensitive to moisture deficiency than *A. hybridus*, as a significant decline in germination percentage occurred at -500 kPa in the former, while a significant decrease in germination percentage only occurred at -1250 kPa in *A. hybridus*.

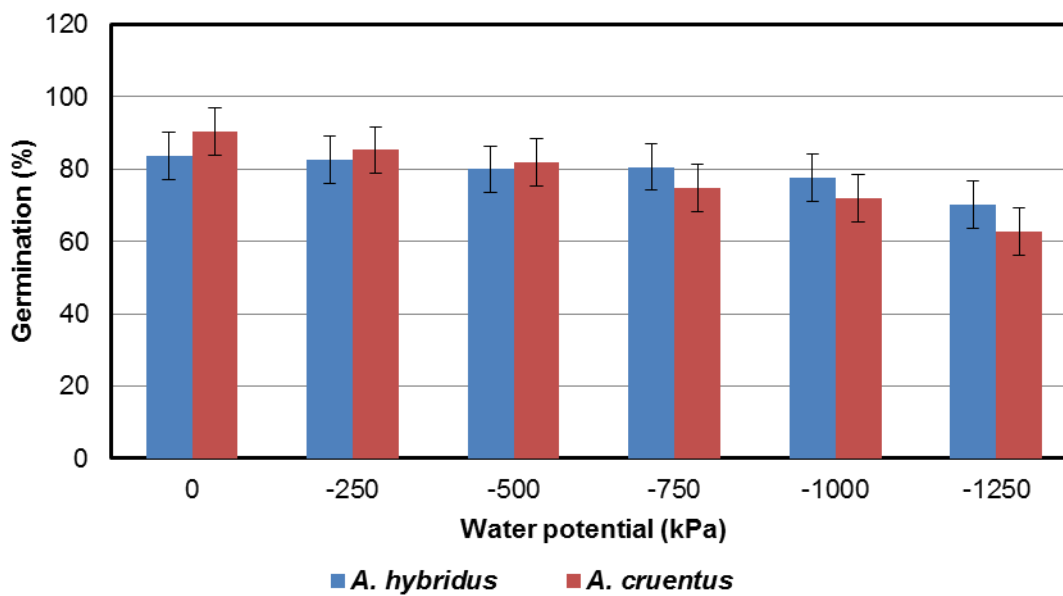


Figure 3.2 Differences in germination percentage of two amaranth species at different water potential levels ($LSD_{T(0.05)}=6.51$).

3.3.2 Hypocotyl length

The analysis of variance revealed that hypocotyl growth was significantly influenced by the interaction between temperature and amaranth species, as well as the interaction between temperature and water potential. However, the three way interaction between moisture, temperature and amaranth species did not significantly affect hypocotyl length growth.

The interaction effect of temperature and species showed no significant difference in hypocotyl length between the species at 25, 30 and 40°C (Figure 3.1). At 35°C, however, seedlings of *A. hybridus* showed a significantly better hypocotyl growth than those of *A. cruentus*. From Figure 3.3 it can be seen that no significant differences in hypocotyl growth occurred in *A. hybridus* until 40°C, where a significant decrease was noted. *Amaranthus cruentus* on the other hand, already shows a significant decrease in hypocotyl growth at 35°C, indicating that *A. hybridus* seedlings are less sensitive to this temperature than those *A. cruentus* and will grow better.

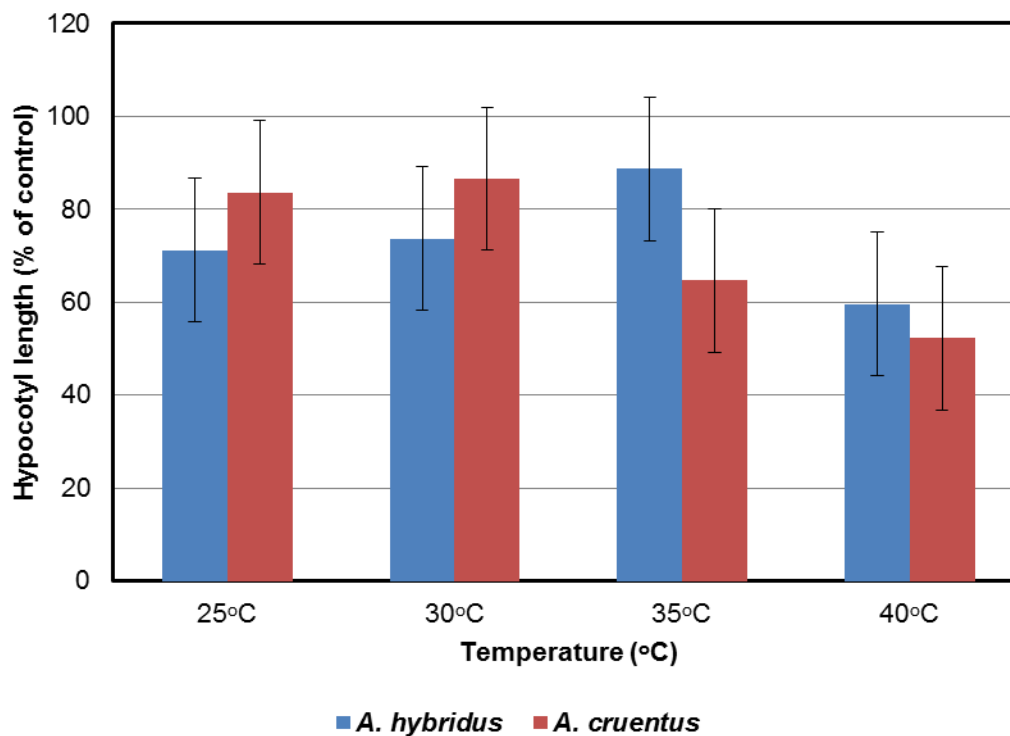


Figure 3.3 Effect of temperature on the hypocotyl length growth of two amaranth species ($LSD_{T(0.05)}=15.41$)

The temperature by moisture interaction was significant at the 5% level of significance indicating that both factors played a role in hypocotyl growth (Table 3.2). From Table 3.2 it can be seen that hypocotyl growth decreased significantly as the water potential decreased, irrespective of temperature.

Hypocotyl growth was significantly greater at 35 and 40°C than at 25 and 40°C up to a water potential of -500 kPa after which it was significantly lower. When sufficient water is available (0 to -500 kPa) the best growth is obtained at 35°C. However, when water become limiting (-750 to -1250 kPa) significantly better hypocotyl growth was obtained at 30°C while that at 40° was worse than at other temperatures. It follows that hypocotyl growth should follow the pattern 30>25>35>40°C.

Table 3.2: Combined effect of decreasing moisture potential and increasing temperature on the hypocotyl length growth of amaranth seedlings.

Water potential (kPa)	Temperature (°C)				Mean
	25	30	35	40	
0	100 ^a	100 ^a	100 ^a	100 ^a	100
-250	86.03 ^{de}	87.82 ^{cd}	93.85 ^b	92.66 ^b	90.09
-500	82 ^f	82.12 ^f	88.32 ^c	84.96 ^e	84.35
-750	74.41 ^h	77.17 ^g	54.24 ^l	22.82 ^o	57.16
-1000	64.61 ⁱ	72.78 ^h	48.9 ^m	19.41 ^p	51.43
-1250	57.31 ^k	60.74 ^j	37.22 ⁿ	15.62 ^q	42.72
Mean	77.39	80.11	70.42	55.91	

LSD_{(T)(0.05)} M=1.61 T=1.32 MXT=1.99

Figures followed by the same letter do not differ significantly at the 5% level of significance.

3.3.3 Root length

The second order interaction effect between species, temperature and water potential had a significant effect on root growth at the 5% level of significance (Table 3.3), showing that interdependence between these factors affects this parameter. From Table 3.3 it can be seen that the root length of the seedlings from both species increases as water deficit increases, and that this increase tends to be greater at higher temperatures. This is particularly apparent as the water potential decreases beyond -500 kPa. Seedling root length of both species is greatest at the lowest water potential (-1250 kPa), irrespective of temperature.

In both species root growth at the greatest water deficit (i.e. lowest water potential) reaches a maximum at the higher temperatures. In *A. hybridus* the maximum root growth is found at 40°C and water potential of -500 to -1250 kPa, at which levels the root lengths are generally significantly greater than those at lower temperatures and water deficiencies. In *A. cruentus*, on the other hand, the greatest root length occurred at a temperature of 35°C at the same water potentials. In this species no significant increase was noted as the temperature increased to 40°C. This data would appear to indicate that *A. hybridus* seedlings would be better adapted to growth at higher temperatures and lower water potentials than those of *A. cruentus*, so making their seedlings more drought tolerant.

This data (Table 3.3) also confirms the optimum germination temperature for *A. cruentus* at 35°C, and it appears as though this is also the optimal temperature for initial seedling growth of this species.

Table 3.3: Effect of temperature and water potential on seedling root growth of *A. hybridus* and *A. cruentus* (expressed as a % of controls)

Moisture potential (kPa)	<i>A. hybridus</i>					<i>A. cruentus</i>				
	25°C	30°C	35°C	40°C	Av	25°C	30°C	35°C	40°C	Av
	Amaranth species roots length (%)									
0	100 ^u	100 ^u	100 ^u	100 ^u	100.00	100 ^u	100 ^u	100 ^u	100 ^u	100.00
-250	130.56 ^{qrs}	131.12 ^{qrs}	142.16 ^{opqr}	126 st	132.46	115.2 ^t	129.16 ^{rst}	115.16 ^t	115.16 ^t	128.67
-500	136.45 ^{pqr}	134.34 ^{pqrs}	171.05 ^{jk}	154.72 ^{lmno}	149.14	122.92 st	143.99 ^{npqr}	131.31 ^{qrs}	130.89 ^{qrs}	132.78
-750	130.77 ^{qrs}	153.99 ^{lmno}	147.94 ^{mno}	340.71 ^d	193.53	136.37 ^{pqrs}	145.47 ^{nopq}	293.64 ^f	293.59 ^f	217.27
-1000	157.34 ^{lmn}	158.32 ^{lmn}	187.57 ⁱ	444.27 ^b	244.8	165.02 ^{kl}	161.56 ^{klm}	318.77 ^e	318.77 ^e	247.01
-1250	206.21 ^g	206.21 ^g	206.44 ^g	572 ^a	297.77	180.81 ^{ij}	190.27 ^{hi}	360.48 ^c	366.12 ^c	277.39
Av	143.56	147.33	159.19	289.62		136.72	145.08	219.89	220.75	
LSD_{T(0.005)} MxSxT =15.10 TxS=20.94 MxT=15.14 MxS=16.84 T= 12.24 S= 8.66 M=14.99										

Figures followed by the same letters do not differ significantly from each other at the 5% level of significance.

3.3.4 Hypocotyl dry mass

Hypocotyl dry mass was significantly affected by all water potential levels below 0. A steady and linear decrease in seedling dry mass was observed when seeds were allowed to germinate on a series of agar beds with water potentials adjusted between 0 and -1250 kPa (Figure 3.4). This strongly indicated that dry matter accumulation in hypocotyls of both amaranth species was very sensitive to and severely jeopardized by moderate to high simulated drought conditions.

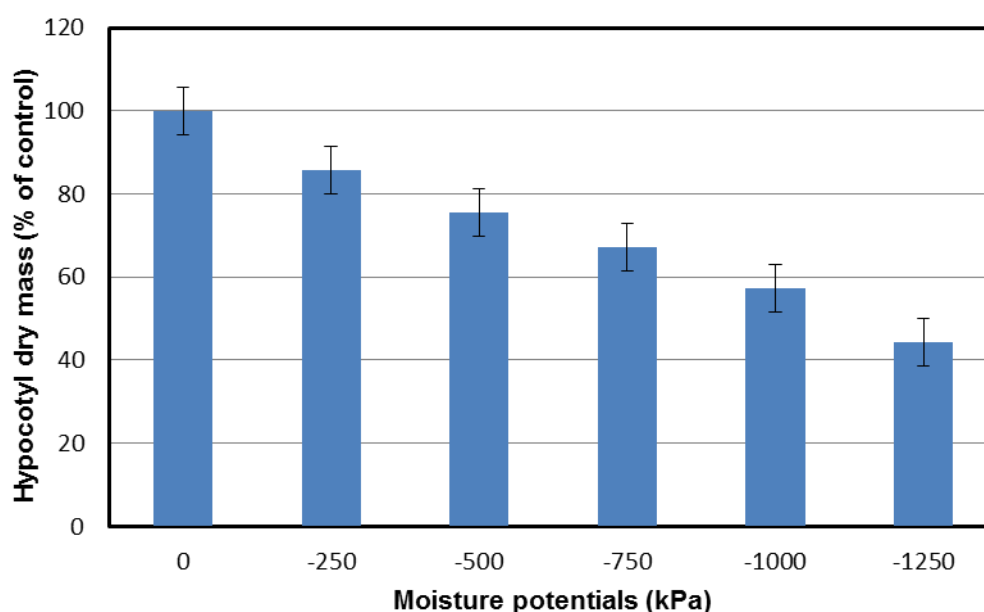


Figure 3. 4 Combined effect of different water potential levels on hypocotyl dry mass of two amaranth species ($LSD_T(0.05) = 5.73$)

No significant differences in hypocotyl dry mass were observed between 25 and 30°C, irrespective of species (Figure 3.5). However, maximum dry matter accumulation in *A. cruentus* seedlings occurred at 30°C while that of *A. hybridis* occurred at 35°C. This was also markedly higher in the case of the latter than the former. At 40°C, compared to the respective temperatures where the two species reached their optimum dry matter accumulation levels, a significant reduction in hypocotyl dry mass occurred (Figure 3.5). Despite this reduction, *A. hybridus* still maintained significantly higher hypocotyl mass

than *A. cruentus*. This may indicate that *A. hybridus* is less sensitive to high temperature (40°C) than *A. cruentus*.

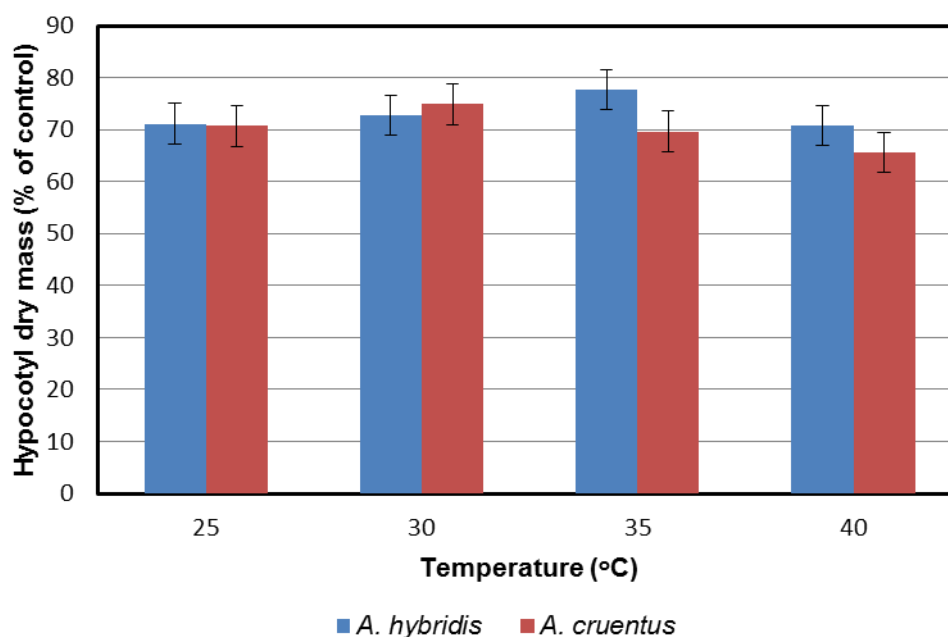


Figure 3.5 Effect of increasing temperature on hypocotyl dry mass of two amaranth species ($LSD_{T(0.05)} = 3.9$)

3.3.5 Root dry mass

The dry mass of amaranth seedling roots was significantly affected in a similar fashion as that of hypocotyls by a series of water potentials below 0 as well as by the interaction between temperature and species. The root dry mass decreased significantly in a linear fashion when seeds were allowed to germinate on a series of agar beds with water potentials adjusted between 0 and -1250 kPa (Figure 3.6). No significant difference was observed between -750 and -1000 kPa. As was the case with hypocotyls, amaranth seedling roots seem to be extremely sensitive to moderate and extreme drought conditions in terms of dry matter accumulation.

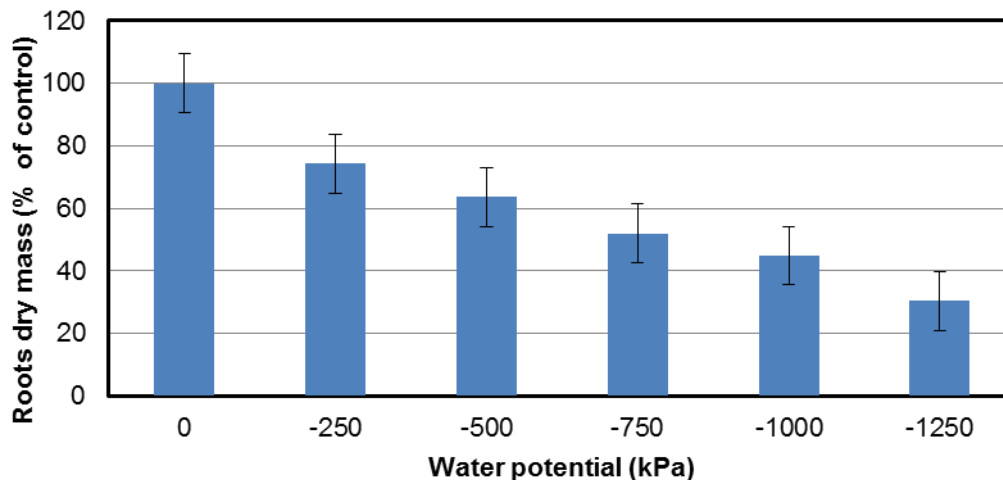


Figure 3.6 Effect of water potential on root dry mass of two amaranth species ($LSD_{T(0.05)} = 11.27$)

The influence of temperature on root dry mass of *A. hybridis* and *A. cruentus* seedlings is shown in Figure 3.7. Dry matter accumulation in roots of *A. hybridis* seedlings is the same at all temperatures tested. Although dry matter accumulation is slightly higher in roots of *A. cruentus* seedlings at 25 and 30°C, a marked decrease occurs at the higher temperature regimes (35 and 40°C). However, no significant differences in root dry mass occurred between cultivars until at 40°C, when the root dry mass of *A. cruentus* seedlings was significantly lower than that of *A. hybridis*.

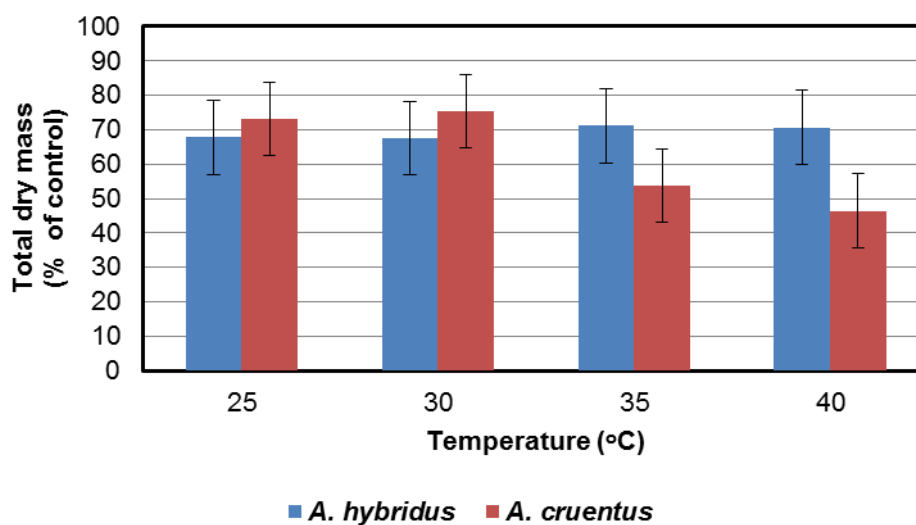


Figure 3.7 Effect of increasing temperature on root dry mass of *A. hybridis* and *A. cruentus* seedlings ($LSD_{T(0.05)} = 10.74$)

3.3.6 Total dry mass

As expected the accumulation of total dry mass in water stressed seedlings followed the same pattern as the hypocotyl and root dry mass when measured separately (Figure 3.8). Total dry mass in amaranth seedlings decreased linearly as the water potential was decreased from 0 to -1250 kPa and this was significant between increments of -250 kPa except between -750 and -1000 kPa.

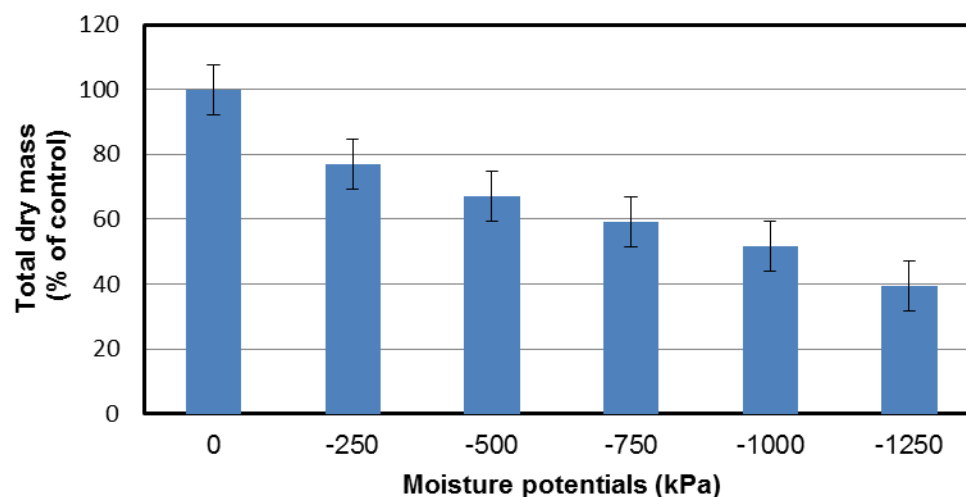


Figure 3.8 Effect of decreasing water potential on the total dry mass of amaranth seedlings (% of control) ($LSD_{T(0.05)} = 7.69$)

Once again the response of the two amaranth species towards different temperatures, in terms of total seedling dry mass, followed the same trend as when hypocotyl and root dry mass were measured separately (Figure 3.9). Total dry mass accumulation in *A. hybridus* seedlings was not significantly affected by any of the incremental temperature increases between 25 and 40°C. The total seedling dry mass of *A. cruentus*, however, significantly decreased at 35 and 40°C. Seedling dry mass in this species was neither statistically different between 25 and 30°C nor between 35 and 40°C.

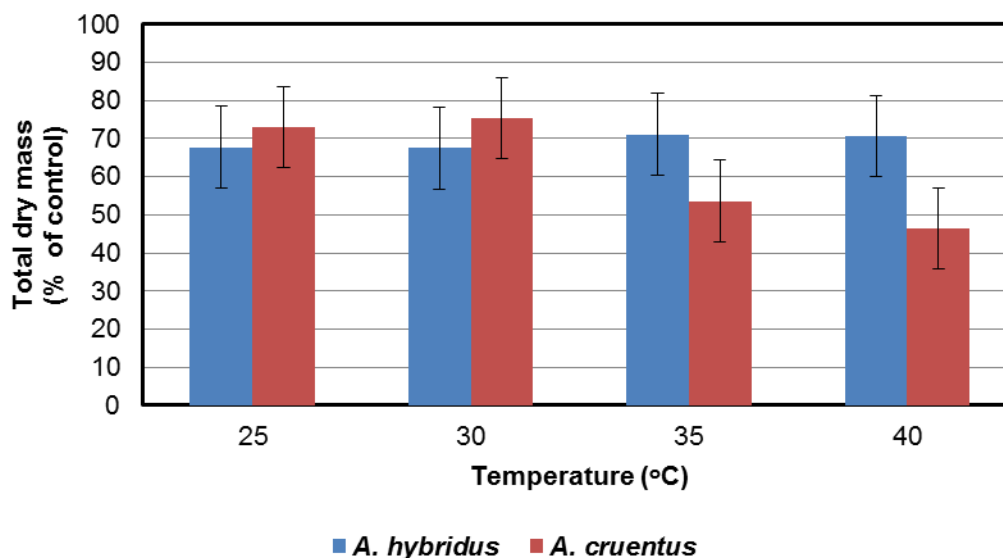


Figure 3.9 Effect of temperature on the total dry mass of the two amaranth species ($LSD_{T(0.05)} = 10.74$)

3.4 Discussion

Seed germination is a stage of plant development that commences with the imbibition of water by the seed and ends when the radicle emerges through the testa. Good germination percentage and seedling establishment is of great concern to all crop farmers as this will determine the stand that is established. Germination requires the availability of sufficient moisture, suitable temperature and sufficient oxygen. High temperature and water deficiency have been shown to affect seed germination and seedling establishment in the field (Horak & Loughin., 2000, Svirskis, 2003).

This study indicated that 25 and 30°C are suitable germination temperatures for both amaranth varieties, although *A. hybridus* seeds exhibited high germination percentages up to 35°C, irrespective of water potential. These results agree with the ISTA recommendations for amaranth germination tests, as they state that the optimum germination percentage for amaranth lies between 20 and 30°C (ISTA, 2010). Germination percentage decreased as the water potential decreased beyond -500 kPa at the higher temperatures (35 and 40°C). Similar findings on the effects of water potential on the germination of amaranths were reported by Mayer & Mayber (1975) as

well as Steckel *et al.* (2004). This also concurs with the findings of Khodarahmpour (2012), who reported that the germination percentage of maize seeds decreased as water potential decreased from 0 to -900 kPa.

The reduction in germination percentage at lower water potentials was probably due to insufficient moisture being available to hydrate the seed sufficiently for successful germination. These findings are in agreement with those of Mayer & Mayber (1975), Weaver & Thomas (1986), Habib & Morton (1987), and Steckel *et al.* (2004), who stated that the poor germination found with increasing water deficit was probably due to the lack of sufficient moisture available to provide adequate hydration to either break dormancy or for embryonic metabolic processes. Khodarahmpour (2012), however, felt that the reduction in germination percentage with increasing water deficit could be due to damage of the seed coat or embryo caused by drought stress.

It is evident that germination of *A. hybridus*, is more tolerant to high temperature than that of *A. cruentus*. It seems that *A. hybridus* which is native to Africa (central Africa) has adapted well to drought stress and high temperature, while *A. cruentus*, which was introduced into the region from northern America, and that has relatively lower temperatures and good rainfall patterns (Chan & Sun, 1997), is not as well adapted to high temperature and low moisture conditions. The differences in germination percentage found with increasing temperature could possibly be due to the differences in climatic conditions in their areas of origin. Each plant species has an optimal temperature at which it's seed will germinate best (Hartman *et al.*, 2011).

The optimum germination temperature for amaranth has been reported to be between 20 and 30°C (ISTA, 2010), and the best hypocotyl growth for *A. cruentus* was also found to occur within this temperature range. However, hypocotyl growth of *A. hybridus* was better at a temperature range of 25 - 35°C. Very high temperatures (40°C) decreased the hypocotyl growth of both species. From these results it can be seen that *A. hybridus* seedlings are more resistant to high temperature than *A. cruentus* in terms of germination and hypocotyl growth. These findings concur with those of Liu & Stuetzel (2002) who reported that *Amaranth* shoot elongation increased with increasing mean temperature up to 32°C and decreased as temperature increased further.

The root length of both species increased as temperature and drought stress increased, with the greatest root length being attained at the highest temperatures and lowest moisture supply. This was possibly due to the seedling attempting to find sufficient moisture to alleviate the stress by utilizing a larger volume of substrate. Young seedlings in the field are hardened by exposing them to short periods of drought stress to encourage root growth (Mayer & Meyber, 1975; Steckel *et al.*, 2004; Hartman *et al.*, 2011). This enables the plants to exploit a larger volume of soil for water, and so increases their tolerance to periods of drought (Turner & Kramer, 1980; Steckel *et al.*, 2004). Amaranths are no exception to this, and both Oryokot *et al.* (1997) and Oyodele (2000) indicated that exposing amaranth seedlings to short periods of drought promoted root growth.

The results from this study indicate that a similar process occurs in very young seedlings, even before they would emerge from the soil. Root growth of these seedlings would also, then, appear to give an indication of the relative tolerance of these species to drought during the emergence and establishment phase. *A. hybridus* produced significantly longer roots than those of *A. cruentus* under conditions of high temperature and low moisture content. It would, therefore, appear as though the former species would establish better in the field than the latter under drought conditions.

Plant dry mass is one of the most effective parameters for assessing plant growth, as it is not affected by temporary fluctuations in environmental moisture content like fresh mass. During this experiment both hypocotyl and root dry mass of amaranth seedlings was significantly affected by water potential and the interaction between temperature and amaranth species.

Both species indicated a constant dry mass accumulation between 30 and 35°C, but when the temperature was increased to the highest (40°C), the dry mass decreased significantly. It is, therefore, apparent that a temperature of between 30 and 35°C is the best temperature for seedling growth of both amaranth species. Temperature is an important factor in determining the vigour of early seedling growth, as the optimal growth temperature has been reported to promote biochemical processes in the

meristematic region, resulting in better cell division, cell elongation, and so overall dry matter production (Akanbi & Togun, 2001).

Hypocotyl and root mass decreased as water deficit increased, demonstrating the negative impact of drought stress on amaranth seedlings. Although water deficit in the growing medium has been shown to promote root growth (Hartmann *et al.*, 2011), it appears as though these elongated roots are thinner than those at higher water potentials, resulting in a lower dry mass than the shorter non-stressed roots. Similar results were reported in drought stressed cotton seedlings by Pace *et al.* (1999). Decreasing the water potential in the medium using different PEG concentrations reduces the accessibility of water by the roots, so reducing the uptake of water by roots. This in turn decreases the total amount of water in the plant, leading to a reduction in the metabolic and physiological processes, the end result of which is slower growth and development. This would then explain why the dry mass of seedlings decreases as the PEG concentration, and so the moisture deficit, in the agar medium increases.

The findings of this study are similar to those of Michael (1983) who reported the role of PEG solutions in reducing crop water uptake from the roots. Petterson (1995) also reported a significant reduction in plant dry matter production of amaranth plants with increasing water deficits. Plant water deficit in established seedlings and plants occurs when the loss of water through transpiration exceeds the amount of water absorbed through the roots. The dry mass of these plants is then reduced due to the lack of water interfering with chlorophyll synthesis, electron transport and photophosphorylation, as well as the synthesis and activities of carboxylating enzymes within the plant, so inhibiting plant growth (Dreesen & Langhans, 1992; Olufolaji & Ojo, 2010).

3.5 Conclusion

The best temperature for germination and early seedling growth ranges between 25 – 30°C for *A. cruentus* and 25 – 35°C for *A. hybridus* respectively. Seedling growth is highly dependent on both moisture and temperature, with *A. hybridus* being more tolerant to high temperature and low moisture conditions than *A. cruentus*.

3.6 References

- AKANBI, W.B. & TOGUN, A.O., 2001.** The influence of maize-Stover compost and nitrogen fertilizer on growth, yield and nitrogen uptake of Amaranths. *Scientia Horticulturae* 93, 1-8.
- AL-KARAKI, G.N., AL-UJAMI, A. & OTHMAN, Y., 2007.** Seed germination and early roots growth of three barley cultivars as affected by temperature and water stresses. *Journal of Agricultural Environmental Science* 2, 112-117.
- ANGELO, V. & TIELBORGER, K., 2006.** Effects of age or germination of dormant seeds. *Oecologia* 70, 1-9.
- AUFHAMMER, W., CHUCZOROVA, H.P.K. & KRUSE, M., 1998.** Germination of grain amaranth (*A hypochondriacus* x *A hybridus*): Effects of seed quality, temperature, light, and pesticides. *European Journal of Agronomy* 8,127-135.
- BOYER, J.S. & WESTGATE, M. E., 2004.** Grain yields with limited water. *Journal of Experimental Biology* 54, 234-139.
- CHAN, K.F. & SUN, M., 1997.** Genetic diversity and relationship detected by isozyme and RAPD analysis of crop and wild species of amaranth. *Theoretical Application of Genetics* 95, 865-873.
- CHAYAN, A.A.I.M., RAHMAN, H.M., SULTAN, R. & ISLAM, M.R., 2010.** Initial moisture content and different moisture container potentially on vigourity of stem amaranth (*Amaranthus oleraceous*) seed. *Bangladesh Journal of Scientific and Industrial Research* 4, 1197-1203.
- DALE, J.E. & EGGLEY, G.H., 1971.** Stimulation of Witchweed Germination by Run-off Water and Plant Tissues. *Weed Science* 19, 678-681.
- DIELEMAN, A., HAMILL, A.S., FOX, G.C. & SWANTON, C.J., 1996.** Decision rules for postemergency control of pigweed (*Amaranthus* spp) in Soybean (*Glycine max*). *Weed Science* 44, 126-132.

- DREESEN, D.R. & LANGHANS, R.W., 1992.** Temperature effects on growth of impatiens plug seedlings in controlled environments. *Journal of the American Society of Horticultural Science* 117(2), 209-215.
- FRANK, A.B., STUART, L.W., DAVID, L.N. & WILLIAM, M.R., 2007.** Seed germination of seabeach amaranth (*Amaranthus pumilus*) as influenced by stratifications, temperature, and light. *Journal of Environmental Horticulture* 23, 33-36.
- GHORBANI, R., SEEL, W. & LEIFERT, C., 1999.** Effects of environmental factors on germination and emergency of *Amaranthus retroflexus*. *Weed Science* 48, 505-510.
- GOODCHILD, N. A. & WALKER, M. G., 1970.** A method of measuring seed germination in physiological studies. *Annals of Botany* 35, 615-21.
- GUO, P. & AL-KATIB, K.A., 2003.** Temperature effects on germination and growth of redroot pigweed (*Amaranthus retroflexus*), Palmer Amaranth (*A. palmeri*), and common waterhemp (*A. rudis*). *Weed Science* 51, 869-879.
- HABIB, S. A. & MORTON, H. L., 1987.** The combined effect of temperature and water potential on side oats grama and redroot pigweed seeds germination. *Journal of Agricultural Science* 5:15-24.
- HARTMANN, H.T., KESTER, D.E., JR, F.T.D. & GENEVE, R.L., 2011.** Plant propagation principles and practices. Prentice Hall Publishers, New Jersey. pp 157-171.
- HORAK, M. J. & LOUGHIN, T. M., 2000.** Growth analysis of four *Amaranthus* species. *Weed Science* 48, 347-355.
- HURRO, J.B. & CEES, M.K., 1991.** The dual role of temperature in the regulation of theseasonal changes in dormancy and germination of seeds of Polygonum. *Oecologia* 90, 88-94.
- ISTA-INTERNATIONAL SEED TESTING ASSOCIATION., 2010.** International Rules for Seed Testing. Bassersdorf, Switzerland.

- JANSEN VAN-RENSBURG, J., AVERBEKE, V.W., SLABBERT, R., FABER, M., JAARVELD, V.P., HEERDEN, V.I., WENHOLD, F. & OELOFSE, A., 2007.** African leafy vegetables in South Africa. *Water SA* 33(3), 317-326.
- KHODARAHMPOUR, Z., 2012.** Evaluation of drought stress effects on germination and early growth of inbred lines of MO17 and B73. *African Journal of Microbiology Research* 6(16), 3749-3754.
- KIGEL, J., OFIR, M. & KOLLER, D., 1994.** Control of germination responses of *Amaranthus retroflexus* L. seed by their parental photo-thermal environment. *Experimental Botany* 106, 1125-1136.
- LEON, R.G., KNAPP, A.D. & OWEN, M.D.K., 2004.** Effect of Temperature on the Germination of Common Waterhemp (*Amaranthus tuberculatus*), Giant Foxtail (*Setaria faberi*), and Velvetleaf (*Abutilon theophrasti*). *Weed Science* 52, 67-73.
- LIU, F. & STUTZEL, H. 2002.** Leaf water relations of vegetable amaranth (*Amaranthus* spp.) in response to soil drying. *European Journal of Agronomy* 16, 137-150.
- MAYER, A.M. & MAYBER, A.P., 1975.** The germination of seeds. *Progress Press*. Toronto, Canada. Pp 129-138
- MICHAEL, B.E., 1983.** Evaluation of the water potentials of solutions of polyethylene glycol 8000 both in the absence and presence of other substances. *Plant Physiology* 72, 66-70.
- MODI, A.T, 2006.** Growth temperature and plant age influence on nutritional quality of amaranthus leaves and seed germination capacity. *Water South Africa* 33, 0378-4738.
- MORAN, P.J. & SHOWLER, A.T., 2006.** *Phomopsis amaranthicola* and *Microsphaeropsis amaranthi* symptoms on *Amaranthus* spp. under South Texas conditions. *Journal of Plant Diseases* 91, 12-16.

- MUTHOMI, J. & MUSYIMI, D.M., 2009.** Growth responses of african nightshades (*Solanum scabrum* mill) seedlings to water deficit. *Journal of Agricultural & Biological Science* 5, 2006-2009.
- MUYONGA, J.H., NABAKABYA, D., NAKIMBUNGWE, D.N. & MASINDE, D., 2008.** Effects to promote Amaranth production and consumption Uganda to fight malnutrition. *In: G.L. Robertson & J.R. Lubien (eds). International Union of Food Sciences & Technology. Landon, UK.*
- OLUFOLAJI, O.A & OJO, O.A., 2010.** Effects of soil moisture stress on the emergence, establishment and productivity of amaranth. *Plant Nutrition* 33, 613-625.
- ORYOKOT, J.O.E., MURPHY, S.D., THOMAS, A.G. & SWANTON, C.J., 1997.** Temperature- and Moisture-Dependent Models of Seed Germination and Shoot Elongation in Green and Redroot Pigweed (*Amaranthus powellii*, *A. retroflexus*). *Weed, Science* 45, 488-496.
- OYODELE, V.I., 2000.** Influence of soil water stress at different physiological stages ondrought and seed yield of amaranth species. *Acta Horticulturae* 357, 114-121.
- PACE, M. L., COLE, J. J., CARPENTER, S. R. & KITCHELL, J. F. 1999.** Trophic cascades revealed in diverse ecosystems. *Trends in Ecology and Evolution* 14: 483–488.
- PETTERSON, D.T, 1995.** Effects of environmental stress on weed/crop interactions. *Weed Science* 43, 483-490.
- SHUKLA, S., PANDEY, V., PACHAURI, G., DIXIT, B.S., BANERJI, R. & SINGH, S.P., 2003.** Nutritional contents of different foliage cuttings of vegetable amaranth. *Plant food for human Nutrition* 85, 1-8.
- STALLKNECHT, G.F. & J.R. SCHULZ-SCHAEFFER., 1993.** Amaranth rediscovered. p. 211-218. *In: J. Janick and J.E. Simon (eds.). New crops. Wiley, New York.*
- STECKEL, L., CRISTY, L.S., EDWARD, W.S. & LOYD, M.W., 2004.** Temperature effects on germinations of nine *Amaranthus* species. *Weed Science* 52, 217-221.

- STEEL, R.G. & TORRIE, J.H. 1981.** Principles and Procedures of Statistics-a Biometrical Approach, 2nd edition. McGraw-Hill Book Company, New York. pp 374-388.
- SVIRSKIS, A., 2003.** Investigation of cultivation and utilization in Lithuania. *Agronomy Research* 1(2), 253-264.
- THOMAS, W.E., BURKE, I.C., SPEARS, J.F. & WILCUT, J.W. 2006.** Influence of environmental factors on slender amaranth (*Amaranthus viridis*) germination. *Weed Science* 54, 316-320.
- TUCKER, J.B., 1986.** The once and future crop.
<http://www.hort.purdue.edu/newcrop/proceedings> (Accessed 28/09/2012)
- TURNER, N.C. & KRAMER, P.J., 1980.** Adaptations of plants to water and high temperature stress. *Wiley-Interscience Publication*, New York.
- WANG, W., VINO CUR, B. & ALTMAN, A., 2003.** Plant responses to drought, salinity and extreme temperatures toward genetic engineering for stress tolerance. *Planta*. 218, 1-14.
- WEAVER S.E. & THOMAS A.G., 1986.** Germination Responses to Temperature of Atrazine-Resistant and -Susceptible Biotypes of Two Pigweed (*Amaranthus*) Species. *Weed Science* 34, 865-870
- WEBB, D. M., SMITH, C. W. & SCHULZ-SCHAEFFER, J., 1987.** Amaranth Seedling Emergence as Affected by Seeding Depth and Temperature on a Thermogradient Plate. *Agronomy Journal* 79, 23-26.

Chapter 4

Physiological response of amaranth seedlings to moisture stress at different temperature regimes

4.1 Introduction

Abiotic stress such as temperature and drought is a serious problem for agricultural and horticultural crops. For example, drought stress simultaneously affects many plant developmental traits through morphological, physiological or metabolic modifications of all plant organs (Baquedano & Castillo, 2006). As a result, crop yield can be compromised (Tian & Lei, 2007). While the sum total of all physiological and biochemical processes during a growing season determines crop growth and final yield, understanding the way these processes are affected by the environment is of great significance.

Amaranth species belong to the C₄ group of plants that flourish in warm, humid and sunny environments (Lal & Edwards, 1996.). According to the author, amaranth plants show lower water loss rates and greater water use efficiency than many other C₄ plants, and more so in dry conditions. Therefore, amaranth has been often described as a drought tolerant crop (Zavitkovski & Ferrell, 1968; Liu & Stutzel, 2002) capable of maintaining normal physiological processes under stress.

Photosynthesis is a crucial physiological process that supports crop growth and development, and is well known to be sensitive to drought stress in higher plants. The effect of low moisture conditions on photosynthesis has been widely researched and several reports have been published that also dealt with the experimental procedures followed (Lal & Edwards, 1996; Wu *et al.*, 2008). Older methods based on CO₂ absorption rate measurements provide handy information based on net photosynthetic activities (Rong-Hau *et al.*, 2006). However, this information is not sufficient to explain possible changes in different aspects of the photosynthesis process under abiotic stress conditions. Measuring related factors such as chlorophyll content and chlorophyll fluorescence under normal and stress conditions contribute towards estimating the

photosynthetic capacity of a plant. Correlation of this data with growth and yield data is of great value since these traits are closely related to the rate of carbon exchange (Wang *et al.*, 2003). According to the authors, these parameters are reliable gauges to evaluate the effects of abiotic stress conditions on photosynthesis and plant growth.

Apart from chlorophyll fluorescence and chlorophyll content data, measurement of carbohydrate content at specific intervals supplies additional information pertaining to photosynthesis capacity and the synthesis of photosynthate (Hong *et al.*, 2001; Liu *et al.*, 2008). Moreover, a direct relationship between sugar content in the source (leaves) and sinks, determining its translocation, and other physiological process such as respiration, exists. More than four decades ago Zavitkovski and Ferrell (1968) already reported that among the soluble carbohydrates, sucrose and fructans play an important role in adaptation of plants to abiotic stressors (Zavitkovski & Ferrell, 1968).

In more recent years, Schellenbaum *et al.* (1999) reported that sucrose acts as a replacement for water in membranes under moisture stress in order to maintain membrane integrity by keeping phospholipids in the liquid crystalline phase and, by doing so, prevent structural changes in soluble proteins. Glucose, on the other hand, can partake in cross linking with proteins by a complex glycosylation reaction between amino acids and carboxyl groups known as the Maillard reaction (Kerepesi & Galiba, 2000). Further, as respiratory substrate, monosaccharides such as glucose and fructose supply energy needed by all resistance mechanisms independent of what it is (Kerepesi & Galiba, 2000; Wu *et al.*, 2008).

Further, fructose is involved in fructan synthesis which in turn is known to be involved in resistance mechanisms towards abiotic and biotic stress conditions (George, 1992). According to the authors, fructans not only act as carbohydrate reserve, but is believed to also play a key role in stress induced metabolic events. Further, treatments that enhance photosynthetic carbon fixation increase fructan accumulation which in turn seems to ameliorate water stress by protecting membranes or other cellular components from the adverse effects of drought (George, 1992). There is a strong indication that another protection point might be the important photosynthetic enzyme Ribulose-1,5-bisphosphate carboxylase (RUBISCO; EC 4.1.1.39). Several reports have

indicated that the decline of photosynthesis rate during water stress can be associated with the inhibition of photochemical reactions due to inactivation of RUBISCO, apart from stomatal closure (Liu & Stutzel, 2002; Li & Wang, 2003; Wang *et al.*, 2003; Hura *et al.*, 2007). Additionally, other photosynthetic enzymes such as ribose-5-phosphate isomerase and ribose-5-phosphate kinase as well as the respiratory enzyme phosphofructokinase were reported to be sensitive to drought stress (Liu & Stutzel, 2002; de Ronde *et al.*, 2004).

For plants to survive hot and semi-arid conditions, they require the ability to withstand extreme drought and heat stress. Mechanisms to accomplish this include water stress avoidance or drought tolerance (Liu & Stutzel, 2002). According to the authors, a change in gene expression of tolerant plants results in the alteration of protein metabolism and changes in protein profiles of a drought tolerant plant assist us in understanding the impact of water shortage on protein synthesis (Liu & Stutzel, 2002). The authors additionally reported that, like other C4 plants, amaranth, which flourishes in warmer, humid and sunny environments, maintains lower respiration rates (Liu & Stutzel, 2002).

Little information on the physiological response of vegetable amaranth species to environmental stress is available in the literature. The results obtained in chapter 3 have indicated that 30 and 35°C are the optimum temperatures for *A. cruentus* and *A. hybridis*, respectively, in terms of growth. However, at these temperatures, concomitant with drought stress, seedling growth of both species were affected. The latter supplied a rationale for following key physiological events that underlie seedling growth in this chapter. The objective was to quantify these physiological events in an attempt to identify the main effect(s) of water stress at two temperature regimes. These included total water soluble protein content, photosynthesis and respiration rates as well as sugar production and the *in vitro* activities of key regulatory enzymes of glycolysis and the oxidative pentose phosphate pathway, namely phosphofructokinase (PFK; EC 2.7.1.11) and glucose-6-phosphate dehydrogenase (G6P-PDH; EC 1.1.1.49) respectively.

4.2 Materials and methods

4.2.1 Materials

Seed from two amaranth species viz. *A. cruentus* and *A. hybridis* were obtained from the Agricultural Research Council of South Africa (ARC-Roodeplaat Vegetable and Ornamental Plant Institute). Selection of these two species was based on their use as food (grain or herb) in many areas of the arid and semi-arid ecological regions of the world (Steckel *et al.*, 2004; Tucker, 1986). The agar (high strength gel plant cell culture) used in execution of germination studies were obtained from Sigma Life Science (Spain) and the Polyethylene glycol (PEG 8000) used to adjust the osmotic potential of the agar was purchased from Sigma Aldrich Chemistry (Germany). The kit to analyze seedling extracts for sucrose, glucose and fructose content were acquired from R-Biopharm (Germany). All other chemicals used were of the purest available and obtained from either Merck or Sigma (Germany).

4.2.2 Trial layout

In all cases seeds were allowed to germinate on agar in 90 mm Petri dishes, which were arranged in a complete randomized factorial design with four replicates, in growth chambers at different temperatures.

4.2.3 Preparation of seedlings

For the purpose of following all the different physiological parameters seedlings were prepared in the same manner. Thirty seeds per replicate for both species were sown in separate Petri dishes on top of agar that was either non-treated (control) or treated with 350 g ℓ^{-1} PEG 8000 (see chapter 3; 3.2.2) to produce an osmotic potential of -1250 kPa (Michael, 1983) and allowed to germinate at two different temperatures, 30 and 35°C. Whole four day old seedlings were removed from the agar using tweezers and used directly.

4.2.4 Total water soluble protein content

Proteins were extracted by pulverizing one gram of seedling material in 10 ml Tris-HCl extraction buffer using a mortar and pestle, by adding the buffer slowly and in increments. The buffer (pH = 6.8) contained 12.5 mM Tris-HCl, 2 mM EDTA, 2 mM PMSF and 14 mM mercapto-ethanol. The crude extracts were transferred to 15 ml plastic capped tubes and centrifuged for 5 minutes at 12 000 rpm at room temperature. Two milliliter aliquots of the supernatant were, subsequently, transferred to clean Eppendorf vials for each replicate of each treatment separately. Protein was determined with a dye-binding technique (Bradford, 1976), using bovine gamma globulin as standard. This was done by transferring 10 μ l aliquots to Elisa plate wells and reading the absorbance spectrophotometrically at a wavelength of 595 nm by means of a Bio-Rad micro plate reader. Absorbance was read four times for each replicate and the average was used in calculations.

4.2.5 Extraction and determination of sucrose, D-glucose and D-fructose content in whole seedlings

Whole seedlings were removed from the agar medium, transferred to test tubes, submerged in 10 ml of an 80% (v/v) aqueous ethanol solution and boiled for 10 min in a pre-heated water bath in order to stop all enzymatic activities. On removal the original ethanol volume was restored. Subsequently, the seedlings were homogenized using an oscillating mill (MM 400; Germany). A 1.5 ml aliquot of the homogenate per replicate was transferred to an Eppendorff vial and centrifuged (Selecta P, Spain) at 12 000 rpm for 5 minutes. One ml of the supernatant was transferred to clean marked Eppendorff vials and the ethanol evaporated slowly in an oven at 40°C until dry. The one ml ethanol was replaced with distilled water and frozen until sugar determinations could be carried out.

The Boehringer Mannheim enzymatic technique (Cat Nr: 1071620035; Bergmeyer and Brent, 1974) was employed to spectrophotometrically determine the sugar content in whole amaranth seedlings using a Bio-Rad Microplate reader (Germany) equipped with GEN 5 software. The absorbance of a 10 μ l aliquot of each replicate was measured at 340 nm following the instructions of the manufacturers based on the following principle:

Principle of the Boehringer Mannheim enzymatic technique for determining sucrose, D-glucose and D-fructose content in solid tissue (Branford, 1976)

The D-glucose concentration is determined before and after the enzymatic hydrolysis of sucrose. D-fructose is determined subsequent to the determination of D-glucose.

Determination of D-glucose before inversion:

At pH 7.6 the enzyme hexokinase (*HK*) catalyzes the phosphorylation of D-glucose by adenosine-5'-triphosphate (ATP) with the simultaneous formation of adenosine-5'-diphosphate (ADP) [1].



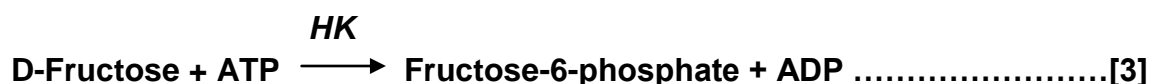
In the presence of *glucose-6-phosphate dehydrogenase (G-6-PDH)*, the D-glucose-6-phosphate (G-6-P) formed is specifically oxidized by nicotine amide-adenine dinucleotide phosphate (NADP) to gluconate-6-phosphate with the formation of reduced nicotine amide-adenine dinucleotide phosphate (NADPH + H⁺) [2].



The NADPH formed in this reaction is stoichiometric to the amount of D-glucose and is measured by means of its absorbance at 340 nm.

Determination of D-fructose:

Hexokinase (HK) also catalyzes the phosphorylation of D-fructose to fructose-6-phosphate (F-6-P) in the presence of ATP [3].



On completion of the reaction [3], **F-6-P** is converted by *phosphoglucose isomerase (PGI)* to glucose-6-phosphate (G-6-P) [4].



G-6-P reacts again with NADP to form gluconate-6-phosphate and NADPH [2]. The amount of NADPH formed is now stoichiometric with the amount of D-fructose.

Enzymatic inversion:

At pH 4.6, sucrose is hydrolyzed by the enzyme β -fructosidase (*invertase*) to D-glucose and D-fructose [5].



The determination of D-glucose after inversion (total D-glucose) was carried out according to the principle outlined above. The sucrose content was calculated from the difference of the D-glucose concentrations before and after enzymatic inversion.

Procedure:

The instructions of the suppliers (Boehringer Mannheim/R-Biopharm) were followed and the sugar content calculated by means of the following equation:

$$c = \frac{V \times \text{MW}}{\epsilon \times d \times v \times 1000} \times \Delta A \text{ g L}^{-1}$$

- Where:
- c = concentration
 - V = final volume (ml)
 - v = sample volume (ml)
 - MW = molecular weight of the substance to be assayed (g mol^{-1})
 - d = light path (cm)
 - ϵ = extinction coefficient of NADPH at 340 nm (= 6.3)

It follows for sucrose:

$$c = \frac{3.02 \times 342.3}{\epsilon \times 1.0 \times 0.1 \times 1000} \times \Delta A_{\text{sucrose}} = \frac{10.34}{\epsilon} \times \Delta A_{\text{sucrose}} \text{ g L}^{-1}$$

for D-glucose:

$$c = \frac{3.02 \times 180.16}{\epsilon \times 1.0 \times 0.1 \times 1000} \times \Delta A_{\text{D-glucose}} = \frac{5.441}{\epsilon} \times \Delta A_{\text{D-glucose}} \text{ g L}^{-1}$$

for D-fructose

$$c = \frac{3.04 \times 180.16}{\epsilon \times 1.0 \times 0.1 \times 1000} \times \Delta A_{\text{fructose}} = \frac{5.477}{\epsilon} \times \Delta A_{\text{fructose}} \text{ g L}^{-1}$$

Sucrose, D-glucose and D-fructose content was expressed as mmol g⁻¹ dry mass (DM).

4.2.6 Determination of chlorophyll and carotenoid content

The chlorophyll content in amaranth seedlings, exposed to 48 h sunlight after removal from the dark following four days of incubation, was determined based on the methods adopted from Boyer (1993). One g seedling material per replicate was ground in 15 ml 80% (v/v) acetone by means of a mortar and pestle. Grinding of the samples was executed carefully by adding small volumes of acetone at a time until all of the acetone was added. Subsequently, the homogenate was centrifuged at 12 000 rpm (Selecta P, Spain) for five minute at room temperature. A Shimadzu UV/VIS PharmaSpec spectrophotometer was used to determine the absorption spectra of the chlorophyll pigments in a quartz cuvette at three different wave lengths (661.6, 644.8, 470 nm). Chlorophyll a and b as well as carotenoid content was calculated based on the procedure adopted by Mackinney (1941) as follows:

Chlorophyll a content

$$\begin{aligned} & [\text{Constant (11.24) x Absorbance (661.6nm)}] - [\text{Constant (2.04) x Absorbance (644.8)}] \\ & = \text{Chlorophyll a (}\mu\text{g ml}^{-1}\text{ extract) / dry mass x15} \\ & = \text{total chlorophyll a content } \mu\text{g-1 dry mass} \end{aligned}$$

Chlorophyll b content

$$[\text{Constant (20.13) x Absorbance (644.8nm)}] - [\text{Constant (4.19) x Absorbance (661.6nm)}]$$

$$= \text{Chlorophyll b } (\mu\text{g ml}^{-1} \text{ extract}) / \text{dry mass x15}$$

$$= \text{total chlorophyll b content } \mu\text{g}^{-1} \text{ dry mass}$$

Total chlorophyll content

$$7.05 (\text{constant}) \times \text{Abs (661.6nm)} + 18.09 (\text{constant}) \times \text{Abs (644.8)}$$

$$= \text{Total chlorophyll content } (\mu\text{g m}^{-1} \text{ extract})/\text{dry mass x15}$$

$$= \text{Total chlorophyll content } \mu\text{g}^{-1} \text{ dry mass}$$

Total carotenoid content

$$\frac{[1000 \times \text{Absorbance (470)}] - [\text{Constant (1.90) x Chlorophyll a}] - [63.14 \times \text{Chlorophyll b}]}{214}$$

$$= \text{Carotenoid } (\mu\text{g ml}^{-1} \text{ extract}) / \text{dry mass x15}$$

$$= \text{total carotenoid content } \mu\text{g}^{-1} \text{ dry mass}$$

4.2.7 Photosynthesis and respiration rates in whole amaranth seedlings

The net photosynthesis and respiration rates were measured at room temperature with a LI-Cor 6400XT portable photosynthesis system (Biosciences, USA), equipped with a whole plant Arabidopsis chamber. One gram fresh mass of whole seedlings per replicate, exposed to 48 h in sunlight following removal from the dark after four days of incubation, were placed in the chamber. Photosynthesis rate was captured in the presence of white light from a light source (6400-18 RGB) while CO₂ was supplied by an equipped CO₂ tank. The system determines photosynthesis rate by establishing the rate at which CO₂ is absorbed by the plant material per second. The photosynthesis rate was expressed as CO₂ absorption m⁻² s⁻¹.

The respiration rate of one g (FW) seedlings was measured in the same chambers by determining the CO₂ release while no CO₂ was supplied. Respiration rate was expressed as CO₂ release m⁻² s⁻¹. Readings for both photosynthesis and respiration rates were taken at five min intervals.

4.2.8 Extraction and *in vitro* assaying of selected regulatory enzyme activities

4.2.8.1 Phospho-fructokinase (ATP-PFK)

ATP-PFK is a regulatory enzyme of the glycolysis pathway. Extraction and assaying of *in vitro* activity were conducted according to modified procedures adopted by Nardi *et al.* (2007). By using a mortar and pestle, one gram of seedling material from each replicate was homogenized in 4 ml of a 100 mM Tris-HCl extraction buffer (pH 8.0) containing 2 mM MgCl₂, 1 mM EDTA, 14 mM Mercapto-ethanol and 10% Glycerol. The buffer was added slowly and in increments. The homogenate was, subsequently, centrifuged (Selecta P, Spain) for 5 min at 12 000 rpm and the supernatant transferred to clean Eppendorf vials. *In vitro* enzyme activity was determined by means of a Shimadzu UV/VIS PharmaSpec spectrophotometer. The final reaction medium, with a total volume of 1 ml, contained 100 mM Tris-HCl assay buffer (500 µl), 5 mM MgCl₂ .6 H₂O (50 µl), 10 mM Fru-6-P (50 µl), 0.1 mM NADH (20 µl), 1 mM ATP (20 µl), 0.3 U Aldolase (50 µl) and distilled water (290 µl) in a quartz cuvette. The 20 µl enzyme extract was added last to start the enzyme reaction. The PFK enzyme activity was determined by measuring the amount of NADH produced over a period of 10 minutes at a wavelength of 340 nm.

4.2.8.2 Glucose-6-phosphate dehydrogenase (G-6-PDH)

Glucose-6-phosphate dehydrogenase (G-6-PDH) is the only regulatory enzyme of the oxidative pentose phosphate pathway. *In vitro* enzyme activity was determined based on modified laboratory procedures adopted from Boyer (1993). One g of amaranth seedling material was homogenized in 4 ml of extraction buffer (pH 7.5) using a mortar and pestle. The extraction buffer contained 100 mM Tris-HCl (pH = 6.5), 2 mM EDTA, 10 mM mercapto-ethanol and 10% glycerol. The extracts were transferred to Eppendorf vials and centrifuged at 12 000 rpm for 1 min at room temperature. Subsequently, the supernatant was transferred to clean Eppendorf vials which were used to determine the enzyme activity.

In vitro Gluc-6-PDH activity was determined spectrophotometrically using a Shimadzu UV-VIS 2450 spectrophotometer following the method described by Liu *et al.* (2007). The final reaction medium (1 ml) in quartz cuvettes contained 100 mM Tris-HCl assay buffer (500 μ l), 1 mM MgCl₂ .6 H₂O (10 μ l), 40 mM NADH (25 μ l), 1 U of the coupling enzyme, 6-phospho gluconate dehydrogenase (6-FGDH; 16 μ l), 125 Mm Gl-6-phosphate (20 μ L) and distilled water (409 μ L). The enzyme extract (20 μ L) was added last to start the reaction. Activity was determined by measuring the reduction of NADP to NADPH over 10 minutes at 340 nm.

4.2.9 Statistical analysis

Statistical analyses were performed on data converted to a percentage of the control treatment in order to negate the inherent growth differences between species. Two factorial ANOVAS were performed on data using the SAS Version 9.1.3 for personal computers. Means of treatments that were statistically significant at the 5% level of significance were separated using the Tukey LSD (least significant difference) procedure (Steel & Torrie, 1981).

4.3 Results

4.3.1 Total water soluble protein

The total water soluble protein content in *A. hybridus* seedlings projected the same tendency, whether non-stressed or drought stressed, namely that the protein content was higher at 30°C than at 35°C (Figure 4.1A). However, under water stress conditions the total water soluble protein content was significantly lower for *A. hybridus* at 35°C compared to both the unstressed control and *A. cruentus* (Figure 4.1B). On the other hand, *A. cruentus* seedlings under water stress contained significantly more total water soluble protein at 35°C than that at 30°C while under non stressed conditions it was similar (Figure 4.1A). For *A. cruentus* seedlings under water stress the percentage change from the unstressed control indicated a highly significant increase in total water soluble protein content at 35°C (Figure 4.1B). This also differed significantly from *A. hybridus* (Figure 4.1B).

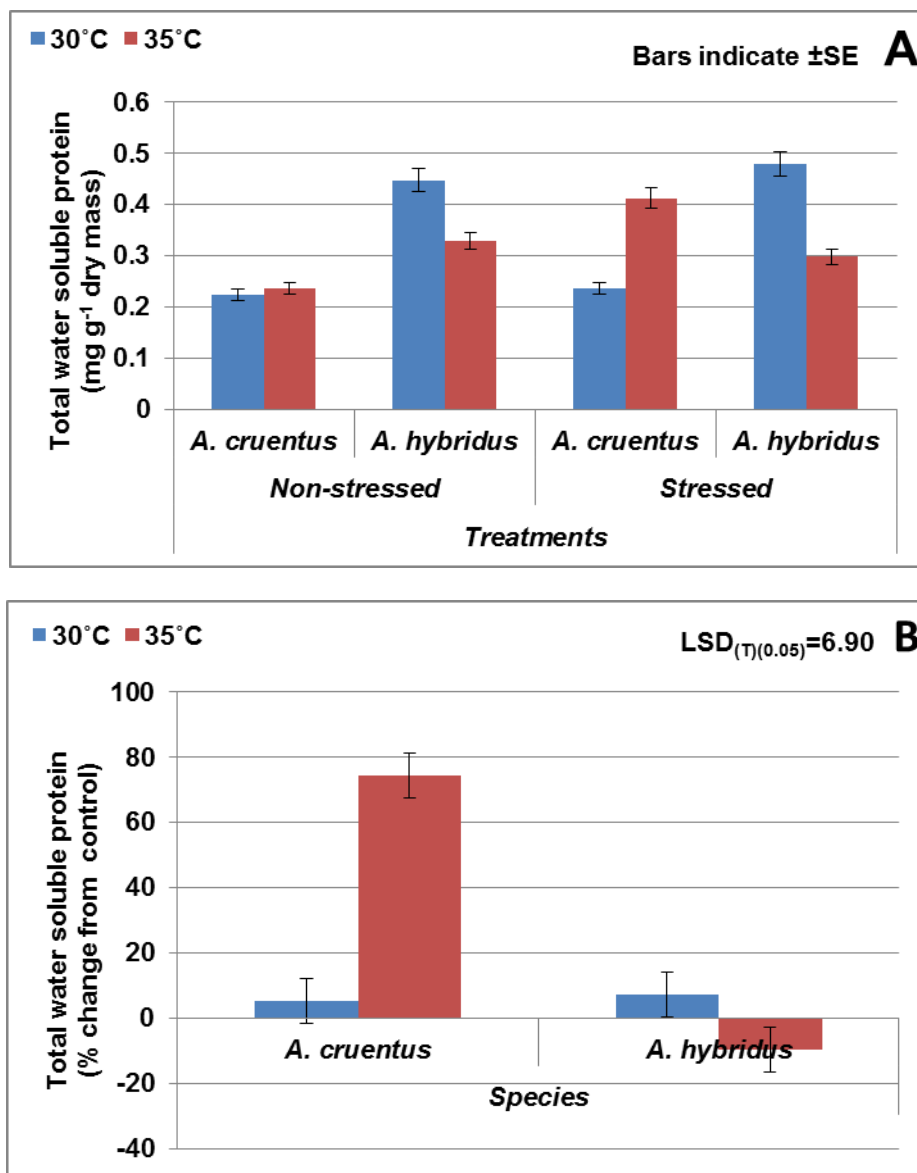


Figure 4.1: Response of 4-day old *A. cruentus* and *A. hybridus* seedlings exposed to different temperatures (30 and 35°C) and water potentials (0 and -1250 kPa) from the seed germination phase in terms of A) mean \pm SE water soluble protein content while B) indicates the % deviation from the non-stressed controls. The $LSD_{(T)(0.05)}$ value is indicated in the graph.

4.3.2 Chlorophyll content

4.3.2.1 Chlorophyll a

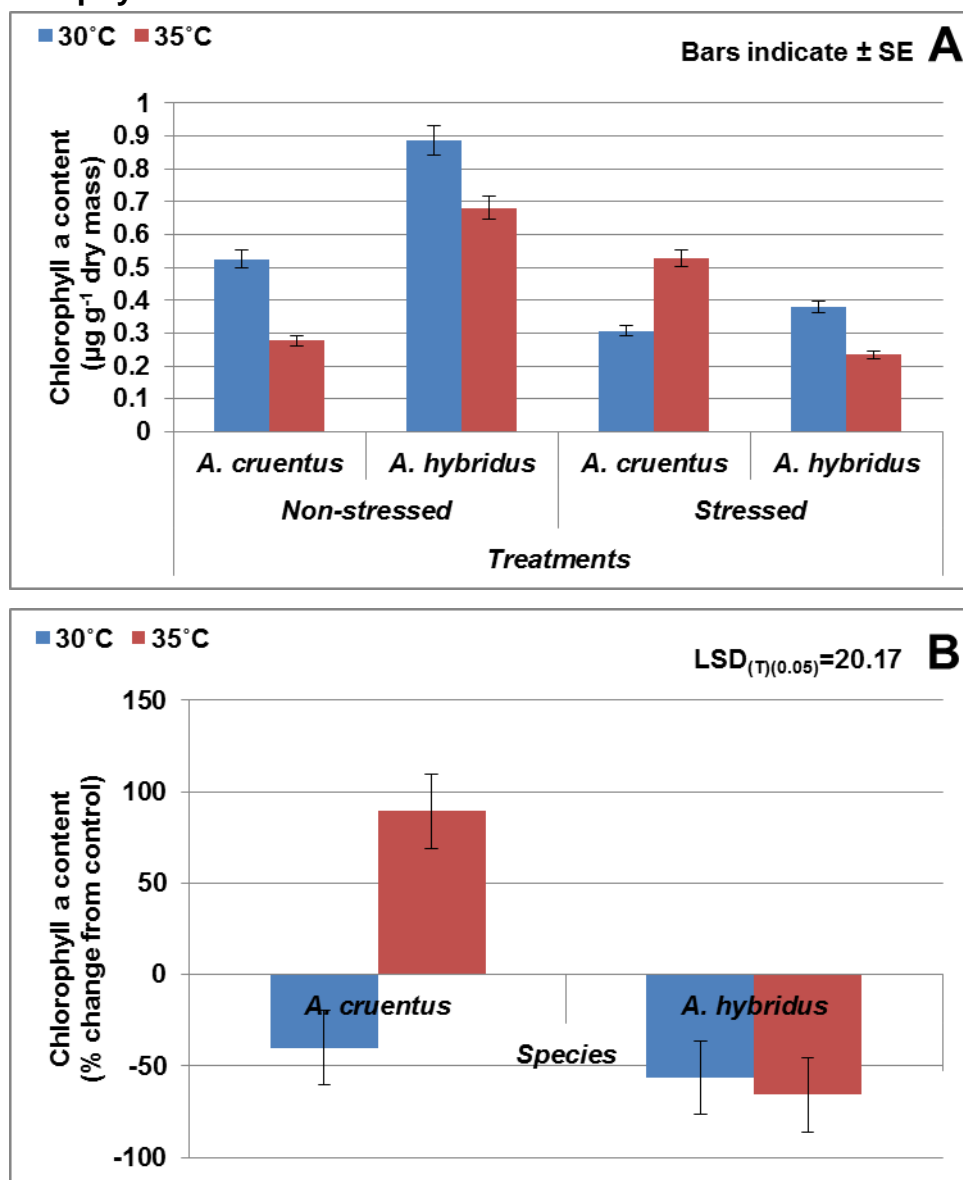


Figure 4.2: Response of 4-day old *A. cruentus* and *A. hybridus* seedlings exposed to different temperatures (30 and 35°C) and water potentials (0 and -1250 kPa) from the seed germination phase in terms of A) mean \pm SE chlorophyll a content while B) indicates the % deviation from the non-stressed controls. The $LSD_{(T)(0.05)}$ value is indicated in the graph.

In *A. cruentus* seedlings the chlorophyll a content tended to be markedly lower at 30°C under stressed conditions, compared to that of non-stressed seedlings, while the opposite was true at 35°C (Figure 4.2A). At both temperatures the % deviation of chlorophyll a content, whether decreasing (30°C) or increasing (35°C), was significantly

($P < 0.05$) different from that of the unstressed control seedlings (Figure 4.2B). In *A. hybridus* seedlings chlorophyll content tended to decrease markedly at both temperature regimes whether stressed or not (Figure 4.2A). In both cases these differences were statistically significant while the percentage deviation did not differ significantly from each other (Figure 4.2B). Comparing the two species, it was clear that *A. hybridus* contained markedly more chlorophyll a under non-stressed conditions at both temperatures than *A. cruentus* (Figure 4.2A). However, under stress *A. cruentus* seedlings retained two fold more chlorophyll a at the higher (35°C) temperature (Figure 4.2A) regime.

4.3.2.2 Chlorophyll b content

Compared to chlorophyll a, the chlorophyll b content pattern was different in *A. cruentus* seedlings. At both 30°C and 35°C a marked increase was observed in stressed seedlings (Figure 4.3A) and this was significantly ($p < 0.05$) different from non-stressed seedlings (Figure 4.3B). In non-stressed *A. cruentus* seedlings the chlorophyll b content was similar at both temperature regimes (Figure 4.3A).

The chlorophyll b (Figure 4.3A) content pattern in *A. hybridus* seedlings was also slightly different from that of chlorophyll a (Figure 4.2A) in the sense that it tended to increase in stressed seedlings at 30°C while the opposite was true at 35°C. The percentage deviation of stressed from non-stressed seedlings was significant in both cases whether increasing (30°C) or decreasing (35°C; Figure 4.3B). However, the intensity of the change from the non-stressed control was also significant. Between species the same tendency as was observed for chlorophyll a applied (Figure 4.3A) namely that *A. cruentus* seedlings retained almost fourfold more chlorophyll b at the higher (35°C) temperature (Figure 4.2A) regime.

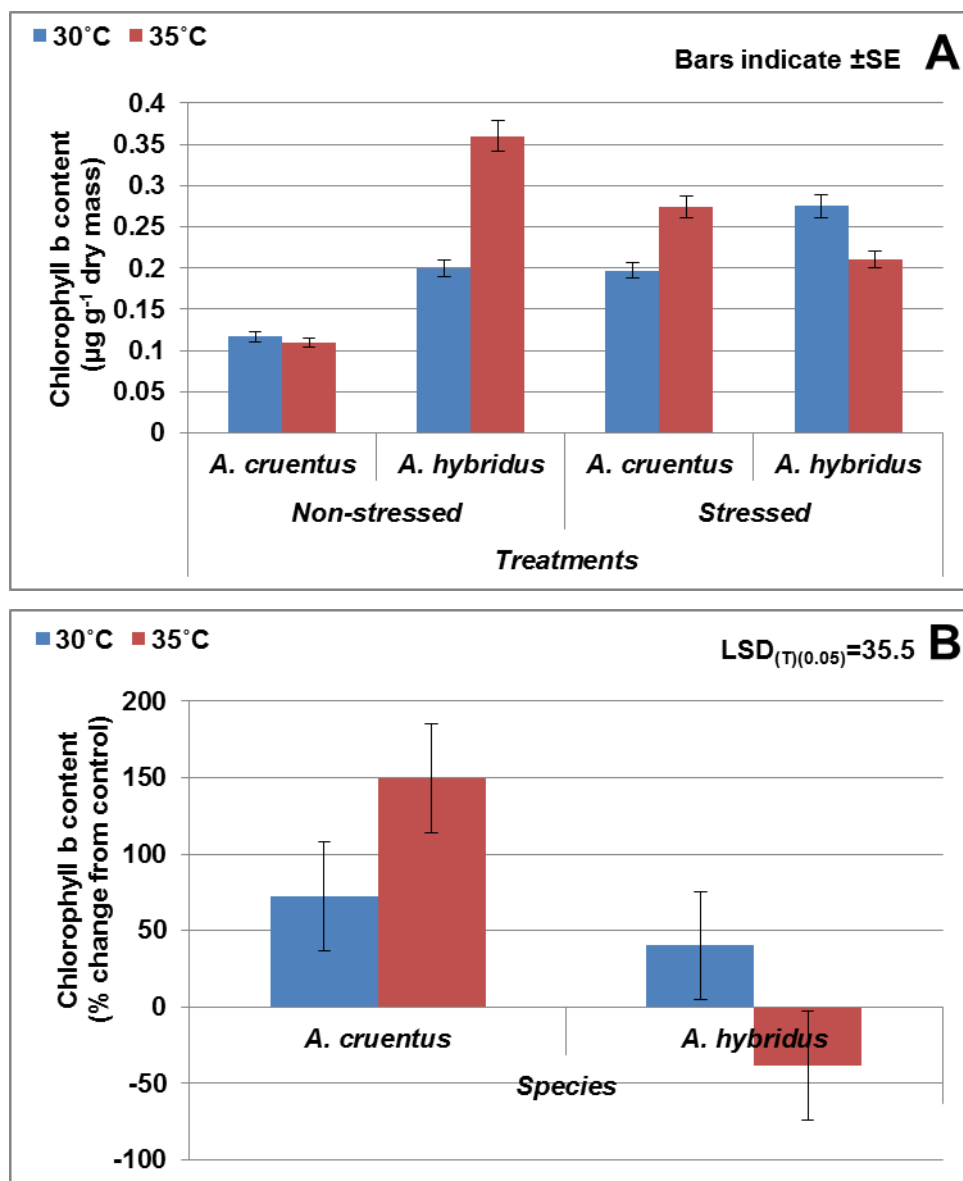


Figure 4.3: Response of 4-day old *A. cruentus* and *A. hybridus* seedlings exposed to different temperatures (30 and 35°C) and water potentials (0 and -1250 kPa) from the seed germination phase in terms of A) mean \pm SE chlorophyll b content while B) indicates the % deviation from the non-stressed controls. The $\text{LSD}_{(T)(0.05)}$ value is indicated in the graph.

4.3.2.3 Total chlorophyll content

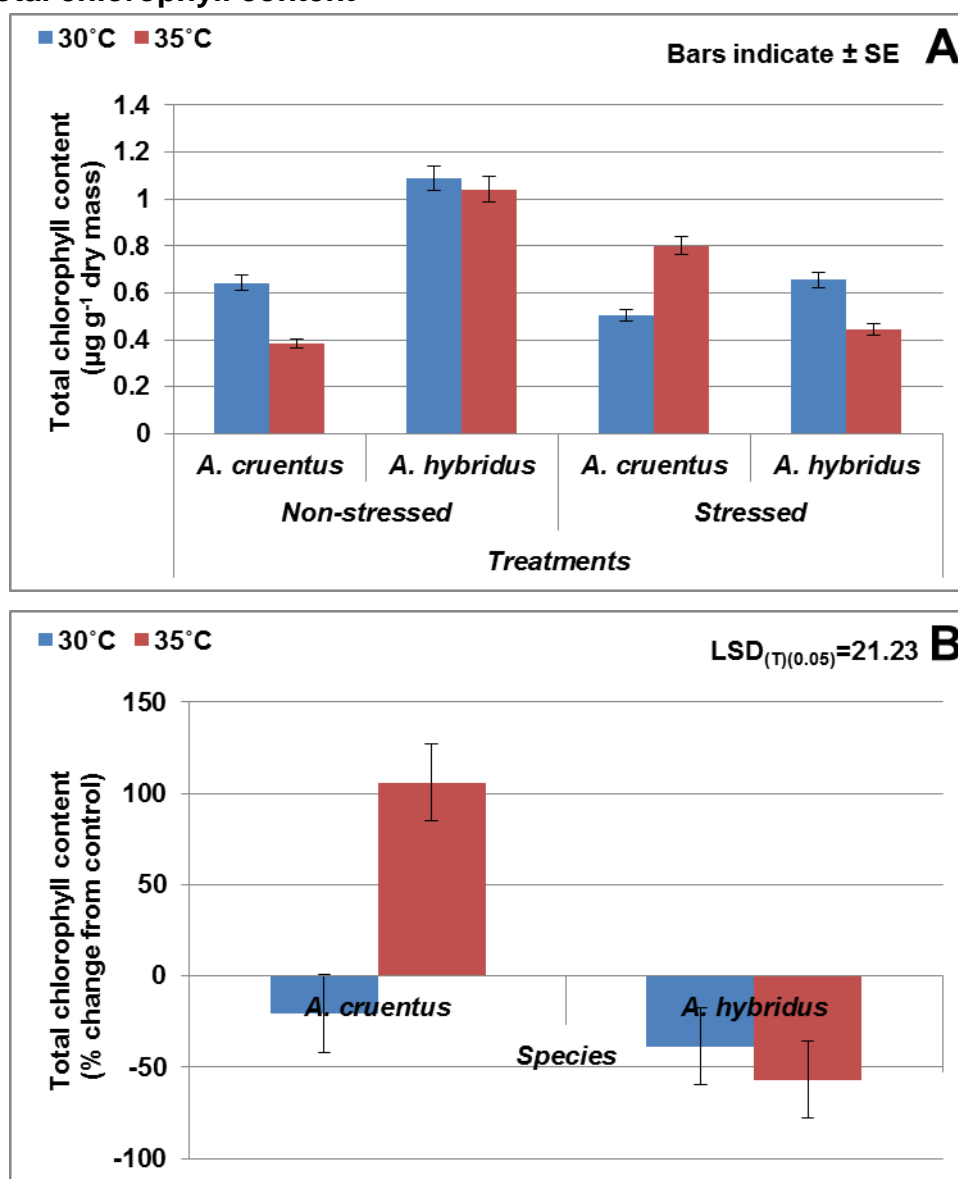


Figure 4.4: Response of 4-day old *A. cruentus* and *A. hybridus* seedlings exposed to different temperatures (30 and 35°C) and water potentials (0 and -1250 kPa) from the seed germination phase in terms of A) mean \pm SE total chlorophyll content while B) indicates the % deviation from the non-stressed controls. The $LSD_{(T)(0.05)}$ value is indicated in the graph.

The total chlorophyll content (Figure 4.4A) in *A. cruentus* seedlings followed exactly the same pattern as did chlorophyll a (Figure 4.2A) namely to decline in stressed seedlings at 30°C and increase at 35°C. Compared to unstressed seedlings both these deviations in stressed seedlings were statistically significant (Figure 4.4B). For *A. hybridus* seedlings the total chlorophyll content pattern (Figure 4.4A) was also the same as for

chlorophyll a (Figure 4.2A), namely that a significant decline (Figure 4.4B) was observed in stressed seedlings at both temperature regimes. However, no significant differences in total chlorophyll content between treatments at 30 and 35°C, whether stressed or not, were observed. Between species the same tendency as was observed for chlorophyll a applied, namely that the drought stress condition contributed to a general decline in total chlorophyll content in the case of *A. hybridus* seedlings at both temperatures while *A. cruentus* seedlings retained markedly more chlorophyll at 35°C, which can be accepted as an additional slight temperature stress condition (Figure 4.4A).

4.3.2.4 Total carotenoid content

In *A. hybridus* seedlings the same tendency of total carotenoid content (Figure 4.5A) to decline markedly in stressed compared to non-stressed seedlings, as was the case with total chlorophyll (Figure 4.4A) content at both temperature regimes, applied. Statistically this decline was significant for both temperatures while the percentage deviation of stressed from non-stressed seedlings did not differ significantly or, in other words, was of the same intensity. Interestingly, in *A. cruentus* seedlings no difference in total carotenoid content was observed at 35°C between non-stressed and stressed seedlings (Figure 4.5A) while a significant increase was observed at 30°C in stressed seedlings (Figure 4.5B). In terms of species specificity *A. cruentus* seedlings significantly increased total carotenoid content while in *A. hybridus* it was significantly decreased.

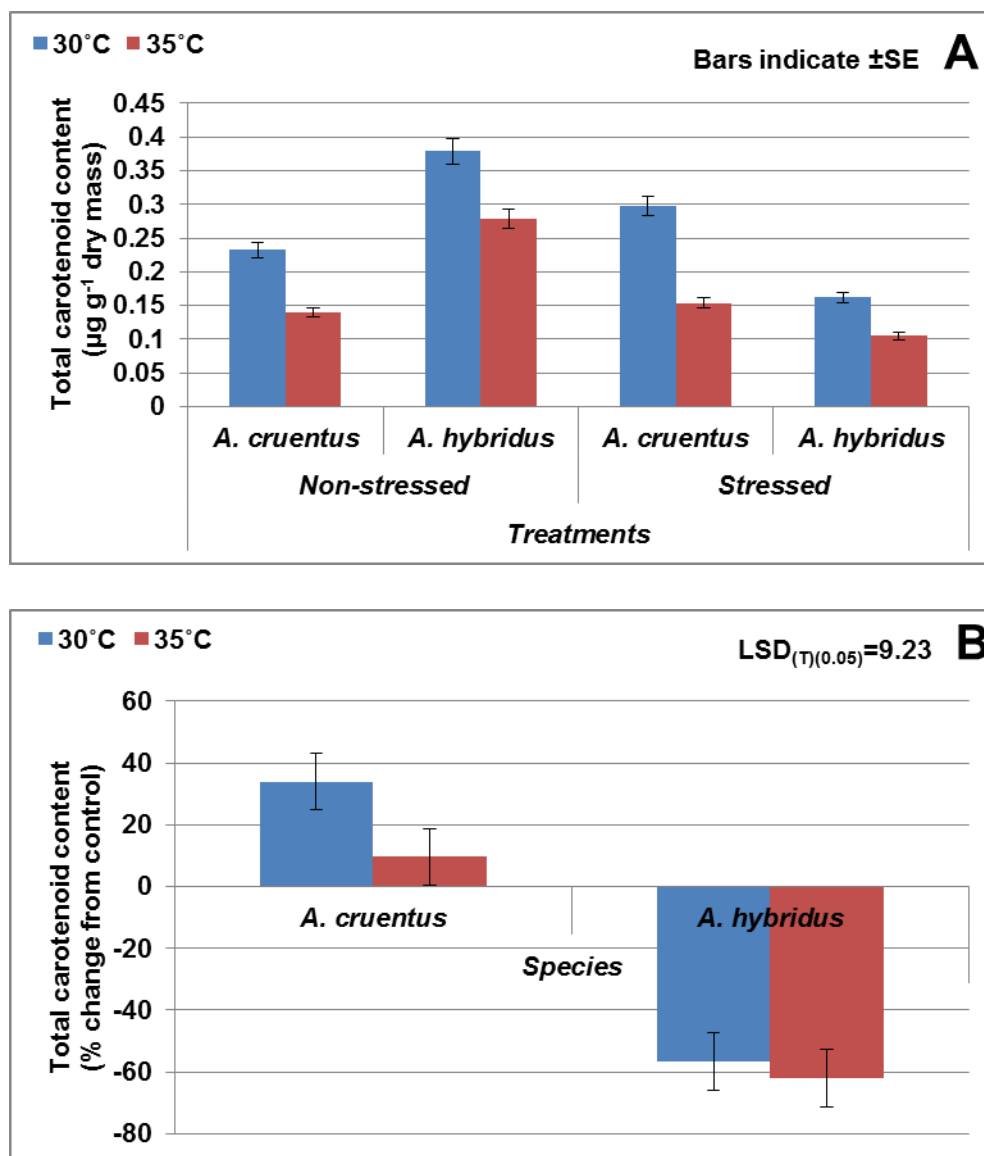


Figure 4.5: Response of 4-day old *A. cruentus* and *A. hybridus* seedlings exposed to different temperatures (30 and 35°C) and water potentials (0 and -1250 kPa) from the seed germination phase in terms of A) mean \pm SE total carotenoid content while B) indicates the % deviation from the non-stressed controls. The **LSD** _{(T)(0.05)} value is indicated in the graph.

4.3.3 Photosynthesis rate

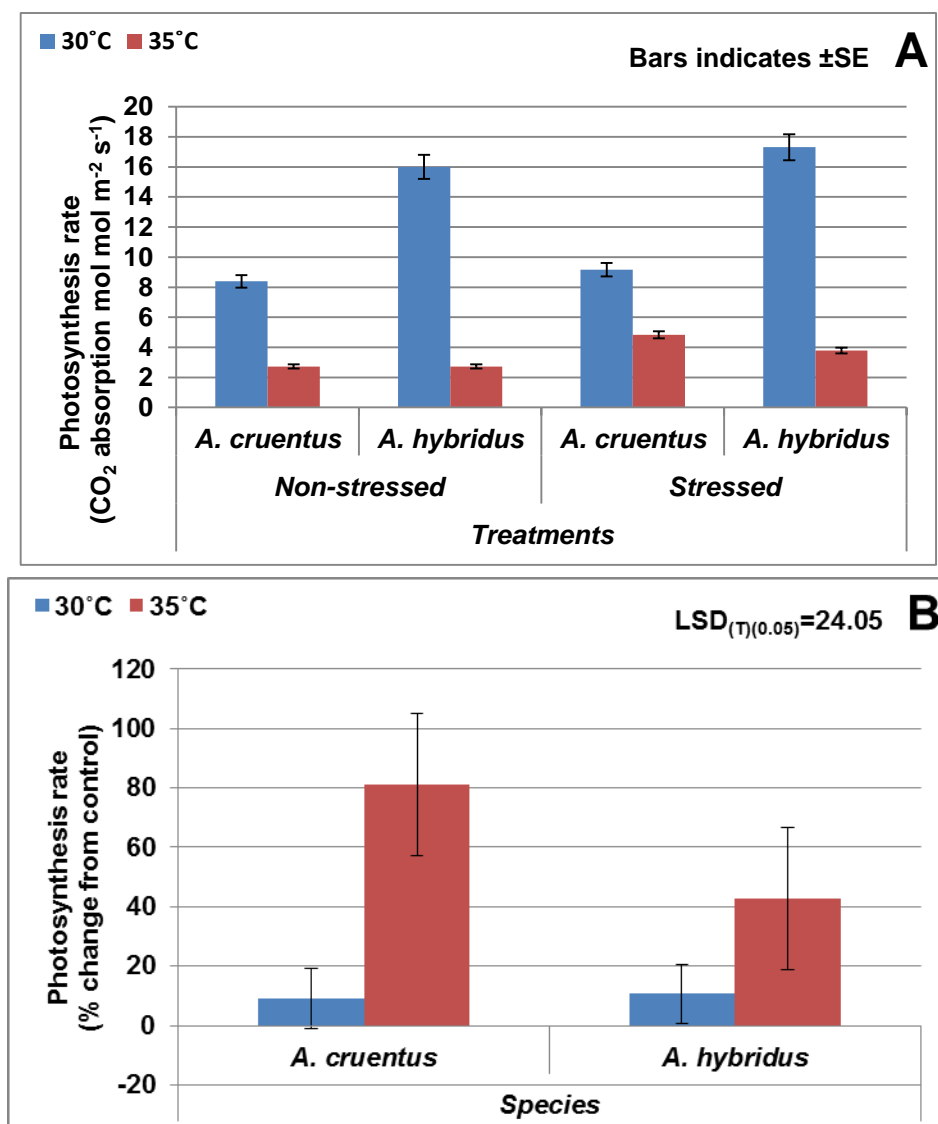


Figure 4.6: Response of 4-day old *A. cruentus* and *A. hybridus* seedlings exposed to different temperatures (30 and 35°C) and water potentials (0 and -1250 kPa) from the seed germination phase in terms of A) mean \pm SE photosynthesis rate while B) indicates the % deviation from the non-stressed controls. The **LSD**_{(T)(0.05)} value is indicated in the graph.

At the onset it must be emphasized that seedlings were exposed to sunlight for 48 h after it was removed from agar plates on day 4. Therefore, only photosynthesis capacity and not the actual rate was measured under both non-stress and drought stress conditions. In this light and compared to *A. cruentus* seedlings, a twofold higher photosynthesis capacity was measured for *A. hybridus* at 30°C in both stressed and non-stressed seedlings (Figure 4.6A), but this was not statistically significant ($P < 0.05$) in

terms of % deviation of the stressed from the non-stressed seedlings (Figure 4.6B). Interestingly, a slight increase in photosynthesis capacity was observed at 35°C for both species under drought stress conditions (Figure 4.6A), but this was significant for both species in terms of % deviation from the non-stressed control (Figure 4.6B). Under drought stress *A. cruentus* seedlings showed a significantly higher photosynthesis capacity at 35°C than did *A. hybridus* seedlings (Figure 4.6B).

4.3.4 Sucrose, D-glucose and D-fructose content

4.3.4.1 Sucrose, D-glucose and D-fructose content in *Amaranthus cruentus* seedlings

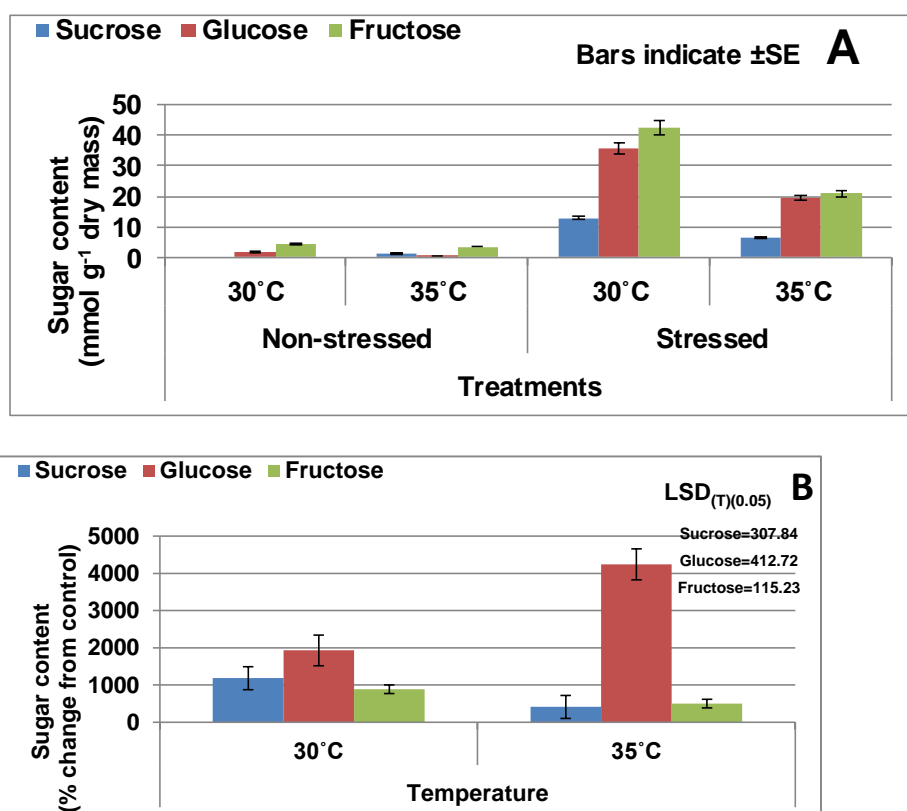


Figure 4.7: Response of 4-day old *A. cruentus* seedlings exposed to different temperatures (30 and 35°C) and water potentials (0 and -1250 kPa) from the seed germination phase in terms of A) mean ± SE sucrose, D-glucose and D-fructose content while B) indicates the % deviation from the non-stressed controls. The **LSD_{(T)(0.05)}** value is indicated in the graph.

It must be emphasized at the onset that sugar measurements were taken directly after seedlings were removed from agar plates that were kept in the dark until the fourth day and not after exposure to sunlight as was the case with photosynthesis capacity measurement. Stressed *A. cruentus* seedlings contained markedly more sugar than non-stressed ones irrespective of the temperature it was exposed to (Figure 4.7A). Interestingly, no sucrose could be detected in non-stressed seedlings exposed to 30°C while it was also extremely low at 35°C. Moreover, under stressed conditions all three sugars were approximately twofold more in seedlings at 30°C than at 35°C while the glucose content increased significantly in stressed seedlings at both temperatures (Figure 4.7B). Sucrose and fructose level changes in stressed seedlings were not significantly different at both temperature treatments (Figure 4.7B).

4.3.4.2 Sucrose, D-glucose and D-fructose content in *Amaranthus hybridus* seedlings

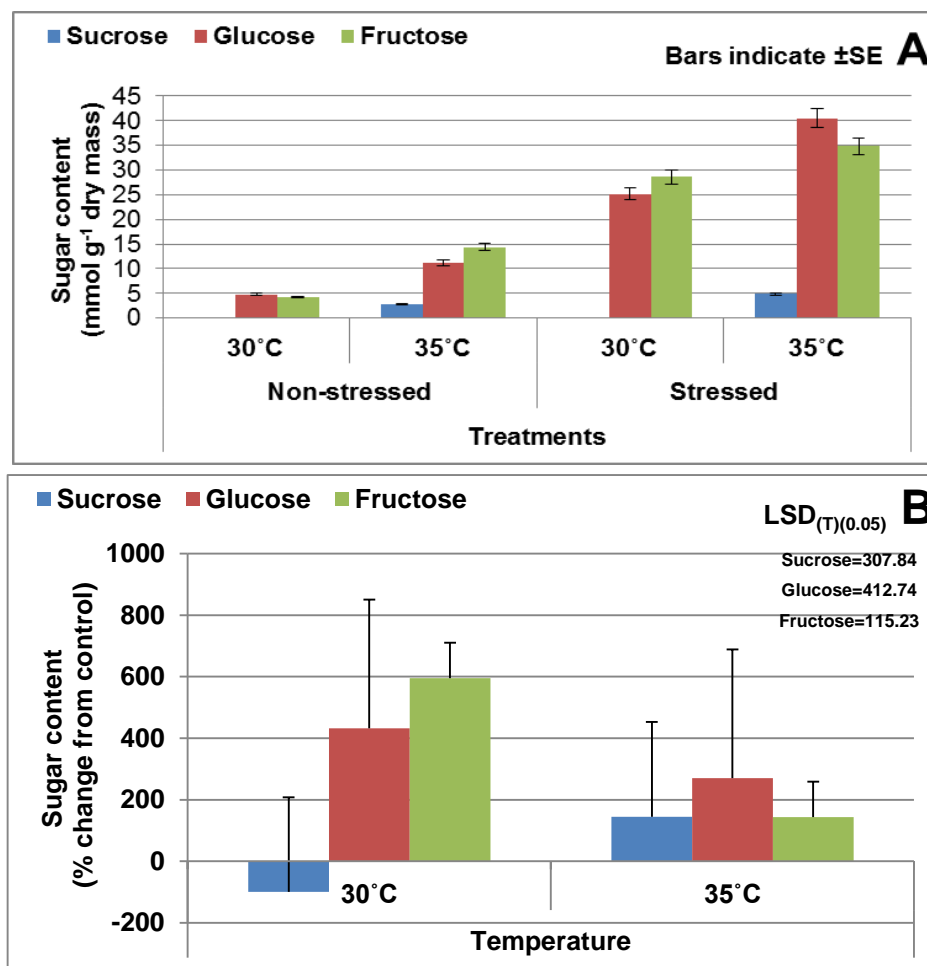


Figure 4.8: Response of 4-day old *A. hybridus* seedlings exposed to different temperatures (30 and 35°C) and water potentials (0 and -1250 kPa) from the seed germination phase in terms of A) mean \pm SE sucrose, D-glucose and D-fructose content while B) indicates the % deviation from the non-stressed controls. The **LSD_(T)(0.05)** value is indicated in the graph.

With the exception of sucrose that could not be detected, the available glucose and fructose in non-stressed *A. hybridus* (Figure 4.8A) seedlings was markedly higher than that of *A. cruentus* (Figure 4.7A). But, *A. hybridus* seedlings showed the same tendency to have elevated sugar content levels under water stress conditions as was the case with *A. cruentus*. However, markedly more monosaccharide sugars were observed in water stressed *A. hybridus* seedlings at 35°C than at 30°C and this was the direct opposite of the situation in *A. cruentus*. The percentage deviation of sugar content in stressed *A. hybridus* seedlings from unstressed ones confirmed a non-significant

decrease in already low sucrose levels, but a significant increase in glucose and fructose levels (Figure 4.8B) that coincided with the situation in *A. cruentus* seedlings (Figure 4.7B).

4.3.5 Respiration rate

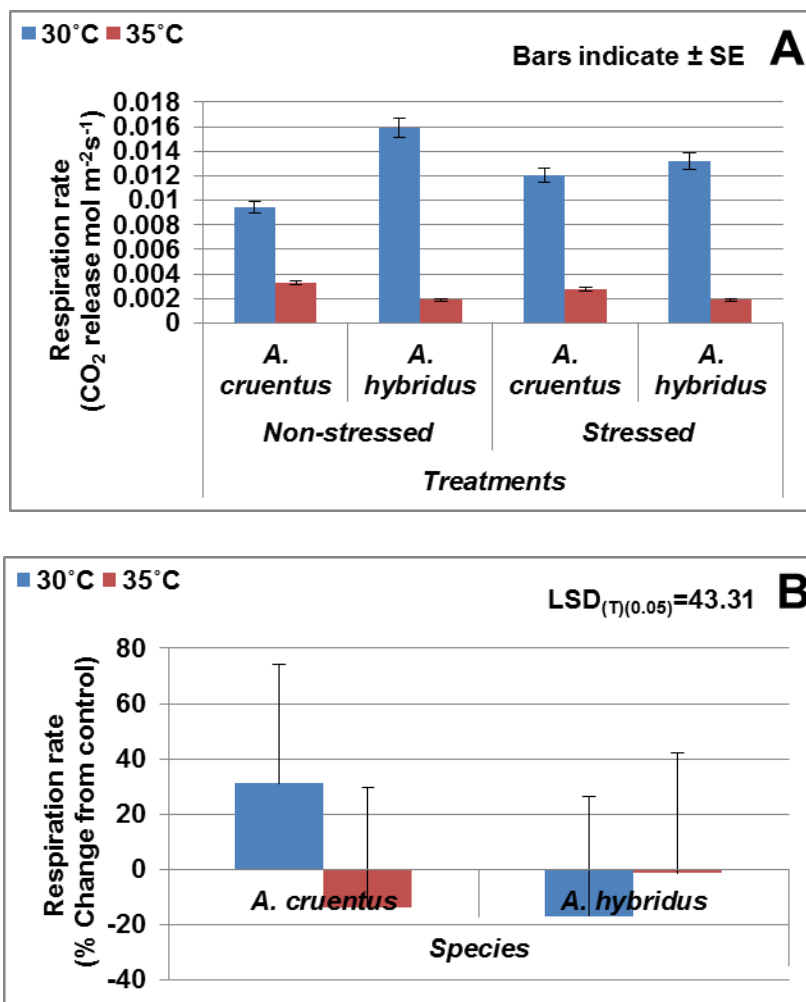


Figure 4.9: Response of 4-day old *A. cruentus* and *A. hybridus* seedlings exposed to different temperatures (30 and 35°C) and water potentials (0 and -1250 kPa) from the seed germination phase in terms of A) mean ± SE respiration rate while B) indicates the % deviation from the non-stressed controls. The LSD_{(T)(0.05)} value is indicated in the graph.

The respiration rate tended to increase slightly in water stressed *A. cruentus* seedlings at 30°C while the opposite was true for *A. hybridus* (Figure 4.9A). At 35°C the respiration rate was the same in both non-stressed and water stressed seedlings. However, the differences were non-significant in all cases (Figure 4.9B).

4.3.6 Respiratory enzyme activities

4.3.6.1 Phospho-fructokinase (ATP-PFK)

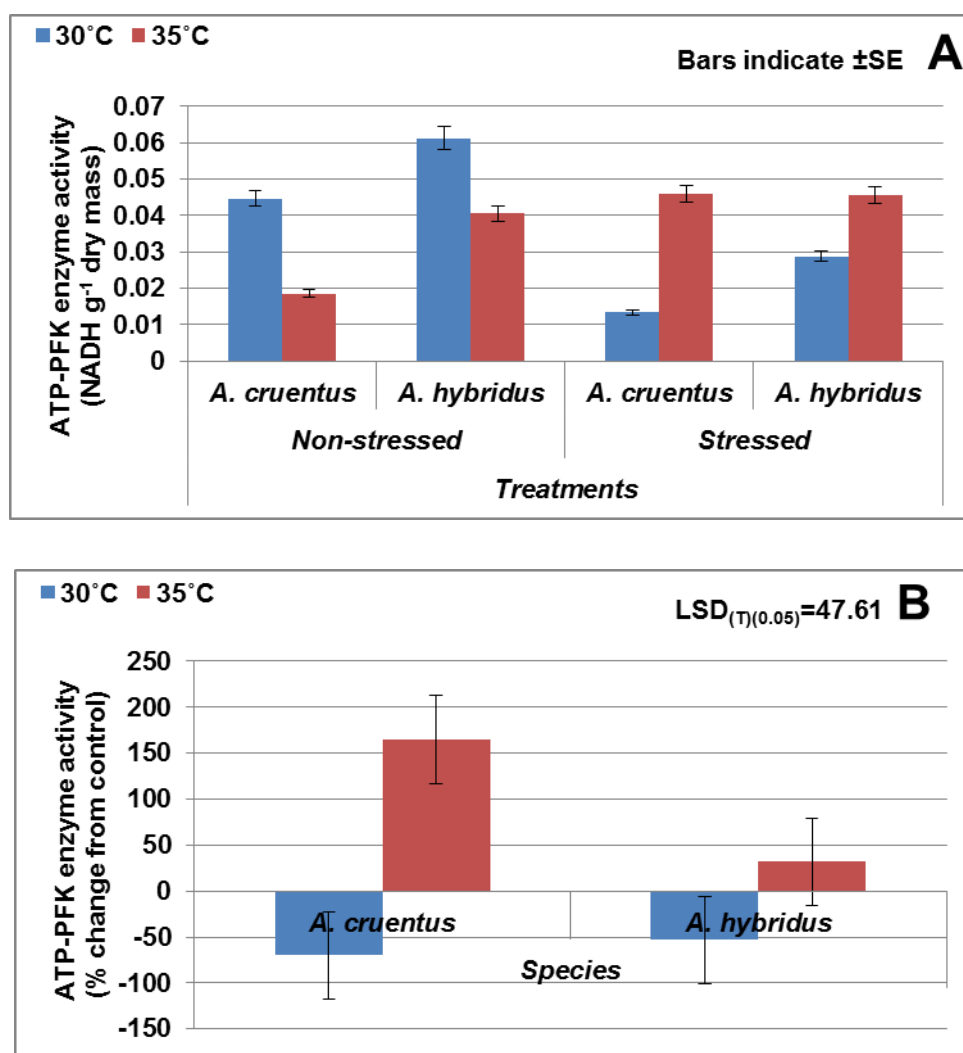


Figure 4.10: Response of 4-day old *A. cruentus* and *A. hybridus* seedlings exposed to different temperatures (30 and 35°C) and water potentials (0 and -1250 kPa) from the seed germination phase in terms of A) mean \pm SE *in vitro* ATP-phosphofructo kinase (PFK) activity while B) indicates the % deviation from the non-stressed controls. The $LSD_{(\tau)(0.05)}$ value is indicated in the graph.

Phospho-fructokinase (PFK) activity in seedlings was markedly higher at 30°C, compared to 35°C, for both species under non-stressed conditions while the opposite was true under water stress conditions (Figure 4.10A). Further, a sharp decline in PFK activity was observed for both species under water stress at 30°C, while a sharp increase for *A. cruentus* and a slight increase for *A. hybridus* were measured at 35°C. The % deviation from the non-stressed control was significant ($P < 0.05$) in the case of *A. cruentus* seedlings, but not for *A. hybridus* (Figure 4.10B).

4.3.6.1 Glucose-6-phosphate dehydrogenase (G-6-PDH)

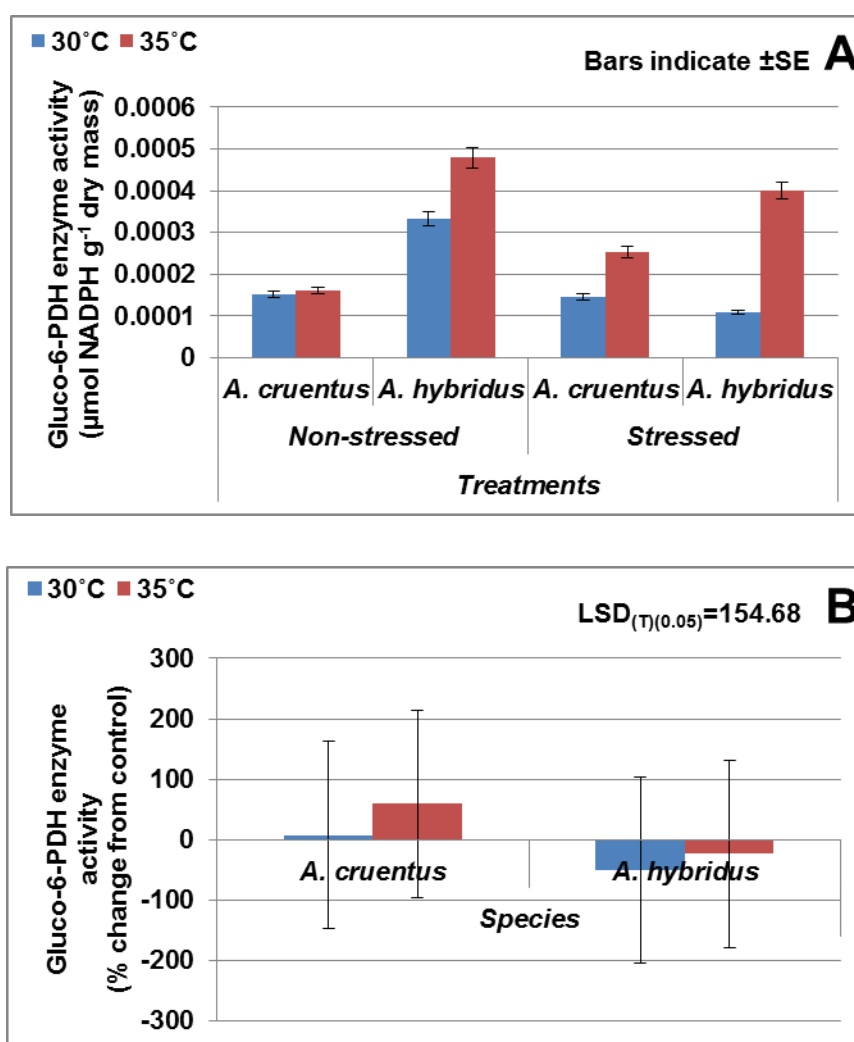


Figure 4.11: Response of 4-day old *A. cruentus* and *A. hybridus* seedlings exposed to different temperatures (30 and 35°C) and water potentials (0 and -1250 kPa) from the seed germination phase in terms of A) mean \pm SE *in vitro* glucose-6-phosphate dehydrogenase activity while B) indicates the % deviation from the non-stressed controls. The $LSD_{(T)(0.05)}$ value is indicated in the graph.

Glucose-6-phosphate dehydrogenase (G-6-PDH) activity revealed the same tendency in seedlings of both amaranth species namely to be slightly higher at 35°C than at 30°C under non-stressed, but markedly higher under water stress conditions (Figure 4.11A). In *A. cruentus* seedlings G-6-PDH activity was very similar at 30°C whether stressed or not, while it increased almost twofold under stressed conditions at 35°C. In water stressed *A. hybridus* seedlings G-6-PDH activity was markedly lower at 30°C and slightly lower at 35°C. However, in all cases the % deviation of G-6-PDH activity in stressed seedlings from non-stressed ones was not significantly different at the 5% probability level (Figure 4.11B).

4.4 Summary of statistical analysis

Table 4.1: Summary of interactions between treatments and species in terms of measured physiological parameters in two amaranth species.

	Temperature (T)	Seed species (S)	Moisture Potential (M)	Replication (R)	TXS	TXM	SXM	TXSXM
Sucrose	**	ns	*	ns	*	*	ns	*
Glucose	***	***	***	ns	***	***	***	***
Fructose	***	***	***	ns	ns	***	***	*
Chlorophyll a	***	***	**	ns	***	***	***	***
Chlorophyll b	ns	***	***	ns	***	ns	***	***
Chlorophyll a+b	***	***	ns	ns	***	***	***	***
Total carotenoid	***	***	ns	ns	***	***	***	***
ATP-PFK enzyme activity	*	ns	ns	ns	ns	ns	ns	*
Glucose-6-PDH enzyme activity	ns	ns	ns	ns	ns	ns	ns	ns
Photosynthesis capacity	***	ns	***	ns	ns	ns	ns	*
Respiration rate	***	ns	ns	ns	*	ns	ns	ns
Total water soluble protein	***	***	***	ns	***	***	***	***

* = significant; ** = highly significant; ns = non-significant

A summary of the analysis of variance carried out for each parameter (Table 4.1) showed that all parameters, with the exception of glucose-6-PDH enzyme activity and respiration, were significantly affected by the interaction between amaranth species, temperature and water potential. This interaction had a highly significant ($P < 0.01$) effect

on glucose, chlorophyll content (a, b and total), carotenoid content and the total water soluble protein. Sucrose and fructose content as well as the ATP-PFK enzyme activity and photosynthetic rate were only significantly affected by this temperature by species by water potential interaction (T x S x M) at the 5% level of significance. Respiration rate was only significantly affected ($P < 0.05$) by the temperature by species (T x S) interaction, although the temperature effect was highly significant ($p < 0.01$) for this parameter. None of the factors tested had any significant effect on the glucose-6-P-DH activity.

4.5 Discussion

Environmental stress is a major limiting factor to crop productivity and a threat to international food security. With the dynamic environmental conditions resulting from the effects of global warming, it is important for plant specialists to fully understand the physiological impact of changing environmental conditions on plant development. Germination and early seedling development are critical stages of every seed propagated crop (Camejo *et al.*, 2005). Therefore, any stress that occurs at either of these developmental stages has a direct impact on vegetative growth, seedling establishment and final yield (Torrecillas & Alarcon, 2005). Water deficit and heat stresses are reported to be the main abiotic hurdles that lead to major crop failures (Du *et al.*, 2008). Hence, this study was undertaken in an attempt to understand their physiological effects during germination and early seedling growth of amaranth.

To quantify the physiological response of amaranth seedlings to drought and heat stress during early development, parameters such as chlorophyll, sugar and total water soluble protein content as well as photosynthesis capacity and respiration rate, including the *in vitro* activity of two regulatory enzymes, were determined. With the exception of chlorophyll content as well as photosynthesis and respiration rates, all other parameters were measured in seedlings that were cultivated from seed kept in a dark incubator for four days. However, seedlings used to determine chlorophyll content, photosynthesis and respiration rates were exposed to light for 48 hours after removal from the incubator. The reason for this, especially in the case of photosynthesis, was

that seedlings removed directly from the dark have not had the opportunity to produce chlorophyll at an optimal rate. It was accepted that, under these conditions, an unrealistic representation of the underlying photosynthesis rate would be obtained. Therefore, at most, photosynthesis and light respiration rates measured in this study merely represent the capacities of these metabolic events under stress conditions.

Although not significant in all cases, interesting differences between species in terms of tendencies in their response towards treatment at two temperature and two water potential regimes were observed in amaranth seedlings. The main purpose of this discussion, therefore, was to ascertain tangency between physiological aspects affected by temperature and water stress conditions in the two amaranth species in order to identify traits in either or both the species in terms of tolerance.

The physiology of plants, including the sum total of all metabolic events, is complex and underlies the growth, development and reproductive potential of a crop. Drought stress and heat shock applied individually on seedlings under controlled research conditions may affect seedling development in different manners. However, it is not entirely clear how they affect physiological mechanism when occurring simultaneously. The response of plants to a combination of drought and heat stress has been reported to include suppressed photosynthesis rate, increased respiration rate, induction of a large number of defense genes including genes involved in sugar and protein synthesis (Rizhsky *et al.*, 2002).

In terms of total water soluble protein, *A. cruentus* seedlings accumulated a significantly higher amount at 35°C under water stress conditions than did *A. hybridus* seedlings while the opposite was true for the latter when non-stressed. The basis for increasing protein content either at higher temperature regimes or water stress conditions is still not well understood, but some authors associated it with natural adaptation to harsh environmental conditions (Dutta *et al.*, 2009). Others, for example Ferguson *et al.* (1994), interpreted increased protein content in peas at a rather high temperature (40°C) as a marker for the expression of new stress protein, and therefore as a measure for the stress condition. It was also reported in drought stressed wheat seedlings that drought stress may stimulate hydrolysis of soluble sugar and protein providing a pool of

compatible osmolytes, which is important in adjusting the plant's regulation in adapting to high temperature and water shortage (Chaves *et al.*, 2008).

More than two decades ago an increase in protein content of plants under temperature or drought stress was attributed to a physiological response (Gurley and Key, 1991). The authors maintained that an increase in total water soluble protein under these circumstances could be due to an increase in key enzyme levels including those responsible for key reactions in metabolic pathways such as glycolysis, the OPP-pathway, the Krebs cycle and lignin synthesis. These have been reported to increase during drought stress. More recently Mohammadkhani and Heidari (2007) supplied evidence supporting the notion that accumulation of drought induced protein in plants is a result of physiological adaptations to water limitation. The authors further indicated that drought stress may accelerate plant development resulting in earlier synthesis of soluble sugars and proteins. Therefore the observed increase in total water soluble protein during drought stress in *A. cruentus* could be the result of a resistance mechanism being triggered, but whether this was successful in contributing to the normal development or even survival of the plant remains to be seen.

To the contrary, a decrease in total water soluble protein in certain plants at a higher temperature regime, as was observed for *A. hybridus* seedlings in this study, was addressed by Riley (1981) more than three decades ago. The author speculated widely on possible reasons. These included that (i) the energy status of seedlings could be too low to fully support anabolic reactions, (ii) there could be a limited supply of amino acids, (iii) the rate of RNA synthesis and RNA turnover could be lower as a result of higher temperature, (iv) decreased protein synthesis rate in heat stressed seedlings could be due to inhibited processing of RNA molecules or their release from the nucleus, (v) the assembly of ribosomes, polysomes or their attachments to membranes could be disrupted and that (vi) key enzymes or other factors involved in polypeptide synthesis could be deficient. From this it is clear that, although total protein level measurement represents a single parameter, the vast physiological impact changes in protein levels might have become evident. Several metabolic events seem to be affected and this probably depends on the specific proteins affected. At most

speculations such as that of Riley (1981) create postulates that can be pursued as a rationale for future research. In this study the protein level increase observed in stressed *A. cruentus* seedlings could be part of changes in metabolic events as pointed out by Riley (1981). Whether this can be associated with differences between the two amaranth species in terms of other measured physiological parameters or even differences in tolerance levels remains to be seen. Moreover, the effect of drought and heat on protein synthesis and especially specific proteins involved seems to be a vast study on its own that needs to be addressed in future.

Of special importance in this study was the photosynthetic response of amaranth seedlings to the abiotic stress conditions it was subjected to. The obvious reason is the fact that photosynthesis is the primary metabolic event that determines the level of combustible substrate and energy supply for growth and development. As mentioned earlier, seedlings used to determine the photosynthesis rate were exposed to light for 48 hours after removal from the incubator. This was done to test the capacity of seedlings cultivated in the dark for four days to (i) produce chlorophyll and (ii) to maintain an optimal photosynthesis rate after being exposed to heat and drought stress.

In the current study, when seedlings were removed after four days incubation in the dark and chlorophyll content measured after exposure to sunlight for 48 h, *A. cruentus* contained significantly more chlorophyll a, b and total chlorophyll at the higher temperature (35°C) regime under drought stress while *A. hybridus* showed the opposite tendency. This is in concert with a study conducted almost three decades ago on the thermo tolerance of three amaranth species (Kerepesi & Galiba, 2000). The authors showed that the chlorophyll content of all three species increased with increasing temperature from 15 to 35°C. They further indicated that the three amaranth species were more tolerant to high alternating temperature since they survived at 40/45°C for more than ten days and recovered well after they were exposed to optimal growth conditions. Although the similar reaction of *A. cruentus* is merely an indication of the potential of the seedlings to synthesize chlorophyll under stress, compared to *A. hybridus* seedlings that showed a significant decrease under stress, it seems that *A. cruentus* seedlings were potentially in a better position to proceed with photosynthesis

even under stress. Total carotenoid content followed exactly the same pattern in both species. Since a direct relationship exists between chlorophyll content and photosynthesis rate, while carotenoids act as chlorophyll protectors (Asada *et al.*, 1998; Loggini *et al.*, 2009), the higher levels measured in *A. cruentus* seedlings points towards a possible advantage under stress compared to *A. hybridus* seedlings. Contrary to earlier arguments in terms of protein content, photosynthetic capacity data might indicate the opposite namely that *A. cruentus* is more resistant towards temperature and drought stress than *A. hybridis*. Alternatively, *A. cruentus* has the ability to recover from the stress when conditions return to normal.

Photosynthesis capacity of seedlings was measured under similar conditions as explained above, namely after exposure to sunlight for 48 h following removal from the dark. Interestingly, despite the difference in chlorophyll content between the two species, the photosynthesis capacity was significantly higher at the higher temperature regime (35°C) in both species under drought stress. However, under non-stressed conditions the opposite was true. This was rather unexpected as photosynthesis has shown a common tendency of decreasing with increasing drought stress and heat shock in many plant species (Jiang & Huang, 2000). Certain drought resistant plant species possess both drought avoidance mechanism and the ability to adapt by means of active osmoregulation (Jie *et al.*, 2012). According to the authors this increased flexibility of plants in response to changing environmental conditions must be considered as advantageous and probably includes adjustment of metabolic and physiological processes to existing environmental conditions that may alter their dry matter production and utilization.

Soluble sugars, the end products of photosynthesis, are crucial components due to their roles as nutrient reserves for energy supply, via respiratory pathways, essential for plant growth and development. In this study sugar levels were measured from seedlings which have been incubated in the dark for a period of four days. This means that the sugars that could be detected in seedlings were not products of photosynthesis, but those translocated from the storage sections of the seed namely plastids of perisperm cells in the cotyledons (Coimbra & Salema, 1994). Importantly, carbohydrate is

translocated from the cotyledons to the axis or seedling section of the embryo in the form of the disaccharide sucrose (Oparka & Turgeon, 1999). During early seed germination and subsequent seedling growth sucrose is broken down to two monosaccharides, glucose and fructose. Both are utilized as substrates for energy release initially via the glycolysis respiratory cycle under anaerobic conditions and finally by the Krebs cycle under aerobic conditions after the radicle penetrated the seed testa.

Interestingly, in this study, extremely low sucrose could be detected in four day old seedlings of both amaranth species under non-stressed conditions and at both temperature regimes directly after removal from the dark. This is attributed to a high rate of sucrose breakdown as the glucose and fructose levels tended to be higher under non-stressed conditions, but especially in *A. hybridus*. This corresponds with the findings of Jie *et al.* (2012) Furthermore, under drought stress conditions the sucrose and monosaccharide levels tended to be high at 30°C and low at 35°C in *A. cruentus* seedlings while the opposite was true for *A. hybridus*. This indicates that either more sugar was translocated to *A. hybridus* seedlings under drought stress or less were utilized during respiration. In *A. cruentus*, the species that appeared to be less stress tolerant, the low sugar levels under stress might indicate a disability to translocate sufficient carbohydrate reserves from the cotyledons to the seedlings (Gill *et al.*, 2001).

The conversion of sucrose to its two monosaccharide moieties, glucose and fructose, is in preparation of supplying combustible soluble sugars to be broken down by respiration to energy, water and CO₂ (Pretorius & Small, 1992; Gupta & Kaur, 2005). Subsequently, the respiration rate of seedlings was measured by quantifying the CO₂ release over time. Once again only the respiratory capacity was measured as seedlings were exposed to 48 h sunlight after removal from the dark. In general the respiration rate was markedly lower in drought stressed *A. hybridus* seedlings compared to *A. cruentus*. This corresponded with the findings of Rizhsky *et al.* (2002) in tobacco (*Nicotiana tabacum*), who reported that the respiration rate decreased markedly in drought stressed plants compared to those that were only subjected to heat stress.

Interestingly, both amaranth species showed a significantly higher respiration rate at 30°C than at 35°C irrespective of whether they were exposed to drought stress or not.

This corresponded with significantly higher phospho-fructokinase (ATP-PFK) enzyme activity at 30°C, but only in seedlings not subjected to drought stress. In seedlings of both amaranth species the activity of this glycolytic regulatory enzyme (Riley, 1981; Byrd *et al.*, 1992) was significantly lower at 30°C than at 35°C indicating that not only this regulatory enzyme was involved in determining the respiratory rate. This is difficult to explain and is in conflict with the findings of Bunce (2007) who reported that increasing temperature can have a decreasing effect on respiratory enzyme activity due to heat shock. The only marked difference observed between species in terms of ATP-PFK activity was that *A. cruentus* seedlings showed significantly higher activity under drought stress and at 35°C than did *A. hybridus* seedlings. However, no clear pattern emerged. Therefore, a need exists for further investigation of other factors responsible for this respiratory behaviour of amaranth species seedlings under heat and drought stress.

Importantly, the oxidative pentose phosphate (OPP) pathway is considered by many as an alternative respiratory route (Kruger & von Schaewen, 2003; Watling *et al.*, 2006; Chen *et al.*, 2010). Subsequently, the *in vitro* activity of glucose-6-phosphate dehydrogenase (G6-PDH), the only regulatory enzyme of the oxidative pentose phosphate (OPP) pathway (Hauschild & von Schaewen, 2003), was measured. Other than ATP-PFK activity, G6-PDH activity tended to be higher at 35°C than 30°C in both amaranth species whether drought stressed or not. Although not significant, the activity of this enzyme tended to be higher in *A. cruentus* seedlings under drought stress and at the higher temperature regime than that of *A. hybridus*.

G6-PDH activity has been positively correlated with environmental stress such as drought (Scharte *et al.*, 2009). Water deficit stress has been correlated with overproduction of reactive oxygen species (ROS), i.e. free radicals that have a negative effect on enzyme activities (Munns, 2002). Tight regulation of excess ROS is an essential protective mechanism in all organisms (Dal Santo *et al.*, 2012). According to the authors a protein of the GSK₃ family up-regulates G6-PDH synthesis in *Arabidopsis* that forms part of a resistance mechanism towards drought stress. In this study the same was not observed for any of the amaranth species except a slight increase in G6-

PDH activity in drought stressed *A. cruentus* seedlings. However, this is a matter that needs to be looked into in future research on amaranth resistance towards abiotic stress factors.

In summary, with the exception of gluco-6-PDH enzyme activity and respiration rate, all measured physiological and metabolic events were either significantly or highly significantly influenced by the interaction effect of temperature, moisture stress and seed species (T x S x M; Table 4.1). Although the effect of individual stress conditions such as drought and heat on plant growth and development have been the focus of research for many years, crops are routinely subjected to a combination of different abiotic stresses in the field (Craufurd & Peacock, 1993; Jiang & Haung 2001; Moffat, 2002). However, Rizhsky *et al.* (2004) pointed out that the metabolic responses of many plant seedlings to a combination of drought and heat stress are unique and cannot be directly extrapolated from the response of each of these different stresses applied individually. The authors also stated that little is known about the mechanisms underlying the acclimatization of plants to a combination of different stress conditions.

Gao *et al.* (2000) and also Suzuki *et al.* (2005) maintained that most of the abiotic stress studies are performed under controlled conditions in laboratories and do not reflect the actual conditions that occur in the field. Therefore, a considerable gap might exist between the knowledge gained by these studies and that required to identify or develop crops that are tolerant to abiotic stress under field conditions. A focus on molecular, physiological and metabolic aspects is therefore crucial to bridge this gap and to facilitate the selection of crops with greater tolerance to field stress conditions (Suzuki *et al.*, 2005). It is, therefore, suggested that an attempt must be made to repeat the underlying study under controlled glasshouse and, preferably, field conditions.

4.6 References

- ASADA, K., ENDO, T., MANO, J. & MIYAKE, C. 1998.** Molecular mechanism for relaxation of and protection from light stress. Saton K, Murata N editors. Stress responses of photosynthetic organisms. Amsterdam. Pp 37–52.
- BAQUEDANO, F. & CASTILLO, J. 2006.** Comparative ecophysiological effects of drought on seedlings of the Mediterranean water-saver *Pinus halepensis* and water-spenders *Quercus coccifera* and *Quercus ilex*. *Trees Structure and Function* 20: 689-700.
- BERGMEYER, H.U. & BRENT, E. 1974.** Sucrose. In H.U. Bergmeyer (Ed.). Methods of Enzymatic Analysis, Vol.3, Academic Press, New York, Pp 1176-1179.
- BOYER, J.S., 1993.** Temperature and growth induced water potential. *Plant cell and environment* 16. 1099-1106.
- BRADFORD, M.M., 1976.** A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 75, 248-254.
- BUNCE, J.A. 2007.** Direct and acclamatory responses of dark respiration and translocation to temperature. *Annals of Botany* 100, 67-73.
- BYRD, G.T., SAGE, RF. & BROWN, R.H. 1992.** A comparison of dark respiration between C₃, and C₄, plants. *Plant Physiology* 100, 191-198.
- CAMEJO, D., RODRÍGUEZ, P., MORALES, M.A., DELL'AMICO, J.M., TORRECILLAS, A. & ALARCON, J.J. 2005.** High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. *Plant Physiology* 162, 281–289.
- CHAVES, M.M., FLEXAS, J & PINHEIRO, C. 2008.** Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Annals of Botany* 103, 551-560.

- CHEN, Y.Y., KO, T.P., CHEN, W.H., LO, L.P., LIN, C.H. & WANG, A.H. 2010.** Conformational changes associated with cofactor/substrate binding of 6-phosphogluconate dehydrogenase from *Escherichia coli* and *Klebsiella pneumoniae*: Implications for enzyme mechanism. *Journal of Structural Biology* 169(1), 25-35.
- COIMBRA, S. & SALEMA, R. 1994.** *Amaranthus hypochondriacus*: Seed structure and localization of seed reserves. *Annals of Botany* 74, 373-379..
- CRAUFURD, P.Q. & PEACOCK, J.M., 1993.** Effect of heat and drought stress on sorghum. *Experimental Agriculture*. 29: 77–86.
- DAL SANTO, S., STAMPFL, H., KRASENSKY, J., KEMPA, S., GIBON, Y., PETUTSCHNIG, E., ROZHON, W., HEUCK, A., CLAUSEN, T. & JONAK, C. 2012.** Stress-induced GSK3 regulates the redox stress response by phosphorylating glucose-6-phosphate dehydrogenase in *Arabidopsis*. *The Plant Cell* 24, 3380-3392.
- DE RONDE, J.A., LAURIE, R.N. & CAETANO, T. 2004.** Comparative study between transgenic and non-transgenic soybean lines proved transgenic lines to be more droughts tolerant. *Euphytica* 138(2), 123-132
- DU, S.T., ZHANG, Y.S., LIN, X.Y., WANG, Y. & TANG, C.X. 2008.** Regulation of nitrate reductase by its partial product nitric oxide in Chinese cabbage pakchoi (*Brassica chinensis* L. cv. Baoda). *Plant Cell Environment* 31, 195–204.
- DUTTA, S., MOHANTY, S. & TRIPATHY, B.C. 2009.** Role of temperature stress on chloroplast biogenesis and protein import in pea. *Plant Physiology* 150, 1050–1061.
- FERGUSON, I.B., LURIE, S. & BOWEN, J.H. 1994.** Protein synthesis and break down during heat shock of cultured pear (*Pyrus communis* L.) cells. *Plant Physiology* 104, 1429-1437.

- GAO, M., SAKAMOTO A., MIURA K., MURATA, N., SUGIURA A. & TAO R. (2000).** Transformation of Japanese persimmon (*Diospyros kaki*) with a bacterial gene for choline oxidase. *Molecular Breeding* 6, 501–510.
- GEORGE, A. F. H. 1992.** Evolutionary origins and natural functions of fructans: a climatological, biogeographic and mechanistic appraisal. *New Phytologist* 123, 2-11.
- GILL, P.K., SHARMA, A.D., SINGH, P. & SUKDEV SINGH BHULLAR, S.S. 2001.** Effect of various abiotic stresses on the growth, soluble sugars and water relations of sorghum seedlings grown in light and darkness. *Bulgarian Journal of Plant Physiology* 27, 72–84.
- GOU, P. & AL-KHATIB, K.A. 2003.** Temperature effects on germination and growth of redroot pigweed (*Amaranthus retroflexus*), Palmer Amaranth (*A. palmeri*), and common waterhemp (*A. rudis*). *Weed Science* 51, 869-879.
- GUPTA, A.K. & KAUR, N. 2005.** Sugar signaling and gene expression in relation to carbohydrate metabolism under abiotic stresses in plants. *Journal of Bioscience* 30, 761-776.
- GURLEY, W.B. & KEY, J.L. 1991.** Transcriptional regulation of the heat-shock response: A plant perspective. *Biochemistry* 30, 1-12.
- HAUSCHILD, R. & VON SCHAEWEN, A. 2003.** Differential regulation of glucose-6-phosphate dehydrogenase isoenzyme activities in potato. *Plant Physiology* 133, 47-62.
- HONG. Z., LAKKINENI, K., ZHANG, K. & VERMA, D.P.S. 2001.** Removal of feedback inhibition of D-1-pyrroline-5-carboxylate syntheses results in increased proline accumulation and protection of plants from osmotic stress. *Plant Physiology* 122, 1129–1136.

- HURA, K., KAJITA, R., TORII, K.U., BERGMANN, D.C. & KAKIMOTO, T. 2007.** The secretory peptide gene EPF1 enforces the stomatal one cell-spacing rule. *Research Communication* 21, 1720–1725.
- JIANG, G.L. & HUANG, S. 2001.** Evaluation of Drought Resistance for Texas Bluegrass, Kentucky Bluegrass, and Their Hybrids. *Soil Science* 44, 1746 – 1753.
- JIE, Z., XIAODONG, J., TIANLAI, L. & ZAIQIANG, Y. 2012.** Effect of moderately-high temperature stress on photosynthesis and carbohydrate metabolism in tomato (*Lycopersico esculentum* L.) leaves. *African Journal of Agricultural Research* 7(3), 487-492.
- KEREPESI, I. & GALIBA, G. 2000.** Osmotic and salt stress-induced alteration in soluble carbohydrate content in wheat seedlings. *Crop Science* 40, 482–487.
- KRUGER, N.J. & VON SCHAEWEN, A. 2003.** The oxidative pentose phosphate pathway: structure and organization. *Current Opinions in Plant Biology* 6(3), 236-246.
- LAL, A. & EDWARDS, G.E. 1996.** Analysis of Inhibition of photosynthesis under water stress in the C4 Species *Amaranthus cruentus* and *Zea mays*: Electron Transport, CO₂ Fixation and Carboxylation Capacity. *Australian Journal of Plant Physiology* 23(4), 403 – 412.
- LI & WANG, 2003.** The deepest divergences in land plants inferred from phylogenomic evidence. *Plant Biochemistry* 23, 1243 – 1251.
- LIU, F. & STUTZEL, H. 2002.** Leaf water relations of vegetable amaranth (*Amaranthus* spp.) in response to soil drying. *European Journal of Agronomy* 16, 137-150.

- LIU, Z.Y., WANG, G.C., ZHOU, B.C. 2008.** Effect of iron on growth and lipid accumulation in *Chlorella vulgaris*. *Bioresource Technology* 99, 4717–4722
- LOGGINI, B., SCARTAZZA, A., BRUGNOLI, E. & NAVARI-IZZO, F. 2009.** Antioxidant defense system, pigment composition and photosynthetic efficiency in two wheat cultivars subjected to drought. *Plant Physiology* 119, 1091–1099.
- MACKINNEY, G. 1941.** Absorption of light by chlorophyll solutions. *Biological Chemistry* 140, 315.
- MICHAEL, B.E., 1983.** Evaluation of the water potentials of solutions of polyethylene glycol 8000 both in absence and presence of other substances. *Plant Physiology* 72, 66-70.
- MOFFAT, A. S. 2002.** Plant genetics. Finding new ways to protect drought- stricken plants. *Journal of Science* 296: 1226–1229.
- MOHAMMADKHANI, N. & HEIDARI, R. 2007.** Effects of water stress on respiration, photosynthetic pigments and water content in two maize cultivars. *Pakistan Journal of Biological Sciences* 10 (22), 4022-4028.
- MUNNS, R. 2002.** Comparative physiology of salt and water stress. *Plant Cell Environment* 25, 239-250.
- MUSTAFA, A., AMAN, P., ANDERSSON, R. & KAMAL-ELDIN, A. 2007.** Analysis of free amino acids in cereal products. *Food Chemistry* 105, 317–324.
- NARDI, S., MUSCOLO, A., VACCARO, S., BAIANO, S., SPACCINI, R. & PICCOLO, A. 2007.** Relationship between molecular characteristics of soil humic fractions and glycolytic pathway and krebs cycle in maize seedlings. *Soil Biology & Biochemistry* 39, 3138-3146.
- OPARKA, K.J. & TURGEON, N. 1999.** Sieve Elements and Companion Cells: Traffic Control Centers of the Phloem. *Plant Cell* 11, 739–750.

- PRETORIUS, J.C. & SMALL, J.G.C. 1992.** The effect of soaking injury in bean seeds on aspects of the oxidative pentose phosphate pathway in embryonic axes. *Seed Science Research* 2, 33–39
- RILEY, G.J.P. 1981.** Effects of High Temperature on Protein Synthesis During Germination of Maize (*Zea mays* L.). *Planta* 151, 75-80.
- RIZHSKY, L., HONGJIAN, L. & MITTLER, R. 2002.** The combined effect of drought stress and heat shock on gene expression in tobacco. *Plant Physiology* 130, 1143–1151.
- RIZHSKY, L., LIANG, H. & MITTLER, R. 2004.** The water-water cycle is essential for chloroplast protection in the absence of stress. *Biological Chemistry* 278: 38921–38925
- RONG-HAU, L., PIE-GUO, G., BAUM, M., GRANDO, S. & CECCARELLI, S. 2006.** Evaluation of chlorophyll content and fluorescence parameters of drought tolerance in barley. *Agricultural Sciences in China* 5(10), 751-757.
- SCHARTE, J., SCHÖN, H., TJADEN, Z., WEISS, E. & VON SCHAEWEN, A. 2009.** Isoenzyme replacement of glucose-6-phosphate dehydrogenase in the cytosol improves stress tolerance in plants. *Proceedings of the National Academy of Sciences* 106, 8061-8066.
- SHELLENBAUM, L., SPRENGER, N., SCHÜEPP, H., WIEMKEN, A. & BOLLER, T. 1999.** Effects of drought, transgenic expression of a fructan synthesising enzyme and of mycorrhizal symbiosis on growth and soluble carbohydrate pools in tobacco plants. *New Phytologist* 142, 67-77.
- STECKEL, L., CRISTY, L.S., EDWARD, W.S. & LOYD, M.W. 2004.** Temperature effects on germination of nine *Amaranthus* species. *Weed Science* 52, 217-221.
- STEEL, R.G. & TORRIE, J.H. 1981.** Principles and Procedures of Statistics-a Biometrical Approach, 2nd edition. McGraw-Hill Book Company, New York. pp 185.

- SUZUKI, N., RIZHSKY, L., LIANG, H., SHUMAN, J., SHULAEV, V. & MITTLER, R. 2005.** Enhanced tolerance environmental stresses in transgenic plants expressing the transcriptional co- activator MBF1. *Plant Physiology* (In press)
- TIAN, X.R & LEI, Y.B. 2007.** Physiological responses of Wheat seedlings to drought and UV-B radiation. Effect of Exogenous Sodium Nitroprusside Application. *Russian Journal of Plant Physiology* 54, 676–682.
- TORRECILLAS, A. & ALARCON, J.J. 2005.** High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. *Plant Physiology* 162, 281–289.
- TUCKER, J.B. 1986.** Amaranth: the once and future crop. *BioScience* 36, 59-60.
- WANG, W., VINOCUR, B. & ALTAN, A. 2003.** Plant response to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta* 218, 1-14.
- WATLING, J.R., ROBINSON, S.A. & SEYMOUR, R.S. 2006.** Contribution of the alternative pathway to respiration during thermogenesis in flowers of the sacred Lotus. *Plant Physiology* 140(4), 1367–1373.
- WU, Q.S., XIA, R.X. & ZOU, Y.N. 2008.** Improved soil structure and citrus growth after inoculation with three arbuscular mycorrhizal fungi under drought stress. *European Journal of Soil Biology* 44, 122–128.
- ZAVITKOVSKI, J. & FERRELL, W.K. 1968.** Effect of drought upon rates of photosynthesis, respiration and transpiration of seedlings of two ecotypes of *Douglas fir*. Two- and three-month-old seedlings. *Botanical Gazette* 129, 346-350.

Chapter 5

General Discussion

More than three decades ago Kigel *et al.* (1977) predicted that food security will become a global concern in future, more specifically in sub-Saharan Africa, due to harsh environmental changes that will limit normal plant growth and development. Although environmental changes have been recorded, e.g. global warming and elevated CO₂ levels, this has not reached catastrophic levels to date. Despite predictions, several environmental factors constantly play a role in crop failures. Of these high temperature and limited water supply are major factors that limit plant productivity and threatens food security in many developing countries of arid and semi-arid ecological regions. Based on the above arguments, it is of great importance for crop specialists to furnish alternative and sustainable approaches in order to improve crop productivity and ensure food security.

Despite crop improvement through various breeding techniques, employing alternative crops with good tolerance characteristics to abiotic stress can be a method to improve food security (Dieleman *et al.*, 1997; Gill *et al.*, 2001). Hence, a search for alternative crops to feed the world is an ongoing process. Amaranth species grew wild for ages, but has been established as an annual vegetable and grain crop through selection. Owing to the tolerance to water deficit and high temperature stress conditions shown by some amaranth species (Gupta & Kaur, 2005), it is seen as a prospective alternative crop with the potential to be cultivated more extensively. Especially it's potential to contribute towards food security, in the event that abiotic stress factors become a threat to traditional vegetable and grain crop production in future, is appreciated as its major attribute (Mustafa *et al.*, 2007).

Seed germination and seedling development are presumed to be the most critical stages in the life cycle of many seed propagated plants, including amaranth, even when environmental factors are optimal (Kaya *et al.*, 2006). Consequently, poor seedling establishment under abiotic stress, leading to crop failure, has been identified as one of the main challenges for researchers and farmers alike (Oryokot *et al.*, 1997; Oyodele,

2000). Furthermore, plant development entails complex metabolic and physiological events that commence as soon as the seed starts imbibing water and continue through the plant's life cycle (Jie *et al.*, 2012). According to the author, seed germination and seedling establishment under limited abiotic conditions has once again attracted the attention of many crop researchers in an attempt to fathom the extent of the problem during these two sensitive growth stages. This served as a rationale for the underlying study on two amaranth species.

In terms of projected changes in the global abiotic environment, Sun *et al.* (2011) recently predicted that the mean annual and global surface temperature will increase by 1.7 and 3.8°C, respectively, by the end of the century. The author further estimated that the annual rainfall will decrease substantially in future as a result of global warming and suggested that alternative crops able to tolerate these stress need to be identified and/or developed. The current study focused on the morphological and physiological response of two amaranth species to different temperature (25, 30, 35, 40°C) and water potential (0, -250, -500, -750, -1000, -1250 kPa) regimes. Seed germination was measured and several growth parameters were employed to quantify the morphological response of seedlings. Physiological parameters included total water soluble protein, chlorophyll and sugar content as well as photosynthesis, respiration and key enzyme rates in seedlings.

The chosen temperatures were based on the fact that the optimum for amaranth seed germination and subsequent seedling growth was reported differently by different authors. For example, Bavec and Mlakar (2002) reported that amaranth species can germinate in a temperature range between 15 and 40°C with the optimum range being between 24 and 35°C. Thomas *et al.* (2006) reported a range between 20 and 40°C while Liu and Stützel (2004) limited it to between 25 and 30°C. However, recently ISTA (2010) published the optimum range as being between 20 and 30°C. For this reason the seed germination and seedling growth response were measured at the four mentioned temperatures (25, 30, 35 and 40°C). Further, amaranth species have been reported to tolerate rather severe drought conditions (Michael, 1983; Liu & Stutzel, 2004; Steckel *et al.*, 2004). Subsequently, seed germination and seedling growth were monitored at six

(0, -250, -500, -750, -1000, -1250 kPa) different water potentials after Michael (1983) and at the four mentioned temperatures.

More specifically, the study was divided into two phases. 1) Assessing seed germination and seedling growth of the two amaranth species under the four mentioned constant temperature and six moisture potential levels. The aim was of dual purpose namely to identify the optimal temperature and moisture potential levels that best support seed germination and early seedling growth and to identify moderate moisture stress conditions where the seedlings still survived, but was clearly under stress. 2) Quantifying the main physiological events in seedlings under two (0 and -1250 kPa) water potential and two (30 and 35°C) temperature regimes guided by results obtained during the first phase. Both phases were conducted under laboratory conditions.

Differences in temperature optima between the two amaranth species in terms of seed germination and seedling growth were observed. The optimum was between 25 and 30°C for *A. cruentus* and between 30 and 35°C for *A. hybridus* indicating that *A. cruentus* is slightly more sensitive to higher temperature than *A. hybridus* in terms of seed germination and early seedling growth. This was confirmed with *A. hybridus* continuing root growth at 40°C and at an extremely low water potential of -1250 kPa while *A. cruentus* reached its maximum growth potential already at 35°C, probably the top of its tolerance level. Interestingly, *A. hybridus* is a wild species genotype found locally while *A. cruentus* is imported possibly indicating a higher level of adaptation to local temperature and moisture potential conditions.

Hypocotyl growth in both species decreased as the water potential was reduced from 0 to -1250 kPa, even at their respective optimum temperatures, while root length growth increased under the same circumstances. This was exaggerated with increasing temperature indicating a strong temperature x water potential interaction, especially at a temperature above 35°C and water potential below -750 kPa.. A recent study by Ntuli (2012), whereby maize seedlings were allowed to germinate at four water potential regimes (0, -300, -600 and -900 kPa), surprisingly showed that germination and seedling growth increased as the water potential was decreased from 0 to -600 kPa. However, at -900 kPa both maize seed germination and subsequent seedling growth

decreased substantially. Increased root length growth could be an indication of the crop's effort to search for moisture to alleviate drought stress (Oryokot, 1997; Oyodele, 2000). This is in concert with the common agronomic and horticultural practice of exposing seedlings to a short period of moderate drought stress in order to encourage root growth (Hartmann *et al.*, 2011). However, although root length increased with decreasing water potential it seems that this happened at the expense of dry matter accumulation. Dry matter in both the hypocotyls and roots decreased linearly and significantly with decreasing water potential, indicating that the sum total of metabolic events, including substrate production and degradation, was affected by the stress condition. It is postulated that, overall, *A. hybridus* seedlings seem to maintain root growth better than that of *A. cruentus* at the higher temperature regimes (35 to 40°C) and at a water potential of -1250 kPa.

The second phase of the study involved the quantification of selected physiological parameters in terms of the response of amaranth seedlings to a rather punitive water deficit level (-1250 kPa), but at two different temperatures (30 and 35°C) that proved to be optimal for *A. cruentus* and *A. hybridus*, respectively. This was an attempt to verify the initial postulate formulated above. Firstly, the sugar content was measured in seedlings directly after removal from the incubator where seeds were allowed to germinate in the dark. In doing so the availability of sugar substrate for seedling growth, translocated from the cotyledons under two different temperature and water potential regimes, was quantified. It is important to note that no photosynthesis was possible at this stage.

In *A. cruentus* seedlings there was a significant increase in sucrose, glucose and fructose content at both 30 and 35°C when placed under water stress, but for all sugars this was about twofold lower at 35 than 30°C. In *A. hybridus* seedlings the same tendency to increase sugar content under water stress at both temperature regimes applied, but the opposite was observed at 35°C where significantly higher sugar levels, especially the monosaccharide sugars, were measured. However, when data was expressed as a percentage difference from the unstressed controls a significant increase in the glucose level was observed in *A. cruentus* compared to *A. hybridus*

seedlings. The latter might be a further indication of an attempt by seedlings of this species to sustain carbohydrate metabolism under water deficit stress, while the situation in *A. hybridus* seedlings was closer to normal or, in other words, less affected by the stress condition.

As total water soluble protein content has often been associated with and used as parameter to measure stress in plants (Ferguson *et al.*, 1994; Gifford, 2003; Dutta *et al.*, 2009), it was quantified in amaranth seedlings. Interestingly, no significant change was observed in *A. hybridus* seedlings at either of the temperature regimes whether under water stress or not. It is postulated that homeostasis in this respect was not affected in *A. hybridus* seedlings or even that this species was rather tolerant to the enforced stress. On the other hand, water soluble protein increased significantly at 35°C and -1250 kPa in *A. cruentus* seedlings. Considering the fact that 30°C proved optimal for seedling growth in this species, it seems that it was already under stress at 35°C and this was aggravated with simultaneous exposure to a rather harsh moisture stress condition. Although one parameter cannot sustain a conclusion, it seems from the above argument that *A. hybridus* seedlings are more tolerant to water stress than that of *A. cruentus*. However, if an induction of water soluble protein under stress forms part of a resistance mechanism (Dutta *et al.*, 2009), the significant increase in *A. cruentus* seedlings can indicate that an attempt was made by the latter to tolerate the stress condition. Alternatively, the latter could merely be an indication of the level of stress experienced by *A. cruentus* seedlings in terms of produced heat shock proteins (Gurley & Key, 1991). Clearly more physiological data is needed to verify this postulate.

Subsequently, the photosynthetic capacity of amaranth seedlings was measured by exposing the stressed and non-stressed amaranth seedlings to sunlight for 48 h and measuring the chlorophyll content as well as photosynthesis rate. Surprisingly, the total chlorophyll content as well as the photosynthesis rate increased significantly in *A. cruentus* seedlings at 35°C and a water potential of -1250 kPa. In the case of the latter the combined effect of 35°C and -1250 kPa at which it was tested proved to be stressful when the degree of seedling growth inhibition is considered. In *A. hybridus* seedlings, however, the chlorophyll content dropped slightly, but not significantly, while the

photosynthesis rate still increased at both 30 and 35°C and when water stressed. Although this is difficult to explain it might be a further indication that the photosynthetic capacity of *A. hybridus* seedlings was not affected severely by the drought stress condition compared to that of *A. cruentus* seedlings, indicating once again a greater ability to tolerate the stress condition in the former. Alternatively, despite the higher sensitivity of *A. cruentus* seedlings to the imposed stress condition, it still possesses the ability to restore its photosynthetic capacity if returned to normal conditions. Carotenoids are known to protect chlorophyll pigments and, therefore, the photosystem under stress conditions (Asada *et al.*, 1998; Loggini *et al.*, 1999). Interestingly, an increase in carotenoid content in *A. cruentus* seedlings under water stress at both 30 and 35°C can partially explain the latter. Further, the fact that the decrease in chlorophyll and carotenoid content observed in *A. hybridus* seedlings at 35°C and a water potential of -1250 kPa did not affect the photosynthesis rate negatively might again be indicative of its tolerance towards the stress condition.

Speculation in terms of the ability of *A. cruentus* seedlings to restore its photosynthetic capacity once returned to normal conditions after a period of stress as well as the tolerance shown by *A. hybridus* is in concert with the findings of Flexas *et al.* (2004). The authors tested a wide range of plants in terms of their photosynthetic response to heat and water stress and concluded that, in general, the photosynthesis process is rather stable in plants at a temperature range of 30-35°C, but this can differ slightly from species to species. A year earlier Chaves *et al.* (2003) reported that a temperature above 40°C significantly diminishes the photosynthetic capacity of plants. Although not measured in this study, it is deduced from growth data that *A. hybridus* seedlings, which showed normal root growth at 40°C while hypocotyl growth was not affected significantly, is more tolerant towards the higher temperature regime than *A. cruentus*, also in terms of photosynthesis capacity. The latter needs to be verified in future research.

Photosynthesis is the process that produces respiratory substrate, which in turn is translocated to all parts of the plant where energy needed for growth and development is released via aerobic respiration and this determines the energy status of a plant

(Pretorius & Small, 1992). As it is important for any plant to sustain its respiratory machinery under stress in order to survive, the respiratory rate in amaranth seedlings was, subsequently, quantified. It is important to note that the respiratory rate data obtained in this study represent respiratory capacity only as the seedlings were exposed to sunlight for 48 h after removal from the incubator and from the dark. In other words, the respiratory rates reported in this study followed a light interval where seedlings were allowed to produce respiratory substrate via photosynthesis and not directly after removal from the dark where the only substrate would have been obtained from the cotyledons.

With the above in mind, a slight increase in the respiration rate of *A. cruentus* seedlings under water stress conditions (-1250 kPa) and a slight decrease in that of *A. hybridus* was observed between 30 and 35°C. However, in neither case were the differences significant at the 5% probability level, indicating that the combined temperature and water stress condition imposed on the seedlings had no severe negative effect on the respiratory capacity of seedlings from either species. Subsequently, the *in vitro* rate of the most important regulatory enzyme of the glycolysis cycle, phospho-fructo kinase; ATP-PFK), was measured in amaranth seedlings.

It was previously reported that high temperature reduces the ATP-PFK enzyme activity as a result of heat shock effects (Tucker, 1986; Bunce, 2007). Almost two decades ago Paulsen (1994) reported that the respiration rate in plants decreases significantly at temperatures above 50°C, supposedly owing to damage to the respiratory mechanism. This was investigated in amaranth seedlings. However, in this study the highest temperature tested was 35°C, but in combination with a rather severe water potential environment of -1250 kPa. Under these circumstances a significant increase in PFK activity was observed in *A. cruentus* seedlings at 35°C and under water stress (-1250 kPa) while no significant change from the unstressed control was observed for *A. hybridus*. Once again it seemed that the latter was less affected by the stress condition

Glucose-6-phosphate dehydrogenase, the only regulatory enzyme of the oxidative pentose phosphate (OPP) pathway (Hauschild & von Schaewen, 2003), has been

positively correlated with drought stress in the past (Scharte *et al.*, 2009). Subsequently, the *in vitro* activity of this enzyme was measured in seedlings of the two amaranth species simultaneously exposed to their respective optimal temperatures (30 and 35°C) and a Ψ of -1250 kPa. According to Dal Santo *et al.* (2012), water deficit stress up-regulates G-6-PDH synthesis in *Arabidopsis* that forms part of a resistance mechanism towards drought stress. In this study this tendency was not observed for either of the two amaranth species tested. The only observation made was a slight, but non-significant, increase in G-6-PDH activity in drought stressed *A. cruentus* seedlings. Data was not convincing enough to suggest any role for this enzyme, and the OPP pathway for that matter, as being part of a possible tolerance mechanism in amaranth seedlings under drought stress.

In conclusion, from the two amaranth species tested in this study, *A. hybridus* showed better seed germination and early seedling growth than *A. cruentus*. Especially the ability of *A. hybridus* seedlings to continue with root length growth at 40°C, five degrees above its optimum, support the general postulate that this species seems to be slightly more tolerant to the higher temperature regime tested in this study. Further, even at a rather low water potential of -1250 kPa this ability was still observed. Comparatively, seedlings of *A. cruentus* did not show the same stringent ability under stress. Surprisingly, albeit sometimes slight and sometimes significant, up regulation of more than half of the quantified physiological parameters was observed in *A. cruentus* seedlings exposed to drought stress while the opposite or even no changes applied in *A. hybridus* seedlings. The fact that the growth of *A. hybridus* seedlings was rather persistent under simultaneous temperature and drought stress pointed towards its inherent tolerance. For this reason the slight, but mostly insignificant, changes in the physiological response of seedlings from this genotype is also interpreted as being in concert with the postulate formulated from growth data.

5.1 References

- ASADA, K., ENDO, T., MANO, J. & MIYAKE, C. 1998.** Molecular mechanism for relaxation of and protection from light stress. Saton K, Murata N editors. Stress responses of photosynthetic organisms. Amsterdam. Pp 37–52.
- BAVEC, T. & MLAKAR, S.G. 2002.** Effects of soil and climate condition on emergence of grain amaranths. *European Journal of Agronomy* 17, 93-103.
- BUNCE, J.A. 2007.** Direct and acclamatory responses of dark respiration and translocation to temperature. *Annals of Botany* 100, 67-73.
- CHAVES, M.M., MAROCO, J.P. & PEREIRA, J.S. 2003.** Understanding plant response to drought: from genes to the whole plant. *Functional Plant Biology* 30, 239–264.
- DAL SANTO, S., STAMPFL, H., KRASENSKY, J., KEMPA, S., GIBON, Y., PETUTSCHNIG, E., ROZHON, W., HEUCK, A., CLAUSEN, T. & JONAK, C. 2012.** Stress-induced GSK3 regulates the redox stress response by phosphorylating glucose-6-phosphate dehydrogenase in Arabidopsis. *The Plant Cell* 24, 3380-3392.
- DIELEMAN, A. HAMILL, A.S., FOX, G.C. & SWANTON, C.J. 1997.** Decision rules for post emergence control of pigweed (*Amaranthus* spp) in soybean (*Glycine max*). *Weed Science* 44, 126-132.
- DUTTA, S., MOHANTY,S. & TRIPATHY, B.C. 2009.** Role of temperature stress on chloroplast biogenesis and protein import in pea. *Plant Physiology* 150, 1050–1061.
- FERGUSON, I.B., LURIE, S. & BOWEN, J.H. 1994.** Protein synthesis and break down during heat shock of cultured pear (*Pyrus communis* L.) cells. *Plant Physiology* 104, 1429-1437.
- FLEXAS, J., BOTA, J., LORETO, F., CORNIC, G. & SHARKEY, T.D. 2004.** Diffusive and metabolic limitations to photosynthesis under drought and salinity in C4 plants. *Plant Biology* 6: 269 – 279.

- GIFFORD, R.M, 2003.** Plant respiration in productivity model: Conceptualization, respiration and issues for global terrestrial carbon cycle research. *Functional Plant Biology* 3, 171-186.
- GILL, P.K., SHARMA, A.D., SINGH, P. & SUKDEV SINGH BHULLAR, S.S. 2001.** Effect of various abiotic stresses on the growth, soluble sugars and water relations of sorghum seedlings grown in light and darkness. *Bulgarian Journal of Plant Physiology* 27, 72–84.
- GUPTA, A.K. & KAUR, N. 2005.** Sugar signaling and gene expression in relation to carbohydrate metabolism under abiotic stresses in plants. *Journal of Bioscience* 30: 761-776.
- GURLEY, W.B. & KEY, J.L. 1991.** Transcriptional regulation of the heat-shock response: A plant perspective. *Biochemistry* 30, 1-12.
- HARTMANN, H.T., KESTER, D.E., JR, F.T.D. & GENEVE, R.L. 2011.** Plant propagation principles and practices. Prentice Hall Publishers, New Jersey. pp 356-420.
- HAUSCHILD, R. & VON SCHAEWEN, A. 2003.** Differential regulation of glucose-6-phosphate dehydrogenase isoenzyme activities in potato. *Plant Physiology* 133, 47-62.
- INTERNATIONAL SEED TESTING ASSOCIATION (ISTA). 2010.** International Rules for Seed Testing. Bassersdorf, Swatzerland.
- JIE, Z., XIAODONG, J., TIANLAI, L. & ZAIQIANG, Y. 2012.** Effect of moderately-high temperature stress on photosynthesis and carbohydrate metabolism in tomato (*Lycopersico esculentum* L.) leaves. *African Journal of Agricultural Research* 7(3), 487-492.
- KAYA, M.D., OKCU, G., ATAK, M., CIKILI, Y. & KOLSARICI, O. 2006.** Seed treatment to overcome salt and drought stress during germination in sunflower (*Helinathus annus*). *European Journal of Agronomy* 24(24), 291-215.

- KIGEL, J., OFIR, M. & KOLLER, D. 1977.** Control of germination responses of *Amaranthus retroflexus* L. seed by their parental photo thermal environment. *Journal of Experimental Botany* 106, 1125-1136.
- LIU, F. & STUTZEL, H. 2004.** Leaf water relations of vegetable amaranth (*Amaranthus* spp.) in response to soil drying. *European Journal of Agronomy* 16, 137-150.
- LOGGINI, B., SCARTAZZA, A., BRUGNOLI, E. & NAVARI-IZZO, F. 1999.** Antioxidant defenses system, pigment composition and photosynthetic efficiency in two wheat cultivars subjected to drought. *Plant Physiology* 119, 1091–1099.
- MICHAEL, B.E. 1983.** Evaluation of the water potentials of solutions of polyethylene glycol 8000 both in absence and presence of other substances. *Plant Physiology* 72, 66-70.
- MUSTAFA, A., AMAN, P., ANDERSSON, R. & KAMAL-ELDIN, A. 2007.** Analysis of free amino acids in cereal products. *Food Chemistry* 105, 317–324.
- NTULI, T.M., 2012.** Drought and Desiccation-Tolerance and Sensitivity in Plants. *Botany*, 67, 435-441.
- ORYOKOT, J.O.E., MURPHY, S.D., THOMAS, A.G. & SWANTON, C.J. 1997.** Temperature and moisture dependent models of seed germination and shoot elongation in green and redroot pigweed (*Amaranthus powellii*, *Amaranthus retroflexus*). *Weed Science* 45, 488-496.
- OYODELE, V.I. 2000.** Influence of soil water stress at different physiological stages on drought and seed yield of amaranth species. *Acta Horticulture* 357, 114-121.
- PAULSEN, G. M. 1994.** High temperature responses of crop plants. In K.J. Boote, J.M Bennett, T.R. Sinclair & G. M. Paulsen (eds). Physiology and determination of crop yield. Pp. 365–389. ASA, CSSA, SSSA, Madison.
- PRETORIUS, J.C. & SMALL, J.G.C. 1992.** The effect of soaking injury in bean seeds on aspects of the oxidative pentose phosphate pathway in embryonic axes. *Seed Science Research*. 2:33–39.

- SCHARTE, J., SCHÖN, H., AND WEIS, E. 2009.** Photosynthesis and carbohydrate metabolism in tobacco leaves during an incompatible interaction with *Phytophthora nicotianae*. *Plant Cell Environment* 28, 1421-1435.
- STECKEL, L., CRISTY, L.S., EDWARD, W.S. & LOYD, M.W. 2004.** Temperature effects on germination of nine *Amaranthus* species. *Weed Science* 52, 217-221.
- SUN, Y., DU, X., ZHANG, W & LI, R. 2011.** Seed germination and physiological characteristics of *Amaranthus* L. under drought stress. *Journal of Advanced Material Research* 1071, 183-185.
- THOMAS, W.E., BURKE, I.C., SPEARS, J.F. & WILCUT, J.W. 2006.** Influence of environmental factors on slender amaranth (*Amaranthus viridis*) germination. *Weed Science* 54, 316-320.
- TUCKER, J.B., 1986.** The once and future crop.
<http://www.hort.purdue.edu/newcrop/proceedings> (Accessed 28/09/2012)

SUMMARY

Several environmental factors constantly play a role in crop failures. Of these high temperature and limited water supply are major factors that limit plant productivity and threatens food security. Hence, a search for alternative crops with good tolerance characteristics towards abiotic stress is an ongoing process. Amaranth has been established as an annual vegetable and grain crop and is seen as a prospective alternative crop. Seed germination and seedling development are presumed to be the most critical stages in the life cycle of many seed propagated crops. This served as a rationale for the underlying study on two amaranth species in terms of seed germination, as well as the morphological and physiological response of seedlings to different temperature (25, 30, 35, 40°C) and water potential (0, -250, -500, -750, -1000, -1250 kPa) regimes.

For *A. cruentus* the optimum temperature was between 25 and 30°C and between 30 and 35°C for *A. hybridus* in terms of seed germination and early seedling growth. *A. hybridus* showed a greater level of adaptation to the higher temperature regimes as well as when simultaneously exposed to a rather stringent water potential of -1250 kPa by maintaining root growth better than *A. cruentus*. Moreover, the imposed temperature/drought stress condition had no significant effect on either of the physiological parameters tested in the former species. These included sugar and total water soluble protein levels as well as photosynthesis and respiratory capacity. Together with the ability of stressed seedlings to maintain growth, the latter strongly suggests that the metabolic events were scarcely affected in *A. hybridus*, supporting the postulate that it showed a higher degree of tolerance towards abiotic stress conditions. Alternatively, more than half of these events were found to be upgraded in *A. cruentus* seedlings and interpreted as an attempt by this species to counteract the stress effects, but not successfully, as measured by its inability to maintain seedling growth under these stress conditions.

Keywords: Seed germination, amaranth, heat stress, drought stress, metabolic response, seedling growth.

OPSOMMING

Verskeie omgewingsfaktore speel 'n rol in oesverliese. Van hierdie is hoë temperatuur en beperkte watervoorsiening die hooforsake wat plantproduktiwiteit beperk en voedselsekuriteit bedreig. Gevolglik is 'n soeke na alternatiewe gewasse met uitstekende weerstandseienskappe t.o.v. abiotiese stremming 'n aanhoudende proses. *Amaranthus* is reeds as 'n eenjarige groente- en graangewas gevestig en word beskou as 'n potensiële alternatiewe gewas. Saadkieming en vroeë saailingontwikkeling is van die mees kritieke ontwikkelingsfases in die lewensiklus van baie saad gepropageerde gewasse. Laasgenoemde het die rasionaal verskaf vir die onderhawige studie op twee *Amaranthus* spesies in terme van saadkieming sowel as die morfologiese en fisiologiese respons van saailinge op verskillende temperatuur (25, 30, 35, 40°C) en water potensiaal (0, -250, -500, -750, -1000, -1250 kPa) reeks.

Vir *A. cruentus* was die optimum temperatuur tussen 25 en 30°C en tussen 30 en 35°C vir *A. hybridus* in terme van beide saadkieming en vroeë saailinggroei. *A. hybridus* het egter 'n hoër mate van aanpassing by die boonste gedeelte van die temperatuurreeks asook tydens die gesamentlike blootstelling aan 'n taamlik streng waterpotensiaal van -1250 kPa getoon deur wortelgroei beter te onderhou as *A. cruentus*. Meer nog het die temperatuur/droogte stremming wat opgelê is geen betekenisvolle effek op enige van die fisiologiese parameters wat getoets is in eersgenoemde spesies getoon nie. Hierdie parameters het suiker en totale wateroplosbare proteïen inhoud asook fotosintese en respirasiepotensiaal ingesluit. Tesame met die vermoë van gestremde saailinge om groei te onderhou, onder hierdie omstandighede, ondersteun laasgenoemde die postulaat dat metaboliese aktiwiteite skaars in *A. hybridus* beïnvloed is en dat hierdie spesies inherent oor 'n hoër mate van weerstand teen abiotiese stremmingsfaktore beskik. Alternatiewelik is meer as die helfte van die fisiologiese prosesse deur die stremming in *A. cruentus* saailinge verhoog wat slegs geïnterpreteer word as 'n poging deur die spesies om die stremming teë te werk maar sonder sukses, gemeet aan die onvermoë om saailinggroei te onderhou onder hierdie omstandighede.

Sleutelwoorde: Saadkieming, *Amaranthus*, hittestremming, droogtestremming, metaboliese respons, saailinggroei.